Plasma 25-hydroxyvitamin D concentration and subsequent risk of total and site specific cancers in Japanese population: large case-cohort study within Japan Public Health Center-based Prospective Study cohort

Sanjeev Budhathoki,[1] Akihisa Hidaka,[1] Taiki Yamaji,[1] Norie Sawada,[1] Sachiko Tanaka-Mizuno,[2] Aya Kuchiba,[3,4] Hadrien Charvat,[1] Atsushi Goto,[1] Satoshi Kojima,[5] Natsuki Sudo,[5] Taichi Shimazu,[1] Shizuka Sasazuki,[1] Manami Inoue,[1] Shoichiro Tsugane,[1] Motoki Iwasaki,[1] for the Japan Public Health Center-based Prospective Study Group

[1]Epidemiology and Prevention Group, Center for Public Health Sciences, National Cancer Center, Chuo-ku, Tokyo, 104-0045, Japan

[2]Department of Medical Statistics, Shiga University of Medical Science, Ohtsu, Shiga, 520-2192, Japan

[3]Division of Biostatistical Research, Center for Public Health Sciences, National Cancer Center, Chuo-ku, Tokyo, 104-0045, Japan

[4]Biostatistics Division, Center for Research Administration and Support, National Cancer Center, Chuo-ku, Tokyo, 104-0045, Japan

[5]CL New Product Development Department, Fujirebio Inc., Hachioji-shi, Tokyo, 192-0031, Japan

Correspondence to: T Yamaji tyamaji@ncc.go.jp

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ABSTRACT

OBJECTIVE

To evaluate the association between pre-diagnostic circulating vitamin D concentration and the subsequent risk of overall and site specific cancer in a large cohort study.

DESIGN

Nested case-cohort study within the Japan Public Health Center-based Prospective Study cohort.

SETTING

Nine public health centre areas across Japan.

PARTICIPANTS

3301 incident cases of cancer and 4044 randomly selected subcohort participants.

EXPOSURE

Plasma concentration of 25-hydroxyvitamin D measured by enzyme immunoassay. Participants were divided into quarters based on the sex and season specific distribution of 25-hydroxyvitamin D among subcohorts. Weighted Cox proportional hazard models were used to calculate the multivariable adjusted hazard ratios for overall and site specific cancer across categories of 25-hydroxyvitamin D concentration, with the lowest quarter as the reference.

MAIN OUTCOME MEASURE

Incidence of overall or site specific cancer.

RESULTS

Plasma 25-hydroxyvitamin D concentration was inversely associated with the risk of total cancer, with multivariable adjusted hazard ratios for the second to fourth quarters compared with the lowest quarter of 0.81 (95% confidence interval 0.70 to 0.94), 0.75 (0.65 to 0.87), and 0.78 (0.67 to 0.91), respectively (P for trend=0.001). Among the findings for cancers at specific sites, an inverse association was found for liver cancer, with corresponding hazard ratios of 0.70 (0.44 to 1.13), 0.65 (0.40 to 1.06), and 0.45 (0.26 to 0.79) (P for trend=0.006). A sensitivity analysis showed that alternately removing cases of cancer at one specific site from total cancer cases did not substantially change the overall hazard ratios.

CONCLUSIONS

In this large prospective study, higher vitamin D concentration was associated with lower risk of total cancer. These findings support the hypothesis that vitamin D has protective effects against cancers at many sites.

WHAT IS ALREADY KNOWN ON THIS TOPIC

As a precursor to a potent bioactive compound with diverse antineoplastic properties, vitamin D has been hypothesised to confer protection against cancer

Although circulating concentration of vitamin D has been associated with a lower incidence of colorectal and lung cancers, evidence for cancer at other sites, as well as for cancer overall, remains inconsistent

Evidence on this question from Asian populations is limited

WHAT THIS STUDY ADDS

In this population based prospective cohort study of a Japanese population, higher vitamin D concentration was associated with a reduced risk of overall cancer in both men and women

A decreased risk of liver cancer was also associated with a high vitamin D concentration

The results support the hypothesis that vitamin D might have beneficial effects in cancer prevention

Introduction

Although the beneficial effects of vitamin D in the prevention of skeletal disorders have long been recognised, accumulating evidence suggests that the benefits may extend beyond bone health to include several chronic diseases, including cancer.[1 2] In vitro studies have shown that vitamin D exerts antiproliferative and pro-differentiating effects on malignant cells through the regulation of multiple signalling pathways involved in cell cycle arrest, apoptosis, angiogenesis, and inflammation.[1 3 4] Experimental animal models have shown that activation of the vitamin D endocrine axis by vitamin D or its analogues inhibits the development and progression of tumours of the colon, breast, prostate, and other tissues, supporting a chemopreventive role of vitamin D in carcinogenesis.[5 6] The mechanisms involved in mediating these anticarcinogenic actions of vitamin D are attributed to 1,25-dihydroxyvitamin D, the active metabolite of vitamin D, which is produced from circulating 25-hydroxyvitamin D by the enzyme 1α-hydroxylase (CYP27B1).[6 7] Because many tissues in the body express 1α-hydroxylase, and because an even wider range of tissues possess receptors for 1,25-dihydroxyvitamin D,[5 7] the anticarcinogenic effect of vitamin D is probably not limited to a single organ or tissue in the body. The inverse correlation observed between exposure to sunlight and mortality from colon cancer, on the basis of which the hypothesis

implicating vitamin D in cancer was first proposed, has been observed with at least 15 other types of cancer.[8] Despite the strong ecological and experimental animal evidence, however, evidence linking circulating concentration of vitamin D to the overall cancer risk in humans is sparse and inconsistent.[9-17]

Among findings from epidemiological studies, circulating vitamin D concentration was associated with the risk of total cancer in one study but not in others.[9-13] These inconsistent findings may be due to differences in mean 25-hydroxyvitamin D concentration, 25-hydroxyvitamin D assay methods and categorisation, and covariate adjustment, although a recent meta-analysis that included some of the abovementioned studies denied such possibilities.[18] Of note, following this meta-analysis, which suggested a moderate inverse association of 25-hydroxyvitamin D concentration with incidence of total cancer, few other studies have been published to date, and no study from an Asian population has yet appeared. Many of the existing studies had relatively few cases of cancer, ranging from 335 to 1134,[10-12] and even the largest two had at most around 2500 cases.[9 13] In contrast to the case of total cancers, epidemiological findings on circulating vitamin D are abundant for site specific cancers, particularly those of the colorectum, lung, breast, and prostate, and meta-analyses seem to show an inverse association with colorectal and lung cancer,[19-22] a suggestive inverse association with breast cancer,[23] and mostly a null or even positive association with prostate cancer.[24-26] However, most of the many prospective studies of circulating vitamin D concentration and site specific cancers were conducted in European or American populations, and only a few studies in Asian populations have been reported.[27-31] Given that vitamin D concentrations and metabolism vary substantially by race/ethnicity,[32 33] whether similar associations would also be observed in non-Caucasian populations remains to be clarified.

Against this background, we designed a case-cohort study that measured plasma 25-hydroxyvitamin D concentration in the largest number of cancer cases to date (n=3301) within a Japanese population of the Japan Public Health Center-based Prospective (JPHC) Study and evaluated whether the circulating concentration of 25-hydroxyvitamin D was associated with the risk of total and site specific cancer.

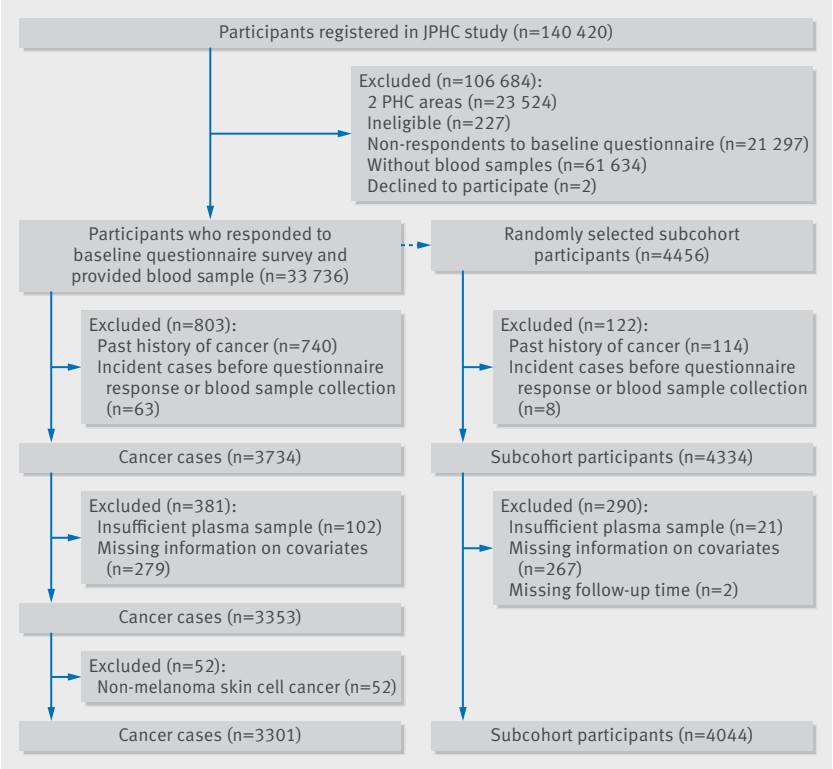

Fig 1 | Selection of cases and subcohort participants in Japan Public Health Center-based Prospective (JPHC) Study. PHC=public health centre

Methods

Study population

The JPHC Study is an ongoing population based cohort study investigating the role of lifestyle and other factors in the risk of cancer and other chronic diseases.[27 34] In brief, the study was started in 1990 with the enrolment of 61 595 registered residents aged 40-59 years from five public health centre areas across Japan (cohort I) and expanded in 1993 with a further 78 825 people aged 40-69 years from six other public health centre areas (cohort II), giving a total study enrolment of 140 420 participants. At baseline, all enrolled participants were encouraged to answer self administered survey questionnaires that included questions on demographic characteristics, past medical history, and lifestyle related factors. Of these, 113 461 (81%) participants also completed a validated food frequency questionnaire, and 49 011 participants also voluntarily donated 10 mL of venous blood during their health check-ups. We excluded 23 524 participants from two public health centre areas (because of a lack of cancer incidence data in one and different inclusion criteria in the second), as well as 227 ineligible participants (non-Japanese nationality, late report of emigration occurring before the start of follow-up, excluded age, and duplicate registration) (fig 1). For this (case-cohort) study, we limited study participants to those who had both responded to the baseline questionnaire and provided blood samples. After excluding a further two participants who declined the use of their blood samples for biomarker measurement, we finally defined a base cohort of 33 736 participants.

Follow-up and case-cohort selection

All cohort members were followed from the start of the study period to 31 December 2009. During the follow-up period, changes in residence status and survival status were ascertained annually through the residential registry of each public health centre area. Incident cancer cases during follow-up were identified by active patient notification from major local hospitals in the study area and data linkage with population based cancer registries. We used death certificates to supplement information on cancer

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incidence. Incidence of total cancer and site specific cancer was coded according to the international classification of diseases for oncology, 3rd edition (ICD-O-3), code C00-C97 (supplementary table A). Among the base cohort population, we excluded a further 740 participants who were found to have a past history of cancer and 63 participants who had incident cancer before the start of follow-up or before blood sample collection. We identified 3734 participants who had a new occurrence of cancer during a median follow-up of 15.9 years. The proportion of cases that were ascertained by death certificates only was 7.9% (294/3734 cases), indicating that the former two methods covered case ascertainment well. After further exclusion of 102 participants with insufficient plasma sample for 25-hydroxyvitamin D assay, 279 with missing information on covariates, and 52 with non-melanoma skin cell cancers, we included 3301 cases of cancer in the analysis (fig 1).

We randomly selected a subcohort sample of 4456 participants from the 33 736 base cohort, accounting for about 13% of the base cohort participants. As with the cases, we excluded 114 subcohort participant with a past history of cancer, eight with cancer before the start of follow-up or before blood sample collection, 21 with insufficient plasma sample, 267 with missing information on covariates, and two with missing follow-up time, leaving 4044 subcohort participants in the analysis (fig 1). We identified 450 cases of cancer in 4044 subcohort participants.

Plasma vitamin D concentration measurement

The blood samples donated at the baseline health check-up were collected into vacutainer tubes containing heparin, centrifuged to obtain the plasma and buffy coat layers, and then stored at −80°C until analysis. Baseline plasma 25-hydroxyvitamin D concentration was determined with the Lumipulse G 25-OH Vitamin D assay (Fujirebio Inc, Japan) based on chemiluminescent enzyme immunoassay by a two step sandwich immunoassay method at Fujirebio Research Laboratories, Japan. Laboratory personnel were blinded to case or subcohort status. Ten samples from two people were interspersed with other study samples on two different days for quality control measures. All intra-assay and inter-assay coefficients of variation were 2.4% or lower. We selected a case-cohort design for this study, which allowed us to evaluate multiple cancer endpoints simultaneously while using the common subcohort samples. In our previous nested case-control studies for colorectal and prostate cancer, we measured plasma 25-hydroxyvitamin D.[27 28] However, we used extended follow-up time and hence an increased number of cases compared with previous reports, and 25-hydroxyvitamin D assays for all samples in this analysis were newly performed.

Statistical analysis

We calculated follow-up time for each participant from the date of response to the baseline questionnaire until the date of diagnosis of cancer, date of moving out of the study area, date of death, or 31 December 2009, whichever occurred first. Participants lost to follow-up were censored on the last confirmed date of presence in the study area. We restricted all cases to the first incident cancer. For analysis of cancer at a specific site, cancers at other sites were excluded from the analysis, whereas analysis for total cancer was based on all cancer events taken as a single endpoint irrespective of site.

Plasma concentration of 25-hydroxyvitamin D tended to be higher in participants sampled during the summer (June-August) and autumn (September-November) months and lower in participants sampled during the winter (December-February) and spring (March-May) months (supplementary figure A). To account for seasonal variability, we created quantile based categories according to sex and season specific distribution of 25-hydroxyvitamin D concentration. We created quartile based categories for analysis of cancer endpoints with a relatively large number of cases, and tertile based categories for endpoints with a smaller number of cases (<130 cases), with the cut-off definitions for both quartile (supplementary table B) and tertile based categories based on 25-hydroxyvitamin D distributions among subcohorts. We used weighted Cox proportional hazards models to estimate hazard ratios and 95% confidence intervals for the association between 25-hydroxyvitamin D and incidence of total and site specific cancer, with the lowest category as reference. To take into account the case-cohort design of the study, wherein all incident cancer cases but only a randomly selected fraction of the cohort participants (namely, subcohorts) were included, we used the inverse subcohort sampling probability weighting method to calculate the pseudolikelihood function and used robust sandwich estimation of the covariance matrix of the model parameters.[35] Two models with a priori selected covariates were fitted: the crude model included age and sex only, and the multivariable model further included body mass index (<21, 21-<25, 25-<30, ≥30), leisure time physical activity (<1 or ≥1 times/week), smoking (never, past/current smoker with <20, 20-<40 or ≥40 pack years of smoking), alcohol drinking (never, occasional, current drinker with <150, 150-<300, 300-<450, or ≥450 g/week of ethanol), family history of any cancer, and history of diabetes. For analysis of breast, ovarian, and uterine cancers, the multivariable model was further adjusted for age at menarche (≤13, 14, 15, ≥16 years), number of births (0, 1, 2, 3, ≥4), exogenous female hormone use (yes, no), menopausal status and age at menopause (premenopausal or postmenopausal at <48, 48-<51, ≥51 years). Women with missing information for these variables were not used in this analysis, including 27 cases for breast cancer, seven cases for ovarian cancer, and two cases for uterine cancer. In an additional analysis for liver cancer, further adjustment was made for hepatitis B and hepatitis C virus infection status (positive or negative) and alanine aminotransferase concentration (<30, 30-<70, ≥70 IU/L) in the multivariable model among participants with available information (110 cases

and 2297 subcohort participants). All models were stratified by public health centre. We tested for linear trend across categories of 25-hydroxyvitamin D concentration by assigning ordinal scores to each quantile based category of 25-hydroxyvitamin D. We did sex specific analyses to assess whether the 25-hydroxyvitamin D/cancer association differed by sexes; we assessed interaction between vitamin D and sex by using multivariate Wald tests of the product terms between 25-hydroxyvitamin D concentrations and sex. We also did several sensitivity analyses to examine the robustness of the findings, including analyses excluding cases of cancer diagnosed in the first three years of follow-up, excluding people with supplemental vitamin use, and alternately excluding all cases of cancer at a specific site from the total cancer cases.

We tested for the proportional hazards assumption by using correlation tests based on Schoenfeld residuals modified for the case-cohort design.[36] We calculated statistical powers for our study to detect an effect of vitamin D on occurrence of cancer by using an extension of the log-rank test for the case-cohort design,[37] the results of which are shown in supplementary table C. P values were two sided, and we defined statistical significance as P<0.05. We used SAS version 9.3 for all statistical analyses, except for the test of proportional hazard assumption, for which we used R version 3.2.3 software (survival package).

Patient involvement

Patients were not directly involved in the setting of the research questions or outcome measures, nor in the design or implementation of the study. Owing to the ongoing and community based nature of the JPHC Study, we have held annual meetings to communicate closely with health practitioners belonging to local municipalities and public health centres in the study area and have appreciated the receipt of many valuable opinions related to their health practice since the beginning of the study. During the same period, we have also sent several newsletters of our research findings to the study participants, which might have helped them to maintain their health, and have provided public lectures on disease prevention for the study communities, which may help to benefit potential patients and care givers. Also, we have updated our research results on our website (http://epi.ncc.go.jp/index.html), which is visited by people from all over the country.

Results

Table 1 shows selected demographic and lifestyle characteristics of cases and subcohorts. Compared with subcohorts, cases seemed to be older, were more likely to be male and heavy smokers and drinkers, and had a higher frequency of a history of diabetes and family history of cancer. Median 25-hydroxyvitamin D concentration was higher in plasma samples collected during the summer and autumn months than in those collected during the winter or spring (P<0.001 among subcohort). Participants in the higher quarters of sex/season specific 25-hydroxyvitamin D (table 2) tended to be older and more physically active in leisure time and were less likely to have diabetes or a family history of cancer than were participants in the lower 25-hydroxyvitamin D quarter. Compared with participants in the lowest quarter of 25-hydroxyvitamin D, the proportion of never smokers was high, whereas that of never drinkers was low in the highest quarter,

Table 1 | Characteristics of cases and subcohort samples. Values are numbers (percentages) unless stated otherwise

Characteristic	Cases (n=3301)	Subcohort (n=4044)	Base cohort (n=33 736)*
Male sex	1730 (52.4)	1384 (34.2)	12 090 (35.8)
Mean (SD) age, years	56.2 (7.5)	53.7 (7.9)	53.9 (8.0)
Mean (SD) body mass index	23.6 (3.0)	23.6 (3.0)	23.6 (3.0)
Physical activity† (≥1 day/week)	623 (18.9)	724 (17.9)	5859/32 493 (18.0)
Smoking:			(n=32 363)
Never smoker	1873 (56.7)	2939 (72.7)	23 363 (72.0)
<20 pack years of smoking	306 (9.3)	380 (9.4)	3000 (9.3)
20-<40 pack years of smoking	652 (19.8)	472 (11.7)	3835 (11.8)
≥40 pack years of smoking	470 (14.2)	253 (6.3)	2265 (7.0)
Alcohol drinking:			(n=31 952)
Never drinker	1724 (52.2)	2508 (62.0)	19 708 (61.7)
Occasional drinker	248 (7.5)	363 (9.0)	2854 (8.9)
<150 g/week	469 (14.2)	499 (12.3)	4028 (12.6)
150-<300 g/week	438 (13.3)	362 (9.0)	2857 (8.9)
≥300 g/week	422 (12.8)	312 (7.7)	2505 (7.8)
History of diabetes	216 (6.5)	168 (4.2)	1355 (4.2)
Family history of cancer	803 (24.3)	908 (22.5)	6994/32 949 (21.2)
Supplemental vitamin use	499 (15.1)	587 (14.5)	4718/29 979 (15.7)
Median (IQR) plasma vitamin D, nmol/L:			
Winter	46.7 (37.7-54.7)	46.4 (36.7-57.7)	-
Spring	48.4 (39.4-60.9)	47.4 (38.6-57.2)	-
Summer	58.4 (47.4-70.4)	55.4 (44.7-66.4)	-
Autumn	58.4 (46.9-70.4)	55.9 (45.7-66.4)	-

IQR=interquartile range.
*Numbers may not add up to total base cohort population for some characteristics owing to missing information for some observations.
†Leisure time physical activity.

Table 2 | Selected characteristics of subcohort participants according to sex and season specific quarters of plasma vitamin D. Values are numbers (percentages) unless stated otherwise

Characteristic	Quarter of plasma vitamin D			
	1 (low; n=1004)	2 (second; n=1000)	3 (third; n=1016)	4 (high; n=1024)
Male sex	342 (34.1)	346 (34.6)	347 (34.2)	349 (34.1)
Mean (SD) age, years	51.9 (7.9)	53.6 (7.8)	54.2 (8.0)	55.2 (7.6)
Mean (SD) body mass index	23.6 (3.1)	23.7 (3.1)	23.5 (2.9)	23.6 (3.0)
Physical activity* (≥1 day/week)	169 (16.8)	180 (18.0)	195 (19.2)	180 (17.6)
Smoking:				
Never smoker	728 (72.5)	715 (71.5)	727 (71.6)	769 (75.1)
<20 pack years of smoking	101 (10.1)	91 (9.1)	102 (10.0)	86 (8.4)
20-<40 pack years of smoking	121 (12.1)	112 (11.2)	127 (12.5)	112 (10.9)
≥40 pack years of smoking	54 (5.4)	82 (8.2)	60 (5.9)	57 (5.6)
Alcohol drinking:				
Never drinker	649 (64.6)	614 (61.4)	622 (61.2)	623 (60.8)
Occasional drinker	95 (9.5)	90 (9.0)	89 (8.8)	89 (8.7)
<150 g/week	118 (11.8)	136 (13.6)	130 (12.8)	115 (11.2)
150-<300 g/week	89 (8.9)	84 (8.4)	89 (8.8)	100 (9.8)
≥300 g/week	53 (5.3)	76 (7.6)	86 (8.5)	97 (9.5)
History of diabetes	43 (4.3)	36 (3.6)	51 (5.0)	38 (3.7)
Family history of cancer	245 (24.4)	242 (24.2)	214 (21.1)	207 (20.2)
Supplemental vitamin use	141 (14.0)	146 (14.6)	168 (16.5)	132 (12.9)
Median (IQR) plasma vitamin D, nmol/L	36.9 (31.2-41.2)	48.4 (43.9-53.9)	56.9 (52.9-63.9)	72.6 (64.6-82.4)
Mean (SD) plasma vitamin D, nmol/L	36.3 (8.4)	49.1 (6.8)	58.3 (7.8)	75.9 (16.6)

IQR=interquartile range.
*Leisure time physical activity.

but proportions did not seem to differ with regard to other characteristics, including body mass index.

Main analyses

A higher 25-hydroxyvitamin D concentration was associated with a lower risk of total cancer (table 3). Compared with participants in the first quarter, the multivariable adjusted hazard ratios for those in the second to fourth quarters were 0.81 (95% confidence interval 0.70 to 0.94), 0.75 (0.65 to 0.87), and 0.78 (0.67 to 0.91), respectively (P for trend=0.001). Reanalysis of the association with total cancer in quintile based categories showed a similar association, with hazard ratios from the second to the fifth categories compared with the first category of 0.93 (0.79 to 1.09), 0.76 (0.65 to 0.90), 0.74 (0.63 to 0.88), and 0.83 (0.70 to 0.98), respectively (P for trend=0.003).

Among the findings for cancers at specific sites (table 3), 25-hydroxyvitamin D concentration showed a significant inverse association with the risk of liver cancer (P for trend=0.006). Further adjustment for dietary factors such as intake of total energy, fruits and vegetables, meat, fish and shellfish, isoflavone, green tea, and coffee slightly attenuated the association, but it remained significant, with hazard ratios from the second to fourth quarters compared with the first quarter of 0.79 (0.48 to 1.28), 0.71 (0.43 to 1.18), and 0.51 (0.29 to 0.90), respectively (P for trend=0.02). In a subset of the population with available information, further adjustment for hepatitis B and hepatitis C virus infection status and alanine aminotransferase concentration in the multivariable model did not appreciably alter the association of 25-hydroxyvitamin D with liver cancer. Compared with the first quarter, the hazard ratios from the second to fourth quarters before adjustment for these factors were 0.43 (0.23 to 0.79), 0.51 (0.27 to 0.96), and 0.40 (0.21 to 0.77) respectively (P for trend=0.03); after adjustment they were 0.37 (0.17 to 0.81), 0.52 (0.22 to 1.19), and 0.20 (0.08 to 0.53), respectively (P for trend=0.004). For other sites, we observed a statistically significant inverse association for premenopausal breast cancer (P for trend=0.03) but not for lung cancer (P for trend=0.06) or prostate cancer (P for trend=0.07). Analysis of prostate cancer by disease progression showed hazard ratios from the second to fourth quarter compared with the first quarter of 0.78 (0.47 to 1.30), 0.81 (0.48 to 1.36), and 0.71 (0.41 to 1.23), respectively, for localised cancer (P for trend=0.27) and 1.19 (0.54 to 2.60), 0.96 (0.43 to 2.13), and 0.59 (0.24 to 1.45), respectively, for advanced cancer (P for trend=0.20). We found no clear associations for cancers with a relatively small number of cases (table 4).

Subgroup and sensitivity analyses

Subgroup analysis by sex showed no evidence of a significant difference in the effect of vitamin D between sexes (supplementary tables D and E). In sensitivity analyses, exclusion of cases of cancer diagnosed in the first three years of follow-up did not appreciably alter the findings for total cancer (hazard ratio for highest versus lowest quarter 0.81, 0.69 to 0.95; P for trend=0.005) and liver cancer (0.43, 0.24 to 0.78; P for trend=0.0045). Likewise, the associations remained unchanged after exclusion of 1836 participants with supplemental vitamin use (data not shown). Additional adjustment for occupational status in the multivariable model did not largely alter the risk estimates (data not shown). To test whether the 25-hydroxyvitamin D/total cancer association was driven by (associations with) any one specific cancer site (in particular by liver, lung, prostate, and breast cancer), we reanalysed

Table 3 | Hazard ratios (HRs) for total and site specific* cancer according to quarters of plasma vitamin D

	Quarters of plasma vitamin D				
	1 (low)	2 (second)	3 (third)	4 (high)	P for trend
All cancer					
No of cases	840	792	795	874	
HR (95% CI)†	1 (reference)	0.84 (0.73 to 0.97)	0.79 (0.68 to 0.91)	0.80 (0.69 to 0.93)	0.003
HR (95% CI)‡	1 (reference)	0.81 (0.70 to 0.94)	0.75 (0.65 to 0.87)	0.78 (0.67 to 0.91)	0.001
Gastric cancer					
No of cases	153	156	157	171	
HR (95% CI)†	1 (reference)	0.98 (0.77 to 1.27)	0.97 (0.75 to 1.26)	1.04 (0.81 to 1.36)	0.78
HR (95% CI)‡	1 (reference)	0.97 (0.75 to 1.25)	0.92 (0.71 to 1.20)	0.99 (0.76 to 1.29)	0.88
Colorectal cancer					
No of cases	134	165	160	178	
HR (95% CI)†	1 (reference)	1.11 (0.86 to 1.42)	0.98 (0.76 to 1.28)	0.97 (0.75 to 1.26)	0.59
HR (95% CI)‡	1 (reference)	1.08 (0.84 to 1.39)	0.96 (0.74 to 1.26)	0.95 (0.73 to 1.23)	0.48
Colon cancer					
No of cases	89	115	115	133	
HR (95% CI)†	1 (reference)	1.12 (0.83 to 1.51)	1.00 (0.73 to 1.36)	1.01 (0.74 to 1.37)	0.80
HR (95% CI)‡	1 (reference)	1.09 (0.80 to 1.47)	0.97 (0.71 to 1.33)	0.98 (0.72 to 1.33)	0.68
Rectal cancer					
No of cases	46	50	47	47	
HR (95% CI)†	1 (reference)	1.08 (0.71 to 1.64)	0.99 (0.64 to 1.54)	0.93 (0.60 to 1.46)	0.68
HR (95% CI)‡	1 (reference)	1.07 (0.70 to 1.64)	1.00 (0.63 to 1.56)	0.92 (0.58 to 1.46)	0.66
Liver cancer					
No of cases	47	43	41	34	
HR (95% CI)†	1 (reference)	0.72 (0.46 to 1.13)	0.65 (0.41 to 1.04)	0.45 (0.27 to 0.77)	0.004
HR (95% CI)‡	1 (reference)	0.70 (0.44 to 1.13)	0.65 (0.40 to 1.06)	0.45 (0.26 to 0.79)	0.006
Lung cancer					
No of cases	109	87	88	112	
HR (95% CI)†	1 (reference)	0.70 (0.51 to 0.96)	0.65 (0.47 to 0.89)	0.75 (0.55 to 1.03)	0.08
HR (95% CI)‡	1 (reference)	0.63 (0.45 to 0.87)	0.56 (0.40 to 0.79)	0.72 (0.52 to 1.00)	0.06
Prostate cancer					
No of cases	67	65	69	62	
HR (95% CI)†	1 (reference)	0.81 (0.54 to 1.22)	0.79 (0.53 to 1.20)	0.64 (0.41 to 1.00)	0.06
HR (95% CI)‡	1 (reference)	0.81 (0.53 to 1.23)	0.81 (0.53 to 1.25)	0.64 (0.41 to 1.02)	0.07
Breast cancer					
No of cases	72	59	46	62	
HR (95% CI)†	1 (reference)	0.82 (0.57 to 1.18)	0.61 (0.41 to 0.92)	0.75 (0.51 to 1.11)	0.08
HR (95% CI)§¶	1 (reference)	0.98 (0.66 to 1.47)	0.69 (0.45 to 1.05)	0.78 (0.51 to 1.21)	0.12
Premenopausal:					
No of cases	35	27	12	12	
HR (95% CI)**	1 (reference)	1.14 (0.62 to 2.09)	0.42 (0.20 to 0.90)	0.56 (0.25 to 1.24)	0.03
Postmenopausal:					
No of cases	29	28	31	40	
HR (95% CI)**	1 (reference)	0.91 (0.51 to 1.60)	0.94 (0.54 to 1.64)	0.97 (0.56 to 1.71)	0.98

*Site specific cancers with case numbers of ≥130.
†Adjusted for age and sex.
‡Adjusted for age, sex, body mass index, smoking, alcohol use, physical activity, family history of cancer, and reported history of diabetes.
§Adjusted for factors in ‡ (except sex) in addition to age at menarche, number of births, use of exogenous female hormones, and menopausal status and age at menopause.
¶Number of cases from first to fourth quarters are 63, 55, 43, and 51, respectively.
**Adjusted for factors in ‡ (except sex) in addition to age at menarche, number of births, and use of exogenous female hormones.

the 25-hydroxyvitamin D/total cancer association by alternately excluding cases of these cancers from total cancer cases—that is, one cancer site at a time in separate models. Hazard ratios in each of these models showed no appreciable difference from the overall model, possibly indicating that the total cancer association is the result of the accumulation of small effects shared across multiple sites.

Discussion

In this prospective study, we measured plasma 25-hydroxyvitamin D in the largest number of cancer cases (n=3301) to date and observed that a higher circulating concentration of vitamin D was associated with a lower risk of total cancer. This finding remained significant after adjustment for known risk factors for cancer and across a variety of sensitivity analyses. To our knowledge, this is the first report involving circulating 25-hydroxyvitamin D concentrations and risk of total cancer in an Asian population. In the site specific analysis, the results indicated a significantly lower risk with higher vitamin D for liver cancer. More importantly, none of the cancer endpoints examined showed an increased risk associated with a higher vitamin D concentration.

Strengths and limitations of study

A major strength of this prospective study is its measurement of plasma 25-hydroxyvitamin D concentration in a large number of participants. The

Table 4 | Hazard ratios (HRs) for site specific cancer* according to thirds of plasma vitamin D

	Thirds of plasma vitamin D			
	1 (low)	2 (middle)	3 (high)	P for trend
Oesophageal cancer				
No of cases	28	14	30	
HR (95% CI)†	1 (reference)	0.48 (0.25 to 0.93)	1.08 (0.57 to 2.04)	0.84
HR (95% CI)‡	1 (reference)	0.39 (0.19 to 0.77)	0.91 (0.47 to 1.75)	0.83
Biliary tract cancer				
No of cases	41	33	41	
HR (95% CI)†	1 (reference)	0.64 (0.40 to 1.02)	0.63 (0.40 to 1.01)	0.07
HR (95% CI)‡	1 (reference)	0.63 (0.40 to 1.01)	0.65 (0.40 to 1.04)	0.09
Pancreatic cancer				
No of cases	42	36	36	
HR (95% CI)†	1 (reference)	0.85 (0.54 to 1.35)	0.85 (0.52 to 1.39)	0.50
HR (95% CI)‡	1 (reference)	0.81 (0.51 to 1.29)	0.80 (0.49 to 1.33)	0.39
Leukaemia				
No of cases	21	38	28	
HR (95% CI)†	1 (reference)	1.48 (0.84 to 2.61)	0.96 (0.50 to 1.83)	0.78
HR (95% CI)‡	1 (reference)	1.55 (0.88 to 2.76)	1.01 (0.53 to 1.92)	0.91
Kidney cancer				
No of cases	20	23	22	
HR (95% CI)†	1 (reference)	1.02 (0.53 to 1.94)	0.87 (0.45 to 1.67)	0.66
HR (95% CI)‡	1 (reference)	0.99 (0.52 to 1.88)	0.85 (0.45 to 1.62)	0.61
Bladder cancer				
No of cases	16	27	17	
HR (95% CI)†	1 (reference)	1.48 (0.78 to 2.79)	0.85 (0.42 to 1.74)	0.62
HR (95% CI)‡	1 (reference)	1.36 (0.71 to 2.64)	0.85 (0.41 to 1.79)	0.64
Lymphoma				
No of cases	30	24	27	
HR (95% CI)†	1 (reference)	0.63 (0.36 to 1.12)	0.60 (0.32 to 1.12)	0.12
HR (95% CI)‡	1 (reference)	0.62 (0.35 to 1.10)	0.60 (0.32 to 1.13)	0.13
Thyroid cancer				
No of cases	24	21	23	
HR (95% CI)†	1 (reference)	0.96 (0.52 to 1.78)	1.16 (0.59 to 2.27)	0.69
HR (95% CI)‡	1 (reference)	1.00 (0.53 to 1.90)	1.22 (0.61 to 2.45)	0.59
Uterus corpus cancer				
No of cases	21	18	14	
HR (95% CI)†	1 (reference)	0.97 (0.52 to 1.83)	0.81 (0.41 to 1.59)	0.55
HR (95% CI)§¶	1 (reference)	1.06 (0.53 to 2.12)	0.82 (0.38 to 1.75)	0.63
Ovarian cancer				
No of cases	13	12	15	
HR (95% CI)†	1 (reference)	0.97 (0.44 to 2.13)	1.08 (0.51 to 2.26)	0.85
HR (95% CI)§**	1 (reference)	0.83 (0.34 to 1.99)	0.96 (0.46 to 2.00)	0.92

*Site specific cancers with case numbers of <130.
†Adjusted for age and sex.
‡Adjusted for age, sex, body mass index, smoking, alcohol use, physical activity, family history of cancer, and reported history of diabetes.
§Adjusted for factors in ‡ (except sex) in addition to age at menarche, number of births, use of exogenous female hormones, and menopausal status and age at menopause.
¶Number of cases from first to third categories are 20, 18, and 13, respectively.
**Number of cases from first to third categories are 12, 9, and 12, respectively.

use of pre-diagnostic plasma samples and long follow-up reduced the possibility of reverse causation. The non-selective inclusion of all incident cases of cancer diagnosed during the study period eliminated concerns about survival bias and selection bias. Because the subcohort populations are representative subsamples of the original cohorts, the findings are expected to be generalisable to the source population without the need to measure biomarkers in the entire cohort. Also, the choice of a case-cohort design allowed us to evaluate multiple cancer endpoints simultaneously while using the common subcohort sample. Measurement of 25-hydroxyvitamin D concentration was done in a single plasma sample collected at one point in time and might therefore not have closely represented the long term circulating range. However, one study reported an intra-class correlation coefficient of 0.71 (95% confidence interval 0.63 to 0.88) in 25-hydroxyvitamin D concentrations across serum samples collected at three time points over a five year period.[38] In addition, the median 25-hydroxyvitamin D concentrations in plasma samples collected at three different time points up to 20 years apart and stored for 10, 20, and 30 years were similar.[9] Moreover, if this misclassification of exposure did occur, then it would be non-differential to both cases and subcohorts and would probably bias the results towards null. However, we were still able to observe a strong association with some endpoints. Because participants in our study were selected from examinees attending basic health examinations, they may have had healthier lifestyle habits than others,[39] in which case caution would

be needed when generalising our findings to other populations. Although the sample size for overall cancer was large, numbers of organ specific cancers were relatively small, particularly for rare cancers, and the analyses may not have been sufficiently powered to capture moderate associations (supplementary table C). Although we adjusted for several potential confounding factors in the statistical model, we cannot fully exclude the possibility of unmeasured or residual confounding.

Comparison with other studies

Only a few studies have evaluated the association between circulating 25-hydroxyvitamin D concentration and risk of total cancer.[9-13] In the Copenhagen City Heart Study, which included more than 2400 cancer events identified during a follow-up of up to 28 years, a 50% decrease in 25-hydroxyvitamin D concentration was associated with a modest 6% increase in the risk of total cancer.[9] In this study, however, the risks for tobacco related cancers were stronger than those for overall cancer (hazard ratio for 50% decrease in 25-hydroxyvitamin D concentration of 1.20, 95% confidence interval 1.13 to 1.28). In contrast, no overall association between 25-hydroxyvitamin D concentration and any cancer risk was seen in other individual studies from the US,[10] Denmark,[11] Germany,[12] and Norway,[13] although the German study showed a suggestive increase in risk with low 25-hydroxyvitamin D concentration among men and other subgroups only. These studies had relatively small numbers of cases of cancer,[10-13] however, and the participants were relatively older.[10 12 13] In the Health Professionals Follow-up Study, an increment in predicted 25-hydroxyvitamin D concentration by 25 nmol/L was associated with a 17% reduction in total cancer incidence.[40] A recent meta-analysis of the above studies suggested an 11% reduction in overall cancer incidence associated with a 50 nmol/L increase in 25-hydroxyvitamin D concentration.[18] The corresponding risk estimates (8% and 16% reduction per 25 nmol/L and 50 nmol/L increase in 25-hydroxyvitamin D concentration) in our study (supplementary methods) was weaker than those of the Health Professionals Follow-up Study but stronger than those produced by the meta-analysis. Moreover, because the inverse association in the Health Professionals Follow-up Study and in the meta-analysis was observed for men only, our study extended similar findings to women. Our study measured plasma 25-hydroxyvitamin D in the largest number of cancer cases reported to date (n=3301) and provides supportive evidence that higher concentrations of plasma vitamin D are associated with a lower risk of total cancer in both men and women.

In one randomised trial of supplemental vitamin D, in which 1179 postmenopausal women were randomised to one of three groups—calcium plus vitamin D_3, calcium plus placebo vitamin D, or both placebo—women in the calcium plus vitamin D_3 group were significantly less likely to develop cancer than women in the placebo group.[14] However, a more recent trial in a similar population with an even larger supplemental dose (2000 *v* 1100 IU of vitamin D_3) failed to produce a significant reduction in total cancer risk.[15] Because baseline 25-hydroxyvitamin D in the recent trial was higher than in the earlier trial (82 *v* 72 nmol/L), the authors argued that the participants with a higher baseline 25-hydroxyvitamin D were likely to have received less benefit from supplemental vitamin D than those with a low baseline 25-hydroxyvitamin D concentration. In other words, the stronger effect of supplementation in participants with low vitamin D is probably diluted by mild or even a lack of effects in those with higher vitamin status, resulting in the modest or null effect sizes seen in trials that included such participants.[41] Data from the Women's Health Initiative Trial showed significant associations between vitamin D and calcium supplementation and a reduced risk of total invasive cancer only in people who did not take personal supplements at baseline, and not in the total population (including personal supplement users at baseline),[16] which may support the argument of Lappe et al.[15] Vitamin D concentrations are known to vary by population characteristics globally.[42] The 25-hydroxyvitamin D concentration in our cohort participants (median 53.2 (interquartile range 42.9-64.6) nmol/L) seemed to be comparable to that of a European population (median 54 (40-69) nmol/L)[13] but much lower than that of a US population (mean 62.3 (95% confidence interval 61.1 to 63.5) nmol/L).[43] Given that both supplement use and food fortification are still not popular in Japan, a future trial of supplemental vitamin D in a Japanese population seems to be promising.

A notable finding of our study is that, compared with the lowest quarter of 25-hydroxyvitamin D concentration, the hazard ratio for total cancer decreased progressively in the second and third quarters but did not further decrease in the highest quarter. When we repeated the analysis in quintile based categories, we observed a similar decreasing pattern in risk estimates, with the smallest hazard ratios in the third and fourth fifths, suggesting a potential ceiling effect. Although a linear trend across quarters of 25-hydroxyvitamin D concentration was statistically significant for total cancer (P for trend=0.001), the hazard ratio per unit increase in concentration was not (supplementary methods; P for trend=0.05). Given that the latter analysis usually requires more stringent assumptions for linearity, a non-linear association between 25-hydroxyvitamin D and total cancer was conversely suggested. This dose-response pattern seems to agree with the observation from the CHANCES consortium for total cancer incidence and those from the ESTHER study, EPIC study, and Framingham Offspring Study for other health outcomes.[13 44-46] Although such a pattern is attributable to some unknown characteristics of people with lower concentrations of vitamin D, which may relate to cancer development at many sites, the accumulation of small effects of vitamin

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D across cancers at many sites might also contribute to producing a similar dose-response pattern. One of our sensitivity analyses showed that the alternating removal of cases of cancer at one specific site from total cancer cases did not substantially change the overall hazard ratios. Given that all cancers share common characteristics of uncontrolled cell growth and disrupted apoptosis, and that vitamin D's proposed anticancer role lies in regulating genes that control/modulate these characteristics, vitamin D may exert a biologically protective effect against cancers at many sites.

A potential ceiling effect observed in our study may suggest that no additional benefit would accrue when a certain concentration of 25-hydroxyvitamin D is exceeded. Together with the findings of the aforementioned trials, this may also suggest that raising a low 25-hydroxyvitamin D concentration to an intermediate concentration may provide protection, whereas raising it to a higher concentration (probably above around 80 nmol/L) may provide no further benefit. One randomised trial, whose participants had relatively lower baseline 25-hydroxyvitamin D (mean serum concentrations of 72 nmol/L), showed a beneficial effect of supplemental vitamin D against cancer development,[14] whereas a second trial, whose participants had relatively higher baseline 25-hydroxyvitamin D (mean concentrations serum of 82 nmol/L), failed to reproduce such a benefit of supplemental vitamin D.[15] These findings may have implications in determining the optimal concentrations of circulating 25-hydroxyvitamin D for prevention of cancer. Nevertheless, because 95% of participants (3839/4044 subcohort participants) had a 25-hydroxyvitamin D concentration less than 85 nmol/L, our results need to be interpreted with caution as our study may not have been sufficiently powered to evaluate further beneficial or even harmful effects at the much higher concentrations. In any event, the beneficial or harmful effects of 25-hydroxyvitamin D and the concentrations that are associated with such effects should be determined in long term randomised trials or on the basis of conclusive evidence from large scale meta-analyses.

Among findings for cancer at specific sites, we observed a strong inverse association between 25-hydroxyvitamin D concentration and risk of liver cancer. Two nested case-control studies within a prospective cohort and a cohort study have examined the association between 25-hydroxyvitamin D and risk of liver cancer.[9 30 47] Our observations are consistent with those of Fedirko et al, who found a 49% lower risk of liver cancer in the highest 25-hydroxyvitamin D group compared with the lowest group in a nested case-control study in a European population.[47] The median 25-hydroxyvitamin D concentration in our highest category (72.6 nmol/L) was comparable to the median in their highest group (69.8 nmol/L). In contrast, the other nested case-control study, which was conducted within the Linxian Nutrition Intervention Trial cohorts in China, showed only a non-significant suggestive decrease in liver cancer risk with higher 25-hydroxyvitamin D concentration.[30] However, this study was conducted among poorly nourished and micronutrient depleted people residing in a region with an elevated incidence of liver cancer, mainly due to a high prevalence of hepatitis infection and high aflatoxin exposure. Of note, however, the decreased risk with higher vitamin D in our study and the European study persisted after adjustment for hepatitis virus infection status. Nevertheless, the overall 25-hydroxyvitamin D concentration in the Chinese study population was also lower (median of 20.1 nmol/L) than in our study (53.2 nmol/L) and the European study (49.9 nmol/L), and this low 25-hydroxyvitamin D might have contributed to the lack of significant association in that study. The cohort study in the Copenhagen City Heart Study, which had a median 25-hydroxyvitamin D concentration of 41 nmol/L, also failed to show clear associations between 25-hydroxyvitamin D and risk of liver cancer.[9] This might be partly explained by the relatively small number of cases (n=55). In addition to anti-proliferative effects, vitamin D is also linked to improvements in inflammation and insulin sensitivity, which are both risk factors for hepatocellular cancer.[45] Vitamin D has also been reported to inhibit hepatic chromosomal aberrations and DNA strand breaks.[5] More recent observations have shown that the activation of vitamin D receptor induces the enzyme CYP3A, which detoxifies lithocholic acid, a hepatotoxic and potentially enteric carcinogenic compound, in the liver and intestine, and that these mechanisms possibly explain the strong finding for liver cancer.[56]

Although we did not observe a clear association between 25-hydroxyvitamin D concentration and risk of colorectal cancer, at least 15 prospective studies (as best described by Garland et al in a recent meta-analysis[20]) have evaluated the association, and several meta-analyses have suggested a lower risk of colorectal cancer in relation to higher 25-hydroxyvitamin D concentration.[19 24 48 49] The major reason for our null finding might be statistical underpower. Nevertheless, evidence is sparse in Asian populations, and only two prospective studies, one each from China and Japan, have been reported to date.[27 31] Whereas the Chinese study, which measured plasma 25-hydroxyvitamin D concentration in 212 case-control pairs (average follow-up of 3.4 years until diagnosis), found that higher 25-hydroxyvitamin D concentration was significantly inversely associated with the risk of colorectal cancer,[31] the Japanese study of 375 case-control trios, which included a subset of the analysis reported here, showed no apparent overall association between 25-hydroxyvitamin D and colorectal cancer.[27] Given that Asians have a higher frequency of the *f* allele in *FokI* polymorphisms in the vitamin D receptor (*VDR*) gene, which is reported to result in longer VDR protein with less transcriptional activity (and possibly less vitamin D related actions than the shorter VDR protein),[50] vitamin D may exert its effect differently in the population. Further studies are needed to examine

how circulating concentrations of 25-hydroxyvitamin D are related to risk of colorectal cancer in Asian populations.

A meta-analysis that included 11 941 cases and 13 870 controls from 21 studies reported a significant 17% elevated risk of prostate cancer in participants with higher 25-hydroxyvitamin D concentration compared with those with low 25-hydroxyvitamin D concentration (odds ratio 1.17, 95% confidence interval 1.05 to 1.30; P=0.004).[26] This meta-analysis did not include subgroup analysis by grade or stage of prostate cancer, but some studies have suggested that the association between 25-hydroxyvitamin D concentration and prostate cancer may differ by disease type.[51 52] For example, although no clear association was observed with overall prostate cancer, significant decreases in the risk of lethal prostate cancer and high grade (Gleason 8-10) prostate cancer associated with higher 25-hydroxyvitamin D concentration were seen in two studies.[51 52] One other study also reported a possible U shaped relation of plasma 25-hydroxyvitamin D concentration with total prostate cancer, which was more evident for high grade (Gleason 7-10) prostate cancers.[53] In our study, however, the hazard ratios associated with higher vitamin D concentration showed no clear association for either localised or advanced prostate cancers.

Although prospective studies of breast cancer have not provided consistent evidence for an association with circulating 25-hydroxyvitamin D, some studies have suggested that the association may differ by menopausal status.[23 54 55] A dose-response meta-analysis (5206 cases and 6450 controls) of plasma 25-hydroxyvitamin D concentrations suggested no association for premenopausal women but a non-linear inverse association for postmenopausal women, in which a 12 nmol/L increase in 25-hydroxyvitamin D was associated with a 12% lower risk beyond a threshold concentration of 67 nmol/L and suggestive flattening above 87 nmol/L.[23] In contrast to this meta-analysis, our study showed a lower risk associated with higher 25-hydroxyvitamin D concentration in premenopausal women but not in postmenopausal women. As renal conversion of 25-hydroxyvitamin D to its active component decreases with increasing age,[56] the anti-carcinogenic effect of vitamin D is likely more evident in premenopausal than postmenopausal women, even though both of them have similar circulating concentrations of 25-hydroxyvitamin D. In line with this speculation, the French E3N cohort (including 636 incident cases of breast cancer and 1272 controls) observed an inverse association between circulating 25-hydroxyvitamin D and breast cancer, which was more pronounced in women aged less than 53 years.[54] Although of interest, this hypothesis should be viewed with caution and needs further confirmatory evidence.

Conclusion

We observed that a higher circulating concentration of vitamin D was associated with a lower risk of subsequent cancer in a large Japanese population. Our findings support the hypothesis that vitamin D may confer protection against the risk of cancer. Nevertheless, the lower risk associated with higher circulating vitamin D concentration seemed to show a ceiling effect, which may suggest that although maintaining an optimal 25-hydroxyvitamin D concentration is important for prevention of cancer, having a concentration beyond this optimal level may provide no further benefit. Future studies are needed to clarify the dose-response pattern and the optimal concentrations for cancer prevention.

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Contributors: ST, MIwasaki, MInoue, SS, TS, AG, NSawada, and TY were involved in study design and data collection. MIwasaki and TY conceived and designed the analysis. SK and NSudo did laboratory 25-hydroxyvitamin D assays. ST-M, AK, and HC provided statistical expertise. SB and AH did statistical analysis. SB drafted the manuscript. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. TY is the guarantor.

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Ethical approval: This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan (approval number 13-021). Although written informed consent was not required, the study participants were informed of the objectives of the study; participants who responded to the questionnaire survey were considered to have consented to participating in the survey.

Transparency statement: SB and TY affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing: No additional data available.

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Supplementary materials