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Low Plasma 25-Hydroxyvitamin D and Risk of Tobacco-Related Cancer

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BACKGROUND: Tobacco smoke chemicals may influence vitamin D metabolism and function, and conversely vitamin D may modify the carcinogenicity of tobacco smoke chemicals. We tested the hypothesis that lower plasma 25-hydroxyvitamin D [25(OH)D] is associated with a higher risk of tobacco-related cancer in the general population.

METHODS: A prospective population-based cohort of 9791 individuals from the Copenhagen City Heart Study who were free of cancer at baseline was followed from 1981–1983 until December 2008 with 100% complete follow-up.

RESULTS: During up to 28 years of follow-up, 1081 participants developed a tobacco-related cancer and 1506 developed other cancers. Decreasing 25(OH)D concentrations, subdivided by clinical categories or by seasonally adjusted percentile categories, were associated with increasing cumulative incidence of tobacco-related cancer (log-rank trend $P = 2 \times 10^{-6}$ and $P = 5 \times 10^{-9}$). Multivariable adjusted hazard ratios of tobacco-related cancer were 1.75 (95% CI, 1.33–2.30) for 25(OH)D <5 vs ≥20 ng/mL, and 2.07 (1.63–2.62) for ≤5th vs >66th percentile. Also, multivariable adjusted hazard ratios for a 50% reduction in 25(OH)D were 1.20 (1.13–1.28) for any tobacco-related cancer, 1.19 (95% CI, 1.09–1.31) for lung cancer, 1.44 (1.19–1.73) for head and neck cancer, 1.28 (1.06–1.54) for bladder cancer, 1.34 (1.04–1.73) for kidney cancer, and 0.95 (0.89–1.01) for other cancers.

CONCLUSIONS: Lower plasma 25(OH)D was associated with higher risk of tobacco-related cancers, but not with risk of other cancers.

Decreased vitamin D concentrations may increase cell proliferation, hinder cell differentiation, and promote tissue invasion, metastasis, and angiogenesis in tumors *(1–7)*. Thus, lower vitamin D concentrations may lead to higher risk of cancer.

Tobacco smoke chemicals may influence vitamin D metabolism and function *(8–10)*, and conversely vitamin D may modify the carcinogenicity of tobacco smoke chemicals *(3, 11–14)*. Such studies led the International Agency for Research on Cancer to suggest that the effect of tobacco smoke on vitamin D may explain a component of the carcinogenicity conferred by smoking *(15)*; however, evidence from later nested case control and prospective cohort studies regarding individual tobacco-related cancers has been conflicting *(16–23)*. It is thus unclear whether lower plasma 25-hydroxyvitamin D [25(OH)D][4] concentrations are associated with the risk of tobacco-related cancers.

We tested the hypothesis that a lower plasma 25(OH)D concentration is associated with higher risk of tobacco-related cancer in the general population. For this purpose, we studied 9791 white individuals from the Copenhagen City Heart Study followed for up to 28 years. Two aspects make this cohort unique: in Northern Europe ultraviolet B radiation from the sun is adequate for sufficient endogenous vitamin D production in the skin only during the summer months from May to September and food has never been fortified with vitamin D in Denmark. Thus, this cohort from the Danish general population allows determination of the natural history of the association of vitamin D deficiency with risk of tobacco-related cancer. For comparison we also studied the risk of other cancers.

Materials and Methods

STUDY DESIGN

The Copenhagen City Heart Study is a prospective cohort study of the Danish general population initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1993, and 2001–2003 *(24, 25)*. Individuals

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[4] Nonstandard abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; HR, hazard ratio.

20–100 years of age were drawn randomly from the national Danish Central Person Register and invited to participate; all inhabitants in Denmark are uniquely identified through their central person registration number that also holds information on age and sex.

The present study included 9791 of 12 175 participants free of cancer at baseline from the 1981–1983 examination. The remaining participants did not have plasma samples for 25(OH)D measurement.

A Danish ethics committee approved the study (KF100.2039/91 and KF01–144/01). Participants provided written informed consent.

MEASUREMENTS OF 25(OH)D

Plasma samples collected at baseline in 1981–1983 were stored at −20 °C until 2009–2010 when 25(OH)D was measured using the DiaSorin Liaison 25(OH)D TOTAL assay *(26–28)*. Assay precision was tested daily with unmasked QCs, while assay accuracy was tested monthly using an external QCl program. The interassay CV was 10% for low-concentration controls [approximately 16 ng/mL (40 nmol/L)] and 8% for high-concentration controls [approximately 54 ng/mL (135 nmol/L)].

COVARIATES

Information on smoking habits was obtained from self-reported questionnaires completed together with an examiner on the day of attendance. Former and current smokers were asked the age they started smoking, and former smokers were also asked the age of smoking cessation. Former and current smokers were asked if they smoked cigarettes, cheroots, cigars, and/or pipe tobacco. Daily tobacco consumption (grams per day) was calculated for current smokers. Cumulative tobacco consumption was calculated for former and current smokers in pack-years; a pack-year was defined as 20 g of tobacco per day for a year.

Participants were also asked about type and amount of weekly alcohol consumption, which was then calculated in grams per week. Furthermore, participants were asked about duration and intensity of leisure time and work-related physical activity, and about years of education including and up to university education. Body mass index (BMI) was calculated as measured weight (kilograms) divided by measured height (meters) squared.

CANCER END POINTS

Diagnoses of invasive cancer from 1943 to December 2008 were obtained from the Danish Cancer Registry that identifies 98% of cancer cases in Denmark *(29, 30)*. Diagnoses from the date of blood sampling and onward were classified according the WHO International Classification of Diseases tenth edition (ICD-10) (see Supplemental Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol59/issue5). In total, 1081 cases were identified; of these, 199 cases (18%) were identified on the basis of postmortem diagnoses.

The end point of this study was incident tobacco-related cancer; however, we also ascertained information on other cancers *(25, 31)*. The combined end point, tobacco-related cancer, consisted of cancers for which smoking has been shown to be a likely causal factor, i.e., lung cancer (n = 507), head and neck cancer (n = 122), bladder cancer (n = 112), kidney cancer (n = 55), liver cancer (n = 55), esophageal cancer (n = 43), stomach cancer (n = 64), pancreatic cancer (n = 109), cervical cancer (n = 32), and myeloid leukemia (n = 21) (see online Supplemental Table 1) *(15, 25)*.

Head and neck cancer was any cancer of paranasal sinuses, salivary glands, nasal cavity, oral cavity, pharynx, and larynx. Three types of tobacco-related cancers had fewer than 50 events (cervical cancer, esophageal cancer, and myeloid leukemia) and were analyzed collectively as "other tobacco-related cancers."

Follow-up time for each study participant began the day of blood sampling in 1981–1983 and ended at the first incident of tobacco-related cancer, death (n = 5102), emigration (n = 55), or December 2008, whichever occurred first. The median follow-up time to the first incident of tobacco-related cancer, death, emigration, or December 2008 was 21 years (range 0.01–28 years). Follow-up was 100% complete, that is, we did not lose track of even a single individual.

STATISTICAL ANALYSES

On the basis of the clinical recommendations derived from the effects of 25(OH)D on bone health *(32)*, we divided baseline 25(OH)D into the following a priori seasonally unadjusted clinical categories of ≥20 ng/mL (50 nmol/L) (sufficient levels), 10–19.9 ng/mL (25–49.9 nmol/L) (insufficient levels), 5–9.9 ng/mL(12.5–24.9) nmol/L (deficient levels), and <5 ng/mL (12.5 nmol/L) (severe deficiency). We also chose to divide 25(OH)D concentrations <25 mmol/L into 2 subgroups to be able to evaluate extremely low concentrations. In addition, because the concentrations of 25(OH)D were expected to vary according to time of year because of the high-latitude geographical position of Denmark, we also used seasonally adjusted 25(OH)D concentrations *(33)* (see the online Supplemental Methods). We divided the seasonally adjusted values into a top (67th–100th percentile) and middle (34th–66th percentile) tertile, whereas the bottom tertile was further subdivided into 3 groups: 11th–33th percentile, 6th–10th percentile, and ≤5th percentile to extract information regarding extreme phenotypes as was done previously *(34, 35)*. These cut points were

Table 1. Baseline characteristics according to clinical cut points for 25(OH)D plasma concentrations.

	Plasma 25(OH)D, ng/mL				
	<5	5–9.9	10–19.9	≥20	Trend, P[a]
Characteristic	n = 470	n = 1824	n = 3936	n = 3561	
Men, %	47	46	44	44	0.09
Age, years	59 (50–65)[b]	58 (49–65)	58 (48–64)	57 (47–64)	<0.001
Ever smoker, %	87	83	79	77	<0.001
Current tobacco consumption, g/day	10 (0–16)	8 (0–16)	6 (0–12)	2 (0–12)	<0.001
Cumulative tobacco consumption, pack-years	27 (13–40)	23 (10–36)	19 (5–33)	18 (3–30)	<0.001
BMI, kg/m^2	25 (22–29)	26 (23–29)	25 (23–28)	24 (22–27)	<0.001
Alcohol consumption, g/week	24 (0–168)	36 (0–144)	48 (0–132)	60 (0–132)	<0.001
High physical activity, leisure, %	26	28	34	40	<0.001
High physical activity, work, %	25	27	25	24	0.05
Education, years	7 (7–9)	7 (7–9)	8 (7–10)	8 (7–10)	<0.001

[a] P values were calculated with Cuzick's nonparametric trend test.
[b] All continuous variables are summarized as median and interquartile range.

chosen to have a reference group with a reasonable size, and to have enough participants in the extreme groups for meaningful analysis. For trend tests, individuals in each group were assigned the median value of their group, either as absolute values or as percentiles.

Cox proportional hazards regression was used to estimate hazard ratios (HRs) with 95% CI for incident cancer. We used age as the time scale, with delayed entry (left truncation). Thus, age differences were automatically adjusted for and referred to in the text, tables, and figures as age adjusted. However, for the test of the interaction of age with 25(OH)D concentrations on cancer risk, we used years of follow-up as the time scale. Multivariable adjusted Cox regression models included age, sex, education, calendar month of blood sampling, and time-dependent covariates from the 1981–1983, 1991–1994, and 2001–2003 examinations, including cumulated tobacco consumption in pack-years, BMI, alcohol consumption in grams per week, and level of leisure time and work-related physical activity. Nonsmokers or never smokers were included in daily and cumulated tobacco consumption with the value zero. All covariates except physical activity were on a continuous scale. The use of different variables describing smoking status or cumulative tobacco consumption gave similar estimates from regression models, so only models adjusted for pack-years are shown. Interactions were tested by use of likelihood ratio tests with Cox regression models, including and excluding multiplicative 2-factor interaction terms. In interaction analyses and stratified analyses, we used $\log_2$ transformed values of plasma 25(OH)D, whereby a 1-U decrease corresponds to a 50% reduction in plasma 25(OH)D. We tested for the proportional hazards assumption in Cox regression models using Schoenfeld residuals; no departures were detected for the different plasma 25(OH)D variables used. Supplementary analyses using competing risk proportional subhazard models, in which competing risk of death was accounted for, were also conducted as were formal analyses for possible mediation or moderation by plasma 25(OH)D (see online Supplemental Methods).

The data were 99% complete; missing data were handled with multiple imputation using BMI, alcohol consumption in grams per week, level of leisure time and work-related physical activity, and education as dependent variables and age and sex as independent variables.

Heterogeneity between estimates was assessed by the Q statistic and its extent was quantified by I^2 (the fraction of between-estimate variability because of heterogeneity) *(36)*. Although these methods assume independence of the estimates, extreme values are interpretable as tests of statistical heterogeneity.

We analyzed the data with the statistical package STATA 11.2.

Results

Table 1 summarizes baseline characteristics by 25(OH)D concentrations. Decreasing concentration of 25(OH)D was associated with increased age, smoking status, increased current tobacco consumption, increased cumulative tobacco consumption, increased BMI, decreased alcohol consumption, decreased leisure time physical activity, and lower duration of education. The dose–response relationship between smoking status or level of tobacco consumption and

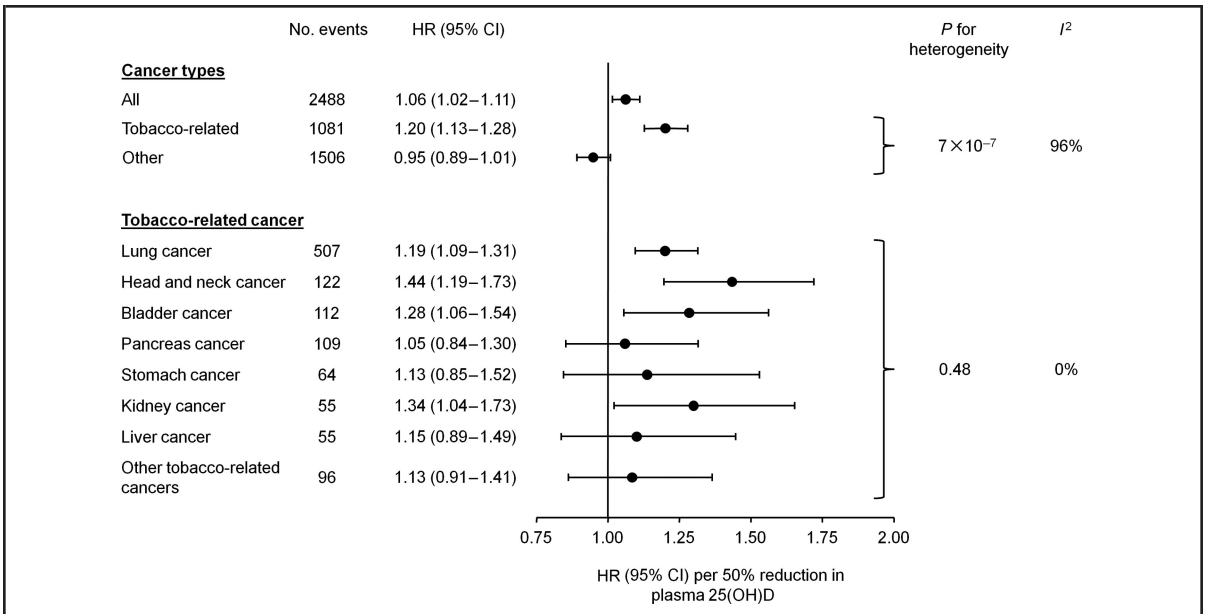

Fig. 1. Hazard ratios for a 50% reduction in plasma 25(OH)D for all cancer types, other cancers, and tobacco-related cancers combined and individually.

Hazard ratios were adjusted for age, sex, pack-years, BMI, alcohol consumption, leisure time and work-related physical activity, and duration of education. Note that some participants developed multiple cancers. Thus, the sum of individual cancers exceeds the number given for all cancer and tobacco-related cancers. The *P* value is for the test of heterogeneity and I^2 quantifies the extent of heterogeneity. Data were derived from study of 9791 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling.

25(OH)D is presented in online Supplemental Fig. 1. The median 25(OH)D concentration was 16.4 ng/mL(41 nmol/L) among all participants and 14.8 (37 nmol/L) among those who later developed a tobacco-related cancer. There was seasonal variation across the months of the year with high concentrations in August–September and low concentrations in January–February (see online Supplemental Fig. 2). A total of 1081 incident tobacco-related cancers and 1506 other cancers occurred among 9791 participants during up to 28 years of follow-up. For 400 healthy participants we had measurements of plasma 25(OH)D from 1981 to 1983 stored at −20 °C, from 1991 to 1994 stored at −80 °C, as well as from 2001 to 2003 stored at −80 °C, which showed that median concentrations were 18 ng/mL, 15 ng/mL, and 17 ng/mL, respectively. Thus, the concentration of 25(OH)D did not vary systematically with storage time or temperature (see online Supplemental Table 2).

RISK FOR ANY CANCER IN RELATION TO 25(OH)D

The multivariable adjusted HR for any type of cancer was 1.06 (95% CI, 1.02–1.11) for a 50% reduction in 25(OH)D. Dividing the end points into tobacco-related and other cancers showed that the association was mainly driven by tobacco-related cancers, indicated by the high degree of heterogeneity between the estimates (Fig. 1). Individual tobacco-related cancers all showed increased risk, with a 50% reduction in plasma 25(OH)D, albeit not significantly so for every cancer (Fig. 1). Nevertheless, the low degree of heterogeneity between the individual estimates, and the fact that all the associations were in the same direction, provides the statistical justification for combining the end points.

Multivariable adjusted HRs for a 50% reduction in 25(OH)D concentration were 1.19 (95% CI, 1.09–1.31) for lung cancer, 1.44 (1.19–1.73) for head and neck cancer, 1.28 (1.06–1.54) for bladder cancer, and 1.34 (1.04–1.73) for kidney cancer (Fig. 1). Corresponding HRs were not significant for pancreas cancer, stomach cancer, liver cancer, and other tobacco-related cancers.

RISK FOR ANY TOBACCO-RELATED CANCER IN RELATION TO 25(OH)D

The cumulative incidence of tobacco-related cancers increased with decreasing concentrations of baseline plasma 25(OH)D expressed in clinical categories (log-rank trend: $P = 2 \times 10^{-6}$) and expressed in seasonally adjusted percentile categories ($P = 5 \times 10^{-9}$) (Fig. 2).

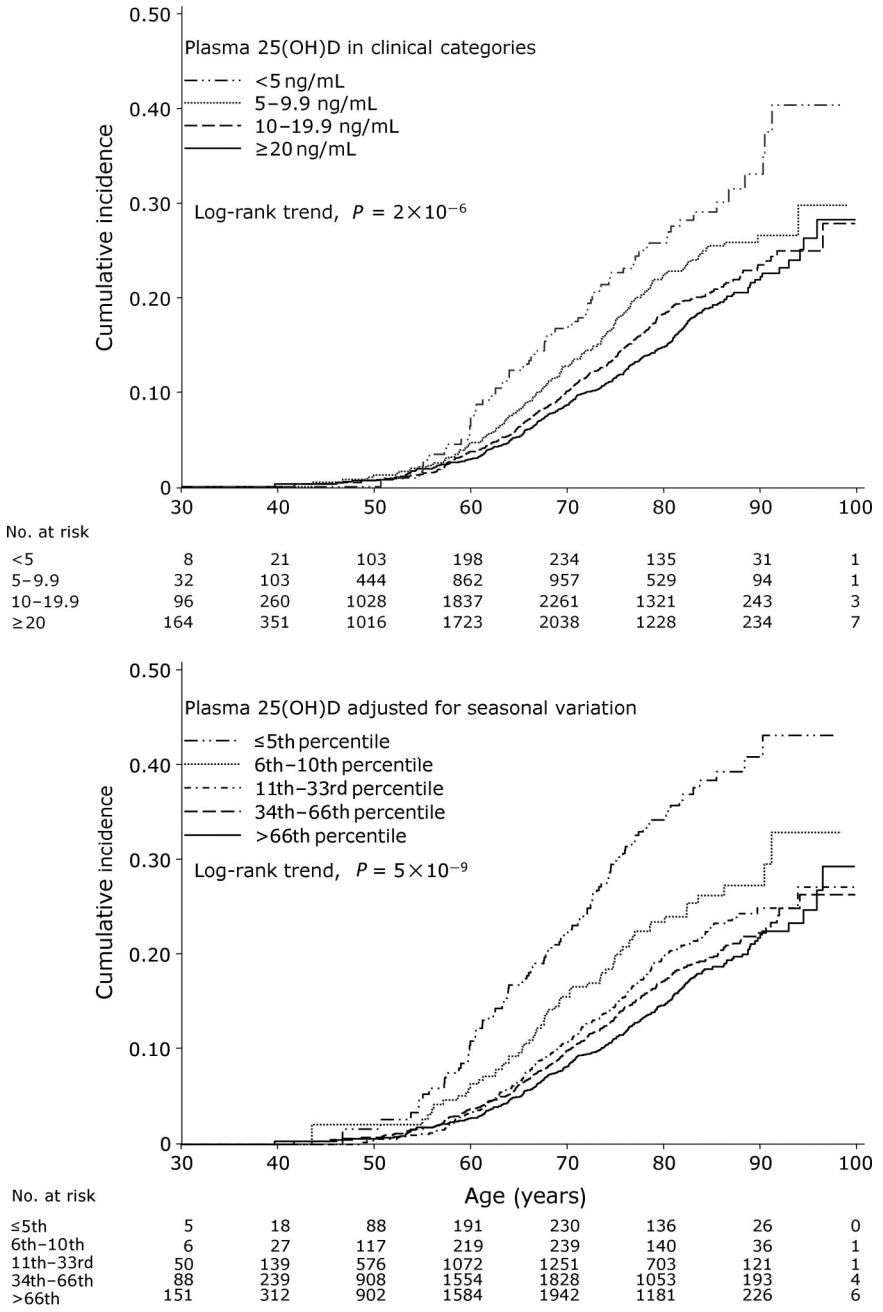

Fig. 2. Cumulative incidence of tobacco-related cancer by plasma 25-hydroxyvitamin D (25(OH)D) in clinical categories and in seasonally adjusted percentiles using the Kaplan-Meier method.

P values for log-rank trend test indicate whether decreasing concentrations of 25(OH)D are associated with increasing cumulative incidence of tobacco-related cancer. Data were derived from study of 9791 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling.

Adjusted HRs for tobacco-related cancers increased with decreasing concentrations of 25(OH)D by clinical categories and by seasonally adjusted percentile categories in the 2 differently adjusted models (Fig. 3). We used 2 different multivariable adjusted models: one including all potential confounders except cumulative tobacco consumption, and the other model also including cumulative tobacco consumption. Multivari-

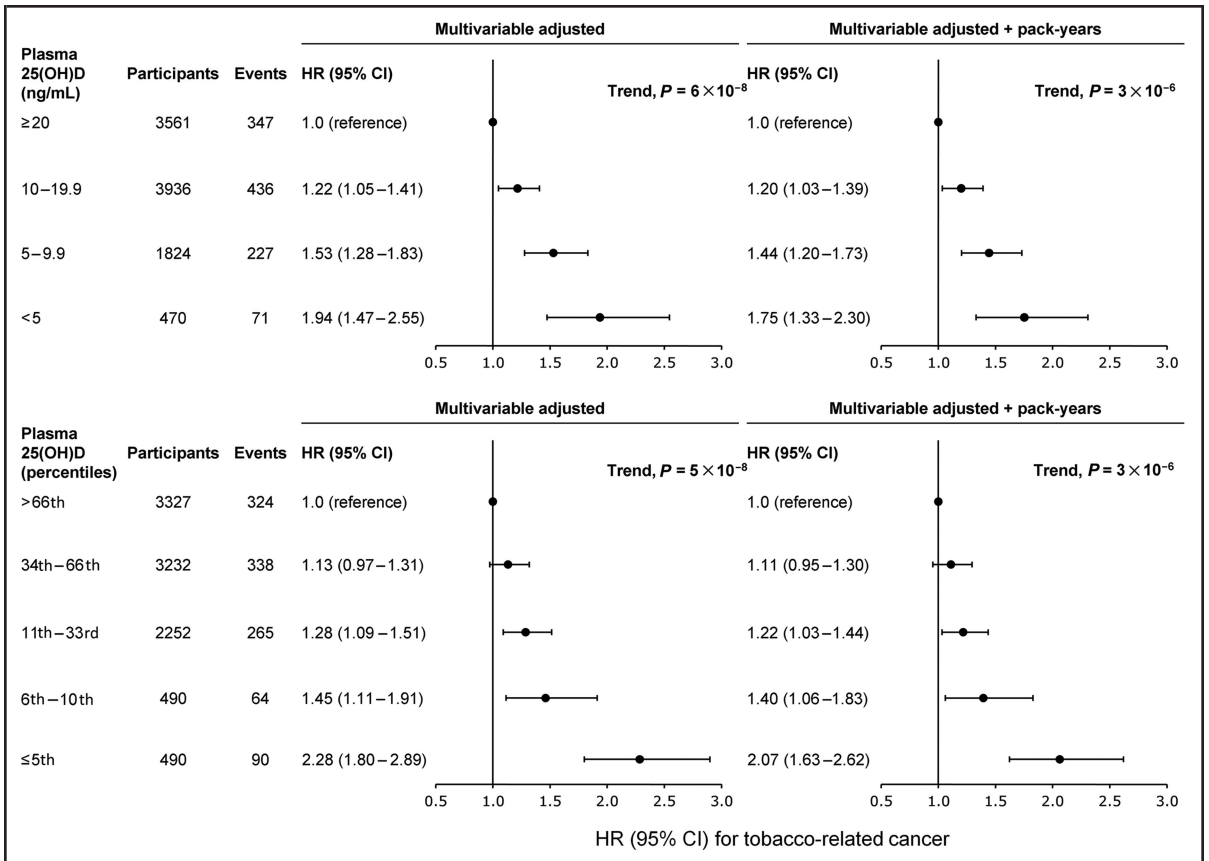

Fig. 3. Hazard ratios for tobacco-related cancer by plasma 25(OH)D in clinical categories and in seasonally adjusted percentiles.

We used 2 different multivariable adjusted models: one including all potential confounders except cumulative tobacco consumption, and the other model also including cumulative tobacco consumption. Multivariable models were adjusted for age, sex, pack-years, BMI, alcohol consumption, leisure time and work-related physical activity, and duration of education. Furthermore, the models using the clinical categories for 25(OH)D were adjusted for month of blood sample. Data were derived from study of 9791 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling.

able adjusted HRs that included adjustment for pack-years were 1.75 (95% CI, 1.33–2.30) for 25(OH)D <5 ng/mL(12.5 nmol/L) vs ≥20 ng/mL (50 nmol/L), and 2.07 (1.63–2.62) for ≤5th vs >66th percentile.

Adjusted HRs for tobacco-related cancers increased with decreasing concentrations of 25(OH)D by clinical categories in analyses stratified by cumulative tobacco consumption among smokers in each of the 3 strata (see online Supplemental Fig. 3). In addition, risk of tobacco-related cancers in smokers increased in each of the 25(OH)D clinical categories with increasing levels of cumulative tobacco consumption (see online Supplemental Fig. 3).

The multivariable adjusted HR for a 50% reduction in 25(OH)D was 1.20 (1.13–1.28) (Fig. 1 and 4). In stratified analyses, a 50% reduction in 25(OH)D was associated with HRs of 1.05–1.28 and no interactions were detected in our models; the only nonsignificant HR was in never smokers with a HR of 1.05 (0.81–1.38) vs that in ever smokers of 1.20 (1.13–1.28) (Fig. 4).

Cumulative incidences and multivariable adjusted sub-HRs for tobacco-related cancers also increased with decreasing concentrations of 25(OH)D by clinical categories and by seasonally adjusted percentile categories in analyses accounting for competing risk of death (see online Supplemental Figs. 4 and 5).

ANALYSES FOR MEDIATION AND MODERATION

We found that 25(OH)D was a significant mediator of the association of higher cumulative tobacco consumption with increased risk of tobacco-related cancer ($z_{\text{mediation}} = 3.38$, $P = 7 \times 10^{-4}$). Several analyses were

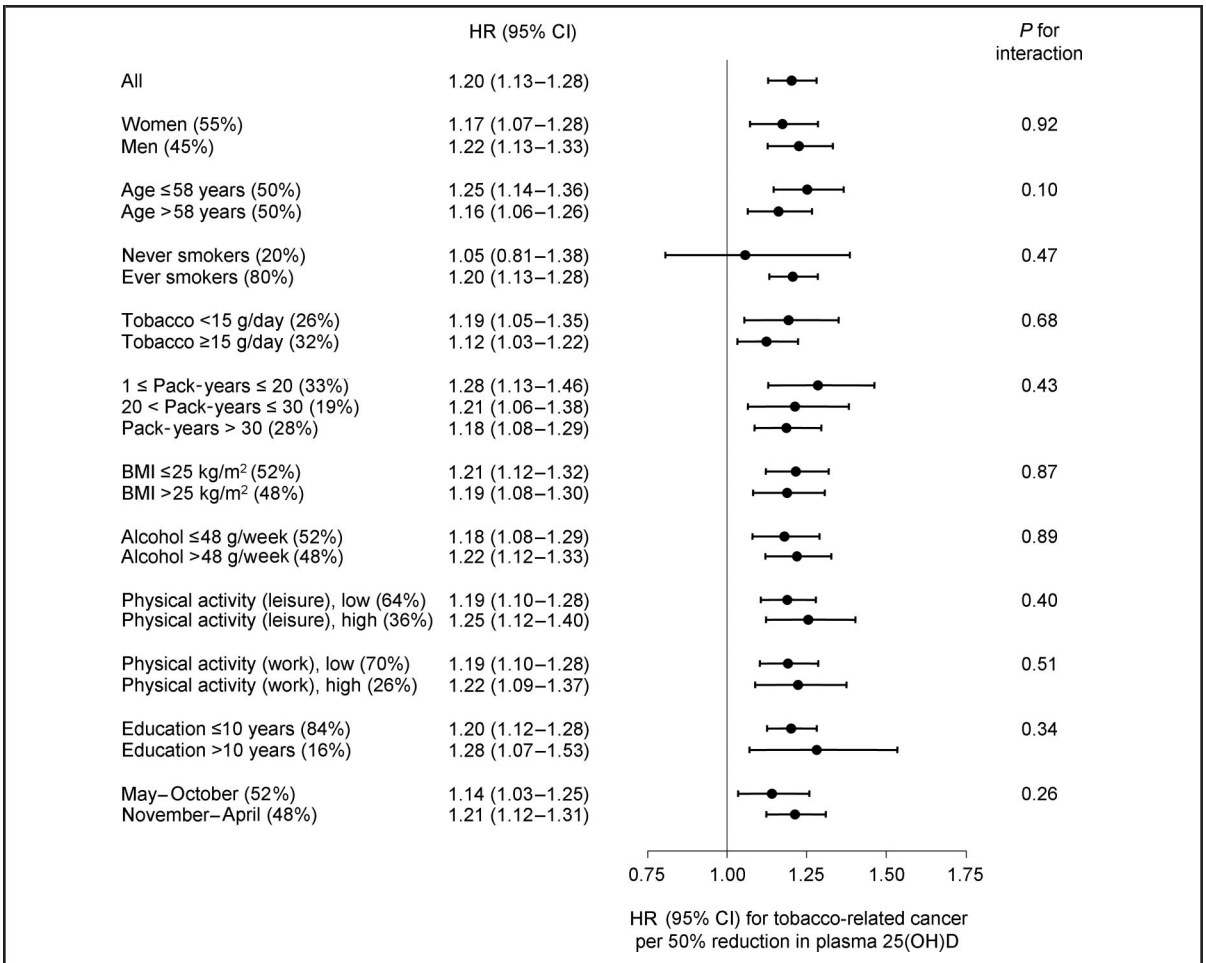

Fig. 4. Hazard ratios for a 50% reduction in plasma 25(OH)D for tobacco-related cancer overall and in strata, adjusted for age, sex, pack-years, BMI, alcohol consumption, leisure time and work-related physical activity, and duration of education.

Age, tobacco consumption, and alcohol consumption were categorized using their respective median values. Never smokers were excluded from the analyses regarding current tobacco consumption and pack-years. Data were derived from study of 9791 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling.

carried out to determine whether decreasing concentration of 25(OH)D is a moderator of the association between cumulative tobacco consumption and tobacco-related cancers (Fig. 4; also see online Supplemental Fig. 3). These analyses did not show a significant interaction of 25(OH)D with smoking on risk of tobacco-related cancers regardless of how we classified smoking status or tobacco consumption ($P = 0.47$; $P = 0.68$; $P = 0.43$; Fig. 4).

RISK OF OTHER CANCERS IN RELATION TO 25(OH)D

Multivariable adjusted HRs for other cancers for decreasing levels of 25(OH)D by clinical categories or by seasonally adjusted percentile categories did not differ from 1.0 (see online Supplemental Figs. 6 and 7). The multivariable adjusted HR for a 50% reduction in 25(OH)D was 0.95 (95% CI, 0.89–1.01) (Fig. 1).

SENSITIVITY ANALYSES

The multivariable adjusted HR for a 50% reduction in plasma 25(OH)D was 1.20 (95% CI, 1.12–1.29) and 1.20 (1.10–1.30) after exclusion of participants who developed a tobacco-related cancer within 5 and 10 years of follow-up, respectively (Fig. 5). Furthermore, when the follow-up was restricted to 10, 15, and 20 years or excluded the largest contributor to tobacco-related cancers, i.e., lung cancer, the analyses led to almost identical effect estimates as the main analyses (Fig. 5).

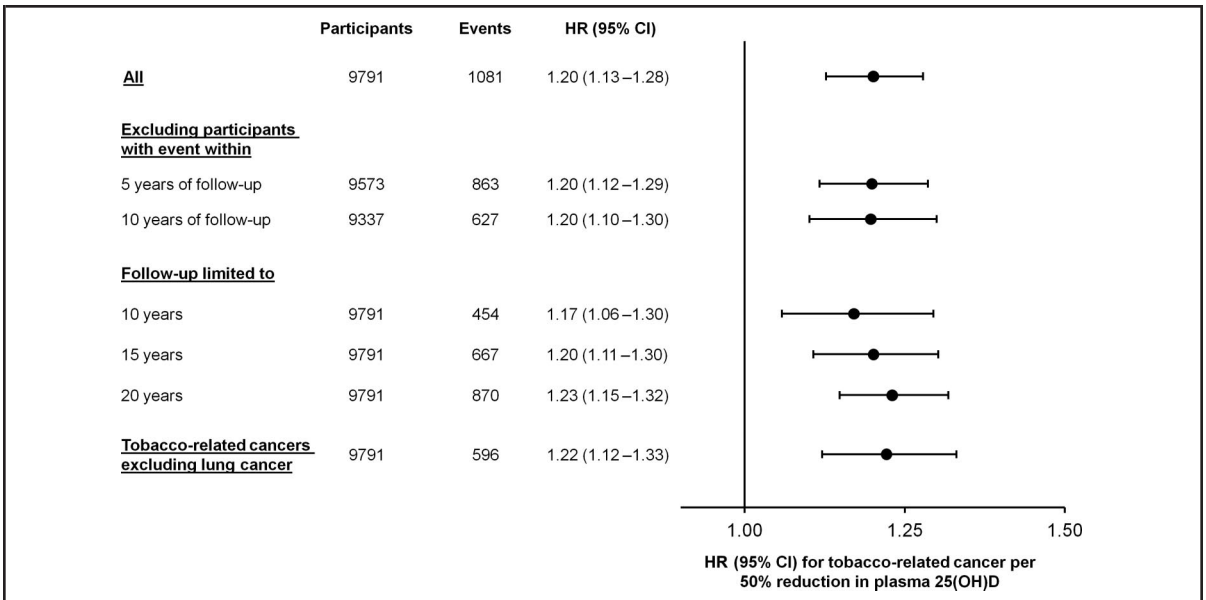

Fig. 5. Sensitivity analyses with HRs for a 50% reduction in plasma 25(OH)D for tobacco-related cancer.
Analyses were adjusted for age, sex, pack-years, BMI, alcohol consumption, leisure time and work-related physical activity, and duration of education.

We analyzed the excluded participants to see whether the incidence of tobacco-related cancers were different between nonresponders, responders without plasma samples, and responders with plasma samples. As shown in the online Supplemental Fig. 8 the cumulative incidence values were similar in these populations. Baseline characteristics were also similar between responders without plasma samples and responders with plasma samples (see online Supplemental Table 3).

Discussion

We found that lower concentrations of 25(OH)D were associated with higher risk of tobacco-related cancer, but not of other cancers in this prospective cohort study with 9791 participants from the general population observed for up to 28 years.

Mechanistically, our results are biologically plausible for several reasons. First, the metabolically active vitamin D derivative, 1α,25-dihydroxyvitamin D, and the pharmacomimetics thereof, decrease tumor invasion, metastasis, and angiogenesis in many in vitro and in vivo cancer models of tobacco-related cancer such as lung, bladder, and oral cancers *(2–4;11–14;37)*. Second, animal models with deficiencies in the 1α,25-dihydroxyvitamin D–signaling pathways show increased susceptibility to chemical carcinogenicity including chemicals related to tobacco smoke carcinogens *(14, 37)*. Finally, smoking is associated with reduced 25(OH)D concentrations *(15)*, and reduced concentrations of vitamin D are associated with increased proliferation, decreased apoptosis, and inhibited differentiation in both normal and neoplastic cells *(1, 5, 6, 14, 37–39)*.

Hitherto, the risk of tobacco-related cancers as a group in relation to lower plasma 25(OH)D concentrations has not been investigated in the general population; however, several individual tobacco-related cancers have been investigated with divergent results *(16–21)*. The association between lower 25(OH)D concentrations and higher risk of bladder and lung cancer is consistent with previous findings *(19, 20, 23)*; however, the associations with higher risk of head and neck cancer and kidney cancer are in contrast to previous studies that did not find any associations *(16, 17)*. The causes of these discrepancies could be that some studies have been conducted on selected subpopulations *(16)*, e.g., only male smokers, or have had shorter follow-up than in our study *(17)*. Alternatively, we had fewer cases of kidney cancer in our population, which could increase the risk of spurious findings. Furthermore, no studies have used the end point, tobacco-related cancer, as a group, so direct comparison with previous work is not possible. However, our analyses show that the association between lower concentrations of plasma 25(OH)D and higher risk of cancer may be driven by tobacco-related cancer as a group,

which has not been shown before. This is important for future studies investigating the association between plasma 25(OH)D and risk of cancer. Furthermore, statistical analyses indicate that low plasma 25(OH)D may be a mediator rather than a moderator of tobacco smoke carcinogenesis, i.e., lower 25(OH)D may be involved in the pathway of tobacco smoke carcinogenesis. However, additional studies are needed to confirm this finding.

Potential limitations of our study include healthy-participant bias because participants are typically healthier than background populations; however, this would tend to weaken the associations rather than strengthening them and thus cannot explain our findings. Also, the cumulative incidence of tobacco-related cancers in responders and in nonresponders of the 1981–83 examination of the Copenhagen City Heart Study was similar. Furthermore, as our cohort consists of white Danes living in Denmark with less sun exposure than those closer to the equator, our findings would be most relevant for individuals with a similar level of sun exposure and for similar 25(OH)D concentrations. The delay in measurement from 1981–1983 to 2009–2010 could raise the concern of potential decay of plasma 25(OH)D, but several observations makes this highly unlikely: we noticed the expected seasonal variation of 25(OH)D concentrations; we found a strong association with skin cancer as expected with a reliable 25(OH)D measurement *(27)*; median concentrations of plasma 25(OH)D across plasma samples from 3 different examinations on the same healthy participants with storage times of 10, 20, and 30 years were similar *(27, 28)*; previous studies have shown high stability during storage *(40)*; the median concentration observed in our study of 16 ng/mL (41 nmol/L) was similar to that in comparable populations *(16, 19)*, and a low sample quality for the 25(OH)D measurement would tend to weaken rather than inflate an association. Finally, residual confounding cannot be ruled out as a potential explanation for our findings, because this is an observational study.

In Northern Europe, ultraviolet B radiation from the sun is adequate for sufficient endogenous vitamin D production in the skin only during the summer months, and food has never been fortified with vitamin D in Denmark. The present study therefore allows for the determination of the natural history of the association of plasma vitamin D concentrations with risk of tobacco-related cancer. However, through the use of supplements, plasma 25(OH)D concentrations might increase. Because in recent years much more attention has been paid to vitamin D, plasma concentrations measured in the early 1980s were likely less influenced by such supplements than presently. Thus, in populations deriving a large part of their vitamin D from supplements, the present observed association may be different, e.g., in more recent cohorts having been collected after the increase in the focus on vitamin D supplementation.

Our study has several strengths: our population was homogenous, we had up to 28 years of complete follow-up, we could account for the time dependency of model covariates, we had detailed smoking history updated at each follow-up, and, finally, we had enough statistical power to examine the associations of very low concentrations of plasma 25(OH)D with tobacco-related cancer risk.

In conclusion, we have demonstrated that lower plasma 25(OH)D was associated with higher risk of tobacco-related cancers, but not with risk of other cancers.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

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