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Cancer Epidemiology, Biomarkers & Prevention

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Research Article

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Serum 25-Hydroxyvitamin D and Cancer Risk in Older Adults: Results from a Large German Prospective Cohort Study

José M. Ordóñez-Mena[1], Ben Schöttker[1], Ulrike Haug[2], Heiko Müller[1], Josef Köhrle[3], Lutz Schomburg[3], Bernd Holleczek[4], and Hermann Brenner[1]

Abstract

Background: Several observational studies assessed the relationship between serum 25-hydroxyvitamin D [25(OH)D] concentrations and the risk of cancer but results were inconclusive.

Methods: We measured 25(OH)D concentrations in a population-based cohort study of 9,949 men and women ages 50 to 74 years in Saarland, Germany. Comprehensively adjusted Cox regression models were applied to estimate HRs and 95% confidence intervals (CI) for the association between season-standardized 25 (OH)D concentrations and total and site-specific cancer incidence.

Results: Overall, during a median of 8 years of follow-up, 873 subjects developed cancer; the most common being prostate (171), breast (137), lung (136), and colorectal (136) cancer. Low season-standardized 25(OH)D (<30, 35, 40, or 36 nmol/L in winter, spring, summer, and autumn, respectively) was neither significantly associated with total cancer incidence (HR, 1.10; 95% CI, 0.93–1.30) nor with site-specific cancer incidence. However, a significantly increased overall cancer risk was observed for low 25(OH)D among men, nonobese subjects and subjects reporting low fish consumption and for high 25(OH)D in nonsmokers and nonobese subjects. Accordingly, restricted cubic splines to investigate dose–response relationships curves showed an inverse association of 25(OH)D levels and total cancer risk in men but not in women.

Conclusions: 25(OH)D concentrations were significantly associated with overall cancer incidence in subgroups of this large cohort from Germany. No significant association was observed with site-specific cancers but this could be due to a limited statistical power for these endpoints.

Impact: Further research should clarify whether and to what extent specific risk groups might profit from vitamin D supplementation. *Cancer Epidemiol Biomarkers Prev; 1–12. ©2013 AACR.*

Introduction

Low vitamin D serum levels, for a long time known to be a risk factor for osteoporosis, falls (1), and fractures (2), have recently been linked to the occurrence of a variety of other chronic diseases at old age such as cancer (3), cardiovascular diseases (4), and diabetes mellitus (5). The potential association of vitamin D concentrations and cancer is of major public health concern in older adults because vitamin D insufficiency is particularly frequent in this age group (6) and cancer incidence strongly increases with age.

The best established biomarker of vitamin D status is serum concentration of 25-hydroxyvitamin D [25(OH)D; ref. 6]. 25(OH)D is generated in the liver and reflects exposure to ultraviolet B radiation and dietary intake of cholecalciferol (Vitamin D_3) (7). The biologically active form of vitamin D, calcitriol, or 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which is generated by the kidneys and other epithelial tissues, reduces cell proliferation, and induces cell differentiation and apoptosis (7–9). In addition, the vitamin D receptor (VDR) is activated by 1,25 $(OH)_2$D and mediates genomic changes in epithelial cells that contribute to a preservation of the differentiated phenotype, resistance to cellular stress, and protection of the genome (10).

Although not the active metabolite, serum 25(OH)D is usually studied because it is the best estimate of a person's usual vitamin D status. It has a longer serum half-life (~3 weeks) than 1,25(OH)D (~4 hours) and is not dependent on the fluctuating calcium needs of the skeletal system (11). A number of longitudinal studies have assessed the relationship between serum 25(OH)D concentrations and cancer risk. The results have been summarized in several

Authors' Affiliations: Divisions of [1]Clinical Epidemiology and Aging Research and [2]Preventive Oncology, German Cancer Research Center, Heidelberg; [3]Institut für Experimentelle Endokrinologie, Charité University Medicine Berlin, Berlin; and [4]Saarland Cancer Registry, Saarbrücken, Germany

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J.M. Ordóñez-Mena and B. Schöttker contributed equally as first authors.

Corresponding Author: Ben Schöttker, Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Im Neuenheimer Feld 581, Heidelberg 69120, Germany. Fax: 496221-421302; E-mail: b.schoettker@dkfz-heidelberg.de

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meta-analyses, which concluded that 25(OH)D concentrations are inversely associated with colorectal cancer incidence (12–16) but are not associated with prostate or breast cancer incidence (14,17–20). Interestingly, a U-shaped association of 25(OH)D with total cancer incidence was reported from a community-based prospective study (21). Interventional studies that assessed the influence of supplementation of vitamin D with or without calcium on the occurrence of cancer (22–26) reported inconclusive or nonsignificant results (27, 28).

The heterogeneous picture from observational and interventional studies underlines that the relationship of serum 25-hydroxyvitamin D and cancer risk is still incompletely understood. We aim to contribute to this important health issue by providing a thorough examination of the association of 25(OH)D concentrations with prostate, breast, lung, colorectal, and total cancer incidence in a large population-based cohort study with particular attention to dose–response relationships.

Materials and Methods

Study design and study population

Our analyses are based on the ESTHER study, an ongoing population-based cohort study conducted in Saarland, a small state (1 million inhabitants) located in South-West Germany (29). In total, 9,949 participants, ages 50 to 74 years at baseline, were enrolled during a routine physician health checkup between 2000 and 2002. The distribution of sociodemographic characteristics, prevalent diseases, and major risk factors in the ESTHER study are comparable with those in the German National Health Survey which was conducted in a representative sample of the German population in 1998 (30). Blood samples were drawn during the health checkup, centrifuged, sent to the study center, and finally stored at −80°C until measurement.

Follow-up questionnaires were sent to study participants and their physicians 2, 5, and 8 years after recruitment (8-year follow-up questionnaires in the years 2008–2011). The questionnaires included information on incident diseases including cancer. Only self-reported cancer diagnoses that were confirmed by information from the physician's questionnaire were considered as cases. In addition, a systematic follow-up with respect to total and cause-specific mortality, as well as total and site-specific cancer incidence was done by record linkage with data from population registries and public health authorities, as well as the Saarland Cancer Registry, a population-based cancer registry that has been operating at high levels of completeness since the 1970s. Data on incident cancer cases from the Saarland Cancer Registry were available until end of 2009.

Endpoint definition

Incident cancer cases were ascertained from physician questionnaires, supplemented and validated by records from the Saarland Cancer Registry. In case of deviations, priority was given to the information from the cancer registry. Total (excluding nonmelanoma skin neoplasms) and site-specific cancer incidence was classified according to International Classification of Diseases (ICD)-10 codes as follows: total (C00-C43, C45-C97), lung (C34), breast (C50), prostate (C61), and colorectal (C18–21) cancer. Only primary incident cancers were considered in the analyses.

Measurement of serum 25(OH)D concentrations

The automated Diasorin-Liaison immunoassay (Diasorin Inc.) was used to measure 25(OH)D concentrations in women from stored baseline serum samples in the central laboratory of the University Clinic of Heidelberg (Heidelberg, Germany) in the framework of a project on women's health in 2006. Funding was obtained in 2009 to measure 25(OH)D also in men from stored baseline serum samples. The Diasorin-Liaison method used for women was unavailable at this date and therefore the IDS-iSYS immunoassay (IA) was used for the men's blood samples. Within-assay and between-assay coefficients of variation for both methods are described elsewhere (31). The lower detection limits were 15 nmol/L for Diasorin-Liaison and 9 nmol/L for IDS-iSYS. Both IA measurements were standardized to a liquid chromatography/tandem mass spectrometry (LC/MS-MS) method (32) which is considered one of the most precise and accurate methods to measure 25(OH)D, as described in detail elsewhere (33, 34). In brief, ordinary least-squares linear regression equations were fitted between the IA measurements and LC/MS-MS results in random subsamples of 97 men and 97 women, and results were used for standardization of 25(OH)D concentrations in the total cohort. Standardization on average increased the 25(OH)D levels by 10.3 nmol/L in women and decreased 25(OH)D levels by 2.9 nmol/L in men (33).

Covariates assessment

Data on sociodemographic characteristics, lifestyle, diet, and hormone replacement therapy (HRT) of the study participants were retrieved from an extensive standardized questionnaire completed by the study participants at baseline. For this analysis, family history of cancer (yes/no) was coded for parents, siblings, and children. School education was classified as $\leq$9, 10–11, and $\geq$12 years, respectively. Smoking status was categorized as never, former, and current smoking with additional distinction of current smokers who smoked more or less than 20 cigarettes/d. Physical activity categories were defined as follows: "Inactive" (<1 h of physical activity/wk); "Medium or high" (2 h of vigorous and $\geq$2 h of light physical activity/wk); and "Low" (other). Fish and red meat intake were dichotomized as "less than once a week" or "at least once a week," fruits and vegetables intake as "daily" or "not daily," and multivitamin intake as "regularly" or "less than regularly." Height and weight were assessed and documented on a standardized form by the physicians during the health checkup and used to calculate the body mass index (BMI). Furthermore, prescribed

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drugs were documented by the physicians. Classification of HRT (yes/no) was based on self-reported HRT or prescribed HRT drugs. Furthermore, the date of the blood sample donation was documented. Four seasons were defined according to calendar weeks in which blood donation took place: "winter" (weeks 1–13), "spring" (weeks 14–26), "summer" (weeks 27–39), and "autumn" (weeks 40–52).

Statistical analyses

Participants of the ESTHER baseline examination ($n = 9{,}949$) were included in this investigation if their 25(OH)D serum level could be determined in a serum aliquot provided for this investigation, which was possible for 9,580 baseline participants. Subjects who had developed any cancer before baseline ($n = 573$, 6.0%) were excluded from the analysis on total cancer incidence, leaving $n = 9{,}007$ for analysis. For analyses on specific cancer types, only those subjects who had a diagnosis of the specific cancer of interest before baseline were excluded. Furthermore, prostate cancer was analyzed only in men and breast cancer only in women, resulting in the following sample sizes for the respective outcomes: prostate cancer, 4,124; breast cancer, 5,261; lung cancer, 9,561; and colorectal cancer, 9,482.

All analyses were conducted with 25(OH)D concentrations standardized with LC/MS-MS. Serum 25(OH)D concentration was season-standardized by the residual method and divided into quartiles as described in the Supplementary Fig. S1. In addition, in sensitivity analyses, clinically defined categories of vitamin D status: "vitamin D deficiency" [<30 nmol/L 25(OH)D] and "vitamin D insufficiency" [30–50 nmol/L 25(OH)D] were compared with the group of subjects with "sufficient vitamin D status" [>50 nmol/L 25(OH)D] (35).

Differences in baseline characteristics across quartiles of season-standardized 25(OH)D levels were assessed by χ^2 test (categorical variables) or Wilcoxon rank-sum test (continuous variables). Cox proportional hazards models were used to estimate HRs with 95% confidence intervals (CI) with respect to total, lung, breast, prostate, and colorectal cancer incidence. Two models were built with an increasing number of established cancer risk factors and determinants of serum 25(OH)D concentrations as covariates. The first model comprised age and sex as covariates. The second model was adjusted for the covariates of the first model and additionally BMI, school education, physical activity, smoking, family history of total or specific cancer, and nutritional variables (multivitamin supplements, fish, red meat, vegetables and fruit consumption). In a sensitivity analysis, use of HRT was also included in the multivariate models for breast, colorectal, and total cancer incidence. Potential interactions of season-standardized 25(OH)D levels and the covariates were tested for statistical significance by adding pertinent product terms to the fully adjusted model 2 in all participants. Finally, dose–response relationships were plotted with restricted cubic splines using the 5th, 25th, 75th, and 95th percentile of season-standardized 25(OH)D levels as knots and the 50th percentile as the reference (36). Multiple imputation was used to adequately deal with missing covariate values (Supplementary Table S1) as described in detail in the Supplementary Data. All statistical tests were 2-sided using an α-level of 0.05. All analyses were conducted with the software package SAS, version 9.2.

Results

Distribution of baseline characteristics across 25-hydroxyvitamin D categories

Baseline characteristics of the study population, stratified by season-standardized 25(OH)D level quartiles, are shown in Table 1. Subjects in the first quartile of season-standardized 25(OH)D were more frequently older, smokers, obese, physically inactive, consumed less multivitamin supplements and less fish and fruits, and had less frequently a family history of cancer than subjects in quartiles 2 and 3. Women in the first quartile were using HRT less frequently than women in the interquartile range (IQR) and women in the fourth quartile had an even higher proportion of HRT use. Subjects in the fourth season-standardized 25(OH)D quartile were more frequently men, former smokers, better educated, consumed more multivitamin supplements, fruits and vegetables, were less frequently older, obese, or inactive, and had a lower BMI than subjects in the IQR.

Association of 25(OH)D concentrations with total cancer incidence

Overall 873 incident cancer cases occurred during a mean follow-up of 8.0 years. HRs for the association between quartiles of season-standardized 25(OH)D levels and total cancer incidence in the total population and in subgroups defined by potential effect modifiers are shown in Table 2. Subjects in the first season-standardized 25(OH)D quartile had a higher cancer risk (on the border to statistical significance) than subjects in quartiles 2 and 3 [HR after adjustment of age and sex, 1.15 (0.98–1.36)]. However, further adjustment in the second model attenuated the HR to 1.10 (0.93–1.30). Sex was an effect modifier of the association ($P_{\text{interaction}} = 0.02$) with the excess risk solely observed in men [HR, 1.33 (1.06–1.68)] and not in women [HR, 0.95 (0.75–1.20)]. The dose–response analysis (Fig. 1) likewise showed a statistically significant increase of total cancer risk at low 25(OH)D concentrations in men only, along with an increase in cancer risk at high 25(OH)D concentrations in both men and women. The latter did though not reach statistical significance.

Furthermore, a significant interaction was observed for obesity with the first season-standardized 25(OH)D quartile ($P_{\text{interaction}} < 0.01$) showing a protective association among obese [HR, 0.65 (0.48–0.90)] and an increased risk of cancer among nonobese subjects [HR, 1.36 (1.12–1.65)]. Fish intake was also an effect modifier ($P_{\text{interaction}} < 0.01$), with a stronger association in subjects consuming fish less than once per week [HR, 1.50 (1.22–2.01)]. Age (older/

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Table 1. Baseline characteristics of the study population stratified by quartiles of season-standardized 25(OH)D levels

	Season-standardized 25(OH)D					
	Quartile 1 (Q_1)		Quartiles 2 and 3 ($Q_2 + Q_3$)		Quartile 4 (Q_4)	
Characteristic	*N*	*n* (%) or median (P_{25}–P_{75})	*N*	*n* (%) or median (P_{25}–P_{75})	*N*	*n* (%) or median (P_{25}–P_{75})
25(OH)D, nmol/L	2,395		4,790		2,395	
Winter (Jan–Mar)		**29.5 (24.4–29.5)**		38.7 (33.5–45.1)		**67.8 (61.3–79.3)**
Spring (Apr–Jun)		**29.5 (28.2–31.5)**		43.9 (38.1–50.1)		**72.1 (64.2–83.6)**
Summer (Jul–Sept)		**35.9 (30.5–40.4)**		54.4 (49.1–61.2)		**86.7 (77.2–101.7)**
Autumn (Oct–Dec)		**29.5 (29.5–32.5)**		46.3 (40.6–52.2)		**75.3 (66.6–92.6)**
Males	2,395	893 (37.3)	4,790	1,769 (36.9)	2,395	**1,535 (64.1)**
Age $\geq$ 65 y	2,395	**1,037 (43.3)**	4,790	1,854 (38.7)	2,395	**819 (34.2)**
Scholarly education	2,329		4,662		2,348	
$\leq$9 y		1,766 (75.8)		3,571 (76.6)		**1,668 (71.0)**
9–11 y		330 (14.2)		639 (13.7)		**344 (14.7)**
$\geq$12 y		223 (10.0)		452 (9.7)		**336 (14.3)**
Smoking	2,279		4,593		2,318	
Never		**1,182 (51.9)**		2,496 (54.3)		**1,031 (44.5)**
Former		**594 (26.1)**		1,453 (31.6)		**982 (42.4)**
Current <20 cig/d		**212 (9.3)**		312 (6.8)		**152 (6.6)**
Current $\geq$20 cig/d		**291 (12.8)**		332 (7.2)		**153 (6.6)**
Prevalent cancer	2,395	142 (5.9)	4,790	290 (6.1)	2,395	141 (5.9)
Family history of cancer	2,338	**1,052 (45.0)**	4,721	2,266 (48.0)	2,384	**1,081 (45.3)**
BMI, kg/m^2	2,389	27.5 (24.8–30.7)	4,787	27.4 (24.9–30.1)	2,391	**26.7 (24.4–29.2)**
Obesity (BMI $\geq$ 30 kg/m^2)	2,389	**717 (30.0)**	4,787	1,280 (26.7)	2,391	**447 (18.7)**
Physical activity	2,384		4,779		2,388	
Inactive		**659 (27.6)**		1,058 (22.1)		**344 (14.4)**
Low		**1,084 (45.5)**		2,234 (46.8)		**1,035 (43.3)**
Medium or high		**641 (26.9)**		1,487 (31.1)		**1,009 (42.3)**
Regular multivitamin supplement use	2,341	**276 (11.8)**	4,676	665 (14.2)	2,360	**414 (17.5)**
Fish intake less than once a week	2,213	**801 (36.2)**	4,517	1,504 (33.3)	2,297	718 (31.3)
Red meat intake at least once a week	2,221	272 (12.3)	4,501	501 (11.1)	2,296	224 (9.8)
Fruit intake less than daily	2,285	**1,391 (60.9)**	4,638	2,596 (63.7)	2,329	**1,403 (60.2)**
Vegetable intake less than daily	2,307	830 (36.0)	4,669	1,700 (36.4)	2,346	**791 (33.7)**
HRT (women only)	1,502	**359 (23.9)**	3,021	939 (31.1)	860	**358 (41.6)**

NOTE: Values in bold are $P < 0.05$ for comparisons of characteristics with the interquartile range of season-standardized 25(OH)D concentrations as the reference.

Abbreviations: *N*, number of participants with data for the characteristic (does not always add up to the total because of missing values); *n*, number of participants with the characteristic; P, percentile; *Q*, quartile.

younger than 65 years old), physical activity (yes/no), multivitamin use (yes/no), red meat consumption (less than once a week/at least once a week), daily fruits and vegetables consumption (yes/no), and HRT use (yes/no) were also tested as potential effect modifiers, but no significant interactions were observed in the association of 25(OH)D levels and cancer risk (data not shown).

Besides subjects in the first quartile, also subjects in the fourth season-standardized 25(OH)D quartile had a (non-significantly) higher cancer risk than subjects in quartiles 2 and 3 [fully adjusted HR, 1.12 (0.95–1.32)]. However, a statistically significant increase in the risk of total cancer was seen among nonsmokers in the fourth quartile [HR, 1.20 (1.00–1.43)], but the *P* value for the test for interaction with smoking was not statistically significant ($P_{interaction} = 0.09$). Furthermore, among nonobese, higher season-standardized 25(OH)D levels were associated with an excess of cancer risk [HR, 1.29 (1.07–1.57)].

Associations of 25(OH)D concentrations with site-specific cancer incidences

Overall, 171, 137, 136, and 136 cancers of the prostate, breast, lung, colon, and rectum were observed during follow-up, respectively. None of the cancers showed

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Table 2. HRs and 95% CIs for the associations of quartiles of season-standardized 25(OH)D levels with total cancer incidence in the total population and subgroups

					Model 1[b]	Model 2[c]	
Group	**25(OH)D[a]**	***N***	**I**	**IR**	**HR (95% CI)**	**HR (95% CI)**	**$P_{interaction}$**
All	Q_1	2,253	235	13.2	1.15 (0.98–1.36)	1.10 (0.93–1.30)	
	$Q_2 + Q_3$	4,500	396	11.2	Ref	Ref	
	Q_4	2,254	242	13.7	1.09 (0.92–1.28)	1.12 (0.95–1.32)	
Men	Q_1	846	128	19.8	**1.40 (1.12–1.75)**	**1.33 (1.06–1.68)**	**0.02**
	$Q_2 + Q_3$	1,668	187	14.4	Ref	Ref	
	Q_4	1,443	181	16.2	1.14 (0.93–1.40)	1.20 (0.97–1.47)	0.84
Women	Q_1	1,407	107	9.5	0.98 (0.78–1.24)	0.95 (0.75–1.20)	
	$Q_2 + Q_3$	2,832	209	9.4	Ref	Ref	
	Q_4	811	61	9.4	1.05 (0.78–1.39)	1.07 (0.80–1.44)	
Smokers	Q_1	525	81	20.1	1.36 (0.99–1.86)	1.31 (0.95–1.81)	0.17
	$Q_2 + Q_3$	690	76	14.2	Ref	Ref	
	Q_4	320	31	12.3	0.74 (0.48–1.12)	0.81 (0.53–1.24)	0.09
Nonsmokers	Q_1	1,728	154	11.2	1.02 (0.84–1.23)	1.02 (0.84–1.24)	
	$Q_2 + Q_3$	3,810	320	10.7	Ref	Ref	
	Q_4	1,934	211	13.9	1.19 (0.99–1.42)	**1.20 (1.00–1.43)**	
Obese	Q_1	680	55	10.1	**0.69 (0.50–0.94)**	**0.65 (0.48–0.90)**	**<0.01**
	$Q_2 + Q_3$	1,212	136	14.5	Ref	Ref	
	Q_4	420	45	13.7	0.78 (0.54–1.10)	0.78 (0.55–1.10)	0.17
Nonobese	Q_1	1,573	180	14.6	**1.42 (1.17–1.71)**	**1.36 (1.12–1.65)**	
	$Q_2 + Q_3$	3,288	260	10.0	Ref	Ref	
	Q_4	1,834	197	13.7	**1.25 (1.03–1.51)**	**1.29 (1.07–1.57)**	
Fish intake less than once a week	Q_1	760	88	14.7	**1.62 (1.22–2.16)**	**1.50 (1.12–2.01)**	**<0.01**
	$Q_2 + Q_3$	1,434	101	8.9	Ref	Ref	
	Q_4	682	65	12.2	1.20 (0.87–1.64)	1.21 (0.87–1.67)	0.52
Fish intake at least once a week	Q_1	1,376	133	12.2	0.95 (0.77–1.17)	0.91 (0.74–1.12)	
	$Q_2 + Q_3$	2,899	282	12.4	Ref	Ref	
	Q_4	1,509	175	14.9	1.07 (0.88–1.30)	1.12 (0.92–1.36)	

NOTE: $P < 0.05$ for values in bold.

Abbreviations: I, incident case numbers; IR, incidence rate per 1,000 person-years; *N*, sample size with imputed missing values in imputed data set no. 1; Q, quartile; Ref, reference category.

[a]Cut-off points for season-standardized 25(OH)D quartiles were for winter, spring, summer, and autumn: 30, 35, 45, and 36 nmol/L 25(OH)D, respectively, for Q1; and 55, 60, 70, and 61 nmol/L 25(OH)D, respectively, for Q3.

[b]Multivariate model 1 was adjusted for age and sex.

[c]Multivariate model 2 was adjusted for the covariates in model 1 and multivitamin use, fish consumption less than once a week (yes/no), red meat consumption less than once a week (yes/no), daily fruit intake (yes/no), daily vegetables intake (yes/no), BMI (kg/m^2), scholarly education (≤9/9–11/≥12 years), physical activity (inactive/low/medium or high), smoking (never, former, current and less than 20 cig/d, current with at least 20 cig/d), and family history of cancer (yes or no for parents, siblings, or children).

statistically significant associations with season-standardized 25(OH)D levels after adjustment for covariates (Table 3). For prostate and breast cancer, dose–response relationships were similar to those for all cancers in men and women, respectively (Fig. 2). For lung and colorectal cancer, no major variation in incidence was seen by season-standardized 25(OH)D levels in dose–response analyses (Fig. 3).

Sensitivity analysis

We also analyzed the risk of cancer according to clinically defined cutoff points in multivariate models adjusting for season (data not shown). Overall, findings for vitamin D deficiency (25(OH)D concentration < 30 nmol/L) were very similar to results for the season-standardized first quartile of 25(OH)D concentrations. Vitamin D insufficiency [25(OH)D concentration between 30 and 50 nmol/L] showed no significant associations with total and cancer-specific incidence.

Discussion

Overall, 25(OH)D concentrations were not statistically significantly associated with total, prostate, breast, lung,

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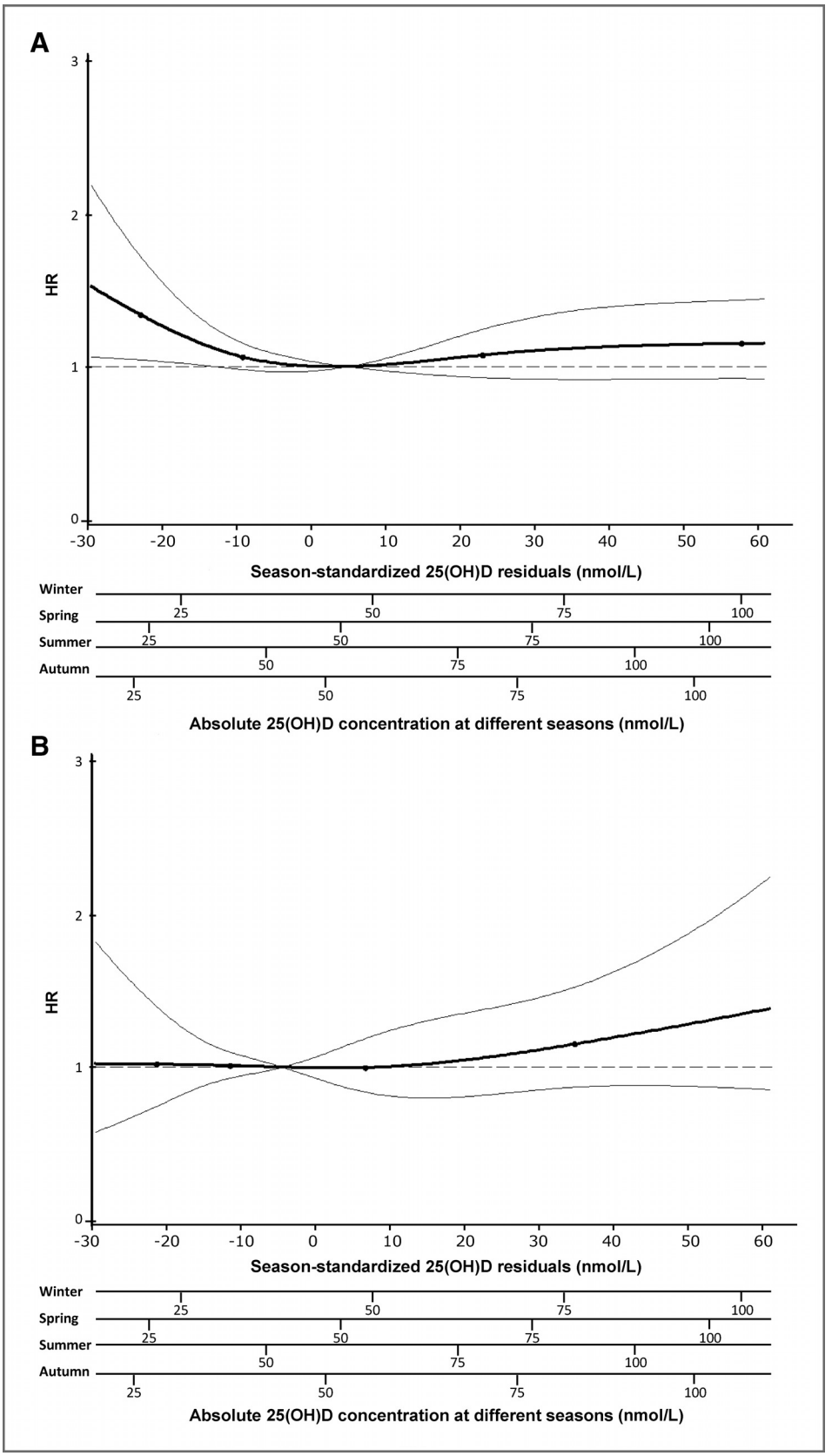

Figure 1. Dose–response relationship between season-standardized 25(OH)D levels and total cancer incidence in men (A) and women (B). Point estimates of HRs (fat line) and 95% CIs (curved thin lines) were obtained by restricted cubic splines Cox regression analysis with knots at the 5th, 25th, 75th, and 95th, percentile [50th percentile (median) as the reference]. The horizontal dashed line marks the HR null effect value of 1.0.

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Table 3. HRs and 95% CIs for the associations of quartiles of season-standardized 25(OH)D levels with site-specific cancer incidence

Endpoint (group)	25(OH)D[a]	*N*	I	IR	Model 1[b] HR (95% CI)	Model 2[c] HR (95% CI)
Prostate cancer (men)	Q_1	882	38	5.4	1.17 (0.78–1.74)	1.16 (0.78–1.74)
	$Q_2 + Q_3$	1,737	66	4.7	Ref	Ref
	Q_4	1,505	67	5.5	1.21 (0.86–1.71)	1.21 (0.86–1.70)
Breast cancer (women)	Q_1	1,464	38	3.2	1.07 (0.72–1.58)	1.08 (0.72–1.60)
	$Q_2 + Q_3$	2,951	71	3.0	Ref	Ref
	Q_4	846	28	4.1	1.37 (0.87–2.10)	1.39 (0.89–2.18)
Lung cancer (all)	Q_1	2,389	45	2.3	**1.52 (1.03–2.25)**	1.21 (0.81–1.80)
	$Q_2 + Q_3$	4,782	57	1.5	Ref	Ref
	Q_4	2,390	34	1.7	0.93 (0.60–1.42)	1.07 (0.69–1.65)
Colorectal cancer (all)	Q_1	2,373	37	1.9	1.03 (0.69–1.53)	1.02 (0.68–1.53)
	$Q_2 + Q_3$	4,741	69	1.8	Ref	Ref
	Q_4	2,368	30	1.5	0.75 (0.48–1.15)	0.77 (0.50–1.20)

NOTE: $P < 0.05$ for values in bold.

Abbreviations: I, incident case numbers; IR, incidence rate per 1,000 person-years; *N*, sample size with imputed missing values in imputed data set no. 1; Q, quartile; Ref, reference category.

[a]Cutoff points for season-standardized 25(OH)D quartiles were for winter, spring, summer, and autumn: 30, 35, 45, and 36 nmol/L 25(OH)D, respectively, for Q1; and 55, 60, 70, and 61 nmol/L 25(OH)D, respectively, for Q3.

[b]Multivariate model 1 was adjusted for age and sex.

[c]Multivariate model 2 was adjusted for the covariates in model 1 and multivitamin use, fish consumption less than once a week (yes/no), red meat consumption less than once a week (yes/no), daily fruit intake (yes/no), daily vegetables intake (yes/no), BMI (kg/m^2), scholarly education (≤9/9–11/≥12 years), physical activity (inactive/low/medium or high), smoking (never, former, current and less than 20 cig/d, current with at least 20 cig/d), and family history of cancer (yes or no for parents, siblings, or children).

and colorectal cancer incidence in this large population-based prospective cohort study of older men and women from Germany. However, both the first and the fourth season-standardized 25(OH)D quartile were significantly associated with increased overall cancer incidence in specific subgroups (i.e., first quartile in men, nonobese and subjects consuming fish less than once a week and fourth quartile in nonsmokers and nonobese).

To our knowledge, only 3 large longitudinal prospective cohort studies have examined the relationship between serum 25(OH)D concentrations and total cancer incidence so far and 2 of them analyzed men only (21, 37, 38). The relative risk for the development of any cancer decreased by 16% in the Health Professionals Follow-up Study (HPFS) for a 25 nmol/L increase in predicted 25(OH)D concentrations [0.84 (0.72–0.98)] (37). In the Uppsala Longitudinal Study of Adult Men (ULSAM), a U-shaped association was found (21) which is in agreement with our findings for men even though the increase in cancer risk at high 25(OH)D levels was not statistically significant in our cohort. In a further prospective, community-based cohort study, low season-standardized 25(OH)D concentrations were not associated with the risk of cancer in adults older than 65 years (38).

In our study, we observed stronger associations of low 25(OH)D levels and total cancer in men, nonobese people, and subjects consuming fish less than once a week. A possible explanation for the stronger association in men could be the influence of vitamin D deficiency on total cancer being more marked in subjects at higher risk of cancer. A recent study found that fish oils increased the antiproliferative effect of $1,25(OH)_2D$ (39). The stronger risk of cancer observed in subjects with lower season-standardized 25(OH)D levels and low fish consumption may thus be explained by this synergistic protective effect that $1,25(OH)_2D$ and fish oils have on cell proliferation and cancer progression.

Among nonobese subjects, the significantly increased risk of cancer associated with low season-standardized 25(OH)D levels is consistent with results of the Women's Health Initiative (WHI) study. In this study, an increased risk of cancer was observed for women with normal waist circumference, whereas no association was discerned in women with abdominal obesity (40). The reasons for these patterns are unclear and deserve further study.

Given that prostate cancer was by far the most common cancer among men, it is not surprising that dose–response relationships with season-standardized 25(OH)D concentrations were similar for total and prostate cancer incidence. However, the CI bands were even wider for the latter due to lower case numbers, and an association of 25(OH)D with prostate cancer incidence could not be confirmed. The latter is also the main conclusion of recent meta-analyses estimating prostate cancer risk by a 25

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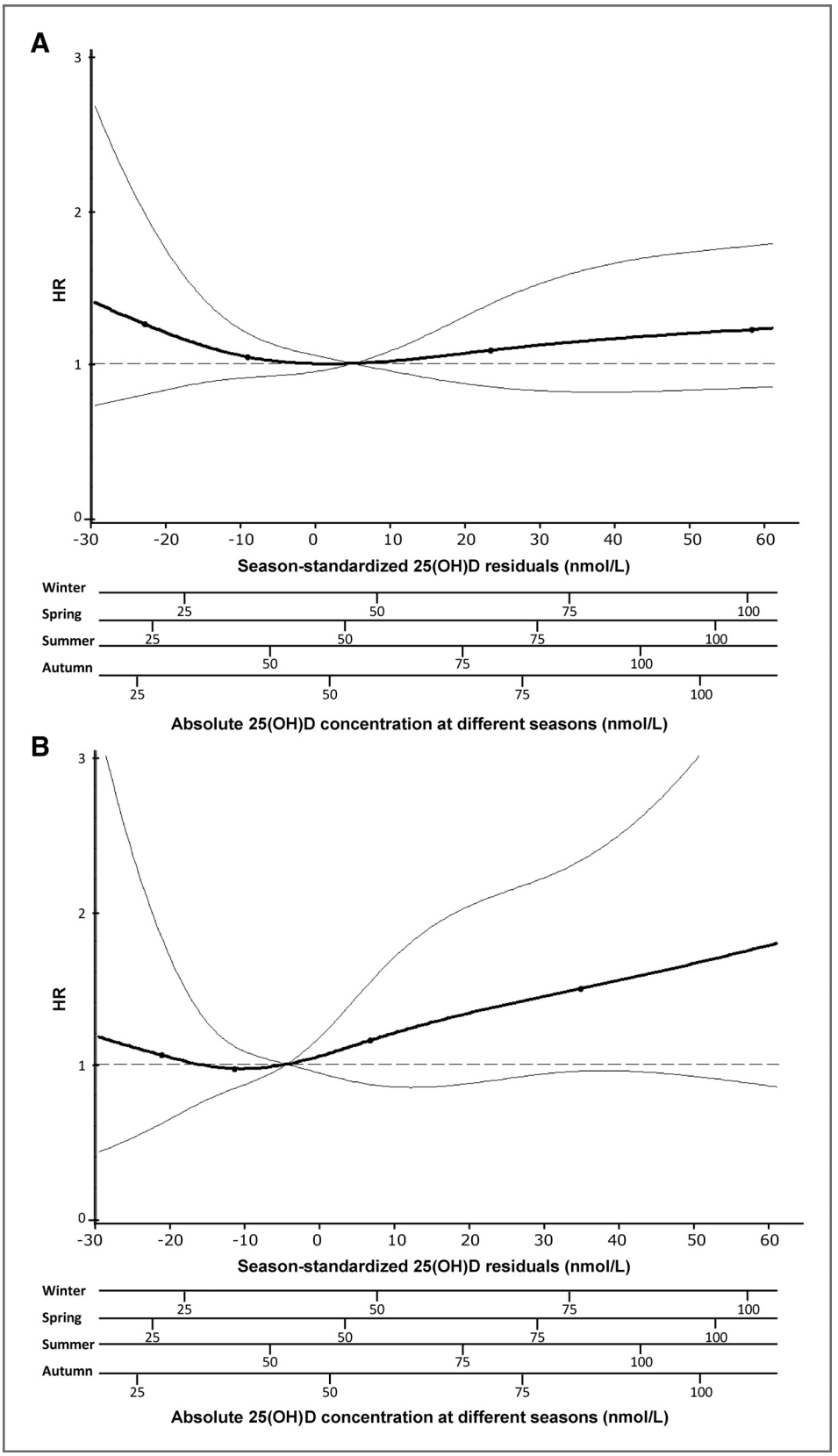

Figure 2. Dose–response relationship between season-standardized 25(OH)D levels and prostate cancer incidence in men (A) and breast cancer incidence in women (B). Point estimates of HRs (fat line) and 95% confidence intervals (curved thin lines) were obtained by restricted cubic splines Cox regression analysis with knots at the 5th, 25th, 75th, and 95th, percentile [50th percentile (median) as the reference]. The horizontal dashed line marks the HR null effect value of 1.0.

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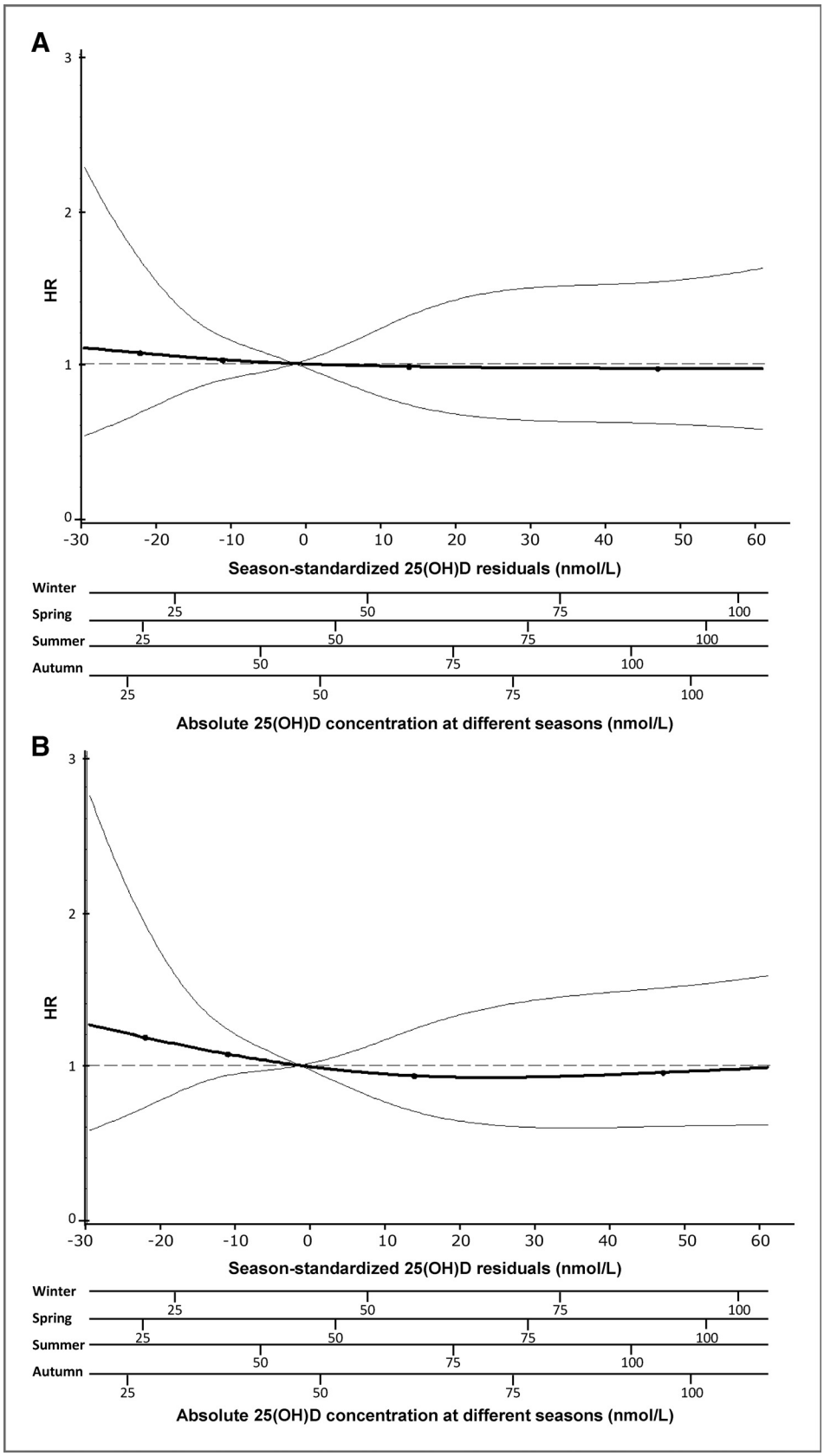

Figure 3. Dose–response relationship between season-standardized 25(OH)D levels and lung (A) and colorectal cancer incidence (B). Point estimates of hazard ratios (fat line) and 95% CIs (curved thin lines) were obtained by restricted cubic splines Cox regression analysis with knots at the 5th, 25th, 75th, and 95th, percentile [50th percentile (median) as the reference]. The horizontal dashed line marks the HR null effect value of 1.0.

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nmol/L increase in 25(OH)D concentrations (14, 17, 18). However, the meta-analyses focused on a linear relationship across the full range of 25(OH)D concentrations. Our dose–response analyses suggest that it could be of interest for further studies to specifically address prostate cancer risk at the low and high end of 25(OH)D levels.

To our best knowledge, no study reported on the association of 25(OH)D concentrations and total cancer incidence in women so far. We observed no increased cancer risk for women at low season-standardized 25(OH)D concentrations. A similar "flat" dose-response curve was observed for total cancer and for breast cancer, the by far most common cancer in women. Our finding of an absence of an association of season-standardized 25(OH)D concentrations with breast cancer risk are in line with previous reports from prospective studies from other countries (14, 20). In contrast, low 25(OH)D concentrations were reported to be associated with increased breast cancer risk in case–control studies (14, 19, 20). However, measurement of 25(OH)D around the time of diagnosis among cases precludes discrimination of a temporal from a causal relationship in these studies.

With respect to lung cancer, we found an increased risk for low season-standardized 25(OH)D concentrations in initial analyses adjusting for age and sex. However, this risk increase was strongly reduced and no longer statistically significant after adjustment for smoking and additional covariates. Our results are in line with the results from the HPFS, in which predicted 25(OH)D concentrations were also nonsignificantly inversely associated with lung cancer incidence (37). Furthermore, a significant protective effect of sufficient 25(OH)D concentrations on lung cancer incidence was observed in the women but not in the men of a study from Finland (41). Taken together, there are indications for a weakly increased lung cancer risk for subjects with low 25(OH)D levels but further, ideally larger studies are required to verify this finding.

For the association of 25(OH)D concentrations and colorectal cancer, several meta-analyses have been conducted to integrate findings from case–control studies, nested case–control studies, and one cohort study. These meta-analyses determined a significant inverse association between 25(OH)D concentrations and colorectal cancer (12–16). This is in agreement with a nonsignificant 23% reduction of colorectal cancer risk in the highest season-standardized 25(OH)D quartile in this population-based prospective cohort study. However, this association was not statistically significant which could be due to a limited sample size or an insufficiently long follow-up time to observe such an association. Previous studies reporting a significant association of 25(OH)D and colorectal cancer included a larger number of cases and longer follow-up periods, whereas those studies with lower numbers of cases and shorter follow-up time usually yielded nonsignificant associations (14). Consequently, additional larger prospective cohort studies are required to investigate the strength of the association of low 25(OH)D status and colorectal cancer.

We restricted cancer site–specific analyses to the most common cancer types in our cohort because of sample size limitations for rarer cancers. The "Vitamin D Pooling Project of Rarer Cancers" observed no association between 25(OH)D levels and non–Hodgkin lymphoma or cancers in the ovaries, kidneys, endometrium, or the upper gastrointestinal tract (42). The differences in risk across cancer types might be the most likely explanation for the low nonsignificant association of 25(OH)D concentrations and overall cancer incidence in our and other prospective studies with this combined outcome.

Some limitations and strengths have to be considered when interpreting our study results. Strengths include the population-based character of the cohort, the LC/MS-MS standardized 25(OH)D measurements, a thorough adjustment for potential confounders and the collaboration with an almost complete regional cancer registry. The main limitation of our study is the sample size. Despite having at least 136 cases for each analysis, the power to detect moderate associations of cancer risk between 25(OH)D concentrations and the risk for overall and site-specific cancers was rather limited. A main reason for the limitations of power may also be that increased risks may be restricted to very low or very high 25(OH)D concentrations (21) which were rare in our study population.

In conclusion, 25(OH)D concentrations were not significantly associated with overall and site-specific cancers in our large population–based cohort of older German adults. However, men, nonobese subjects, and subjects consuming fish less than once a week had an increased risk to develop any cancer if they also had season-standardized 25(OH)D concentrations in the first quartile. For nonobese and nonsmokers, risk was increased when their season-standardized 25(OH)D was in the fourth quartile. Potential interactions between these risk factors and 25(OH)D concentrations could be of relevance for potential interventions and might deserve specific attention in future observational and intervention studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.M. Ordóñez-Mena, B. Schöttker, U. Haug, J. Köhrle, H. Brenner

Development of methodology: J.M. Ordóñez-Mena, B. Schöttker, J. Köhrle, L. Schomburg

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Köhrle, L. Schomburg, B. Holleczek, H. Brenner

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.M. Ordóñez-Mena, B. Schöttker, H. Brenner

Writing, review, and/or revision of the manuscript: J.M. Ordóñez-Mena, B. Schöttker, U. Haug, H. Müller, J. Köhrle, L. Schomburg, B. Holleczek, H. Brenner

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Müller, L. Schomburg

Study supervision: J. Köhrle, H. Brenner

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