Research Article

Cancer Epidemiology, Biomarkers & Prevention

Association of 25-Hydroxyvitamin D with Liver Cancer Incidence and Chronic Liver Disease Mortality in Finnish Male Smokers of the ATBC Study

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Abstract

Background: Although circulating 25-hydroxyvitamin D [25(OH)D] concentrations were linked to liver cancer and chronic liver disease (CLD) in laboratory studies, few epidemiologic studies have addressed the associations.

Methods: Within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, we measured 25(OH)D in baseline serum of 202 incident liver cancer cases and 225 CLD deaths that occurred during nearly 25 years of follow-up, and 427 controls. ORs and 95% confidence intervals (CIs) were estimated using unconditional logistic regression. We examined predetermined clinically defined cut-points, and season-specific and season-standardized quartiles.

Results: Low serum 25(OH)D concentrations were associated with higher risk of liver cancer (<25 nmol/L vs. ≥50 nmol/L: 1.98; 95% CI, 1.22–3.20; P_{trend} across categories = 0.003) and CLD mortality (1.93; 95% CI, 1.23–3.03; P_{trend} = 0.006) in models adjusted for age and date of blood draw. After additional adjustment for body mass index, diabetes, smoking, and other potential confounders, the association remained statistically significant for liver cancer (1.91; 95% CI, 1.16–3.15; P_{trend} = 0.008), but was somewhat attenuated for CLD mortality (1.67; 95% CI, 1.02–2.75; P_{trend} = 0.05). Associations were similar for analyses using season-specific and season-standardized quartiles, and after excluding participants with diabetes, or hepatitis B or C.

Conclusions: Our results suggest a possible preventive role for vitamin D against liver cancer and CLD, although the importance of the liver for vitamin D metabolism and the lack of information about underlying liver disease makes reverse causality a concern.

Impact: Future studies are needed to evaluate associations of vitamin D with liver cancer and liver disease in other populations, particularly those with a different constellation of risk factors. *Cancer Epidemiol Biomarkers Prev; 27(9); 1075–82.* ©2018 AACR.

Introduction

Liver cancer is the sixth most commonly occurring cancer in the world and the second leading cause of cancer death (1). Although a majority of liver cancer occurs in countries where hepatitis B (HBV) and C viruses (HCV) are prevalent, the incidence of liver cancer has dramatically increased in recent years in the United States and other Western countries (2, 3). This rise corresponds with higher rates of HCV infection among birth cohorts at the peak ages for cancer and higher prevalence of obesity and type II diabetes in these countries. With a very poor rate of 5-year survival after diagnosis, it is critically important to identify prevention strategies.

Vitamin D insufficiency may be related to liver cancer and liver disease. For example, vitamin D appears to demonstrate a number of positive benefits for the liver such as antiproliferative, proapoptotic, and antiinflammatory characteristics in cell lines and animal models (4). Some epidemiologic studies have reported evidence of an inverse association (5, 6). However, such associations could reflect reverse causality by undiagnosed underlying fibrosis or cirrhosis. The liver is the main site of synthesis for 25-hydroxyvitamin D [25(OH)D] (7) and the vitamin D binding protein, both of which are essential components of vitamin D signaling.

To obtain further insight into the association between vitamin D, liver disease, and liver cancer, we evaluated the association of serum 25(OH)D with incident liver cancer and chronic liver disease mortality in a case–control study nested within a cohort of Finnish men. Our study has several advantages, including a

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prospective design, exclusion of patients with diagnosed cirrhosis at baseline, available data on HBV and HCV and other known liver cancer risk factors, and nearly 25 years of follow-up.

Materials and Methods

Study population

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a randomized primary prevention trial of α-tocopherol and β-carotene supplementation, which has been described previously (8). In brief, 29,133 Finnish male smokers, ages 50 to 69, were recruited from 1985 to 1988 and randomized to daily supplementation of α-tocopherol (50 mg), β-carotene (20 mg), both, or placebo. Participants with a previous diagnosis of cirrhosis were excluded from the trial. Supplementation ended in April 1993 (median 6.1 years) and participants have been under follow-up since that time. The institutional review boards of the U.S. National Cancer Institute and the National Public Health Institute of Finland approved the study. Written informed consent was obtained from all participants.

Selection of cases and controls

Cases included men diagnosed with incident liver cancer (n = 202; ICD-9 = 155) or who died from chronic liver disease (n = 225; ICD-9 = 571 and ICD-10 = K70, K73, or K74). All liver cancer cases were identified from the Finnish Cancer Registry, whereas all chronic liver disease deaths were identified from the Finnish Register of Causes of Deaths. Both incident liver cancers and chronic liver disease deaths were identified from the time of randomization through December 31, 2009. Alcohol-related liver disease was noted to be the underlying cause of approximately 90% of the chronic liver disease deaths. Each case was matched to a control who was alive and free of cancer at the time of the case's liver cancer diagnosis or death from chronic liver disease. Cases and controls were matched (1:1) on age at randomization (±1 year), date of baseline serum collection (±30 days), and the date of measurement for vitamin D. Case and controls were adjacent in the same batch, in randomized order, with blinded quality control samples interspersed.

Specimen and data collection

At baseline, participants underwent a physical examination by registered nurses to measure height and weight, and to collect an overnight fasting blood sample. Participants also completed a questionnaire detailing their tobacco smoking, diet, lifestyle, and medical history.

Fasting serum samples that were collected at baseline were stored at −70°C. Serum 25(OH)D concentrations were measured using a direct, competitive chemiluminescence immunoassay (Heartland Assays). Most samples for the study were measured at two time points: 175 pairs in January 2008 and 242 pairs in February 2013. The inter- and intrabatch coefficients of variation (CV) were 7.1% and 10.1%, respectively, for the first assay, and 1.4% and 3.9%, respectively, for the second assay. The remaining serum concentrations (n = 13 pairs) were collected and measured from different dates ranging from January 1995 to February 2007. Specific information about the design and methods has been reported previously (9–13). The inter- and intrabatch CVs ranged from 6.3% to 8.6% and 9.3% to 11.0%, respectively.

HBV and HCV status were measured in baseline serum samples for a majority of cases and controls (167 liver cancer cases, 208 chronic liver disease deaths, and 347 controls). Testing for HBV surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), and antibody to HCV (anti-HCV) was performed by the NCI-Frederick National Laboratory. HBsAg was tested using enzyme immunoassay from Bio-Rad Laboratories (Redmond, WA), and anti-HBc and anti-HCV were tested using ELISAs from Ortho-Clinical Diagnostics. To evaluate the accuracy of these assays, we purchased blood samples with known HBV and HCV status from SeraCare. We used panels of 25 samples each (PHA206, PHE203, and PHV206) to evaluate HBsAg, anti-HBc, and anti-HCV, respectively. Concordance of our testing with the results furnished for these panels by SeraCare were 100%.

Statistical analysis

Baseline descriptive characteristics were calculated as medians (continuous variables) or proportions (categorical variables). Logistic regression models were used to estimate ORs and 95% confidence intervals (CIs) of incident liver cancer and chronic liver disease mortality by different 25(OH)D categories. Because of concern about the potential effect of season on vitamin D concentrations, we adjusted for season using three separate approaches. First, we used predefined clinical cut-points for 25(OH)D (<25, 25 to <37.5, 37.5 to <50, 50 to <75, and ≥75 nmol/L; refs. 13–15), adjusting for season in the models. As only 4% of the study participants had concentrations greater than 75 nmol/L, the referent category was defined as ≥50 nmol/L. Second, we used season-specific 25(OH)D quartiles that were based on the distribution of the controls split by season ("sunnier" months: May to October; "darker" months: November to April). Finally, we used season-standardized 25(OH)D quartiles which were determined by regressing log-transformed values of 25(OH)D concentration against calendar week of blood collection, using a locally weighted polynomial regression method. This was performed only among the two larger sets of data (measured in January 2008 or February 2013), as the smaller sets had insufficient numbers to run the polynomial regression. Quartiles were then created from the residuals (13, 16). Results from these later two approaches, season-specific and season-standardized, are presented with the highest quartile as the referent category. Tests for linear trend were conducted by assigning to each category the median value (for clinical cutpoints) or an ordinal value (one to four for season-specific and -standardized quartiles) and then treating this parameter as a continuous variable. Additional information and rationale on the use of these approaches—*a priori* clinically defined cutpoints, season-specific cutpoints, and season-standardized cutpoints—have been previously discussed (13).

We ran logistic regression models adjusted for age and date of blood draw and additionally adjusted for body mass index (BMI; calculated as [weight in kg]/[height in m]^2), history of diabetes, number of years smoked, and daily intake of alcohol and coffee. Additional adjustment for ATBC intervention arm, education, marital status, number of cigarettes smoked per day, serum α-tocopherol, serum β-carotene, serum retinol, serum total cholesterol, urban residence, physical activity, and measurement batch had little effect on the risk estimates and were not included in the final model. Results were similar for both conditional and unconditional logistic regression. Therefore, we present results from unconditional models due to their tighter CIs.

As described earlier, the majority of the serum samples (97.7%) were measured for 25(OH)D at two time points, January 2008 (175 pairs) and February 2013 (242 pairs). For these two time points, the distribution of vitamin D concentrations among the

controls were similar. In the initial measurements, we assessed vitamin D in liver cancer cases and their respective controls. We observed evidence for an association between vitamin D concentrations and incident liver cancer, but with wide CIs. Therefore, we decided to add additional cases of incident liver cancer and, to consider more fully the possible relationship between vitamin D and the liver, cases of chronic liver disease deaths and their respective controls. These samples were measured in February 2013. We also incorporated data from 13 pairs of cases and controls that had 25(OH)D measured previously. We present results from the pooled set for our main results, as these estimates are the most stable. However, the results from each individual set are shown separately in Supplementary Table S1.

Additional subgroup analyses were performed, that is participants who lacked diabetes, participants who tested negative for HBV and HCV, incident liver cancer cases that were classified as hepatocellular carcinoma (ICD-9 = 155.0), participants with complete data on alcohol and diet, participants drinking above or below the median level of daily alcohol intake in controls, and, for lag analyses, among cases occurring after the first 5, 10, and 15 years of follow-up. Effect modification by alcohol drinking was evaluated by using the likelihood ratio test to compare models with and without the cross-product term of 25(OH)D (categorical) and the dichotomous variable for median alcohol intake. For all subgroup analyses, we present results using season-specific quartiles, as these analyses allowed the largest number of controls in each vitamin D category, whereas the numbers of controls in clinically defined categories became sparse in some subgroups.

All statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC) and all *P* values were two-sided.

Results

In Table 1, we present the distribution of vitamin D concentrations by baseline characteristics in controls. Participants with their blood collected in the sunnier months (May to October) tended to have higher concentrations of 25(OH)D, as did those having an urban residence, who were more physically active, who ate more vitamin D–rich foods, and who used vitamin D supplements.

Liver cancer and chronic liver disease cases, compared with controls, drank more alcohol and less coffee at baseline; in addition, liver cancer cases, compared with controls, had a slightly higher BMI and were more likely to have diabetes at baseline (Table 2). Among those with information on HBV and HCV status, very few participants ($\leq$1.2%) were HBV positive (positive for HBsAg) and the prevalence of HCV was also low. Just one control was HCV positive, in comparison to eight (4.8%) of the liver cancer cases and five (2.4%) of participants who later died from chronic liver disease. Serum 25(OH)D concentrations were lower in men who would later develop incident liver cancer (30.4 nmol/L) or die from chronic liver disease (31.4 nmol/L) relative to their respective controls (35.2 and 37.4 nmol/L).

In unconditional logistic regression models that were adjusted for age and date of blood draw, men with 25(OH)D concentrations less than 25 nmol/L had a statistically significant increased risk for incident liver cancer (OR = 1.98; 95% CI, 1.22–3.20, P_{trend} = 0.003 across categories) and for chronic liver disease mortality (OR = 1.93; 95% CI, 1.23–3.03; P_{trend} = 0.006) compared with men with 25(OH)D concentrations at or above 50 nmol/L. Additional multivariable adjustment for alcohol use, BMI, diabetes, smoking, or coffee drinking at baseline did not alter associations for liver cancer (OR = 1.91; 95% CI, 1.16–3.15; P_{trend} = 0.008). Although the associations for chronic liver disease mortality were somewhat attenuated (OR = 1.67; 95% CI, 1.02–2.75; P_{trend} = 0.05; Table 3), primarily due to coffee and alcohol intake, associations remained statistically significant. Similar associations were observed for analyses using season-specific and season-standardized quartiles. For liver cancer incidence, the multivariable adjusted ORs were 1.87 (95% CI, 1.12-3.12; P_{trend} = 0.007) and 1.72 (95% CI, 1.02-2.88; P_{trend} = 0.02) for men in the lowest quartile of vitamin D using season-specific and season-standardized quartiles, respectively, relative to men in the highest quartile. The corresponding ORs for chronic liver disease mortality were 1.69 (95% CI, 1.00-2.88; P_{trend} = 0.06) and 1.40 (95% CI, 0.82-2.38; P_{trend} = 0.18). In analyses of clinical cut-points and season-specific quartiles that were stratified by season, associations were similar, although stronger in the subgroup of men whose blood was drawn in the summer. Similar associations were also observed among samples measured in January 2008 and February 2013 (Supplementary Table S1).

In lag analyses, displayed using season-specific quartiles, generally similar associations were observed in cases occurring five, 10, and 15 years after blood collection (Table 4), although associations were somewhat attenuated among cases occurring many years after blood collection and CIs were wider.

In additional subgroup analyses, we observed similar associations after excluding cases and controls with self-reported diabetes and after excluding the small proportion of HBV and HCV positive participants. For liver cancer, we observed similar associations when limiting the analysis to the 71.8% of cases classified as hepatocellular carcinoma. To address missing data for alcohol and diet in a subset of cases and controls (14 liver cancer cases, 23 chronic liver disease deaths, and 20 controls), we restricted the analyses to those with complete information and observed similar associations.

In analyses stratified by alcohol intake (Table 4), associations persisted for liver cancer among participants who drank both below ($OR_{lowest\ vs.\ highest\ quartile}$ = 1.49; 95% CI, 0.65–3.41) and above ($OR_{lowest\ vs.\ highest\ quartile}$ = 2.13; 95% CI, 1.06–4.28) the median intake (11.3 g/day; $P_{interaction}$ = 0.41). For mortality from chronic liver disease, we observed some evidence that associations may vary by alcohol intake ($P_{interaction}$ = 0.03), with a positive association among the heavier drinkers ($OR_{lowest\ vs.\ highest\ quartile}$ = 2.64; 95% CI, 1.32–5.27) in contrast to no association among lighter drinkers ($OR_{lowest\ vs.\ highest\ quartile}$ = 0.80; 95% CI, 0.32–2.00). We further stratified our analysis by the 75th percentile of alcohol intake in controls (26.7 g/day) and found similar associations for vitamin D with both liver cancer and chronic liver disease mortality in participants who drank 11.3 to <26.7 g/day and $\geq$26.7 g/day (Supplementary Table S2).

Discussion

In our cohort, men with lower 25(OH)D concentrations had a higher risk of incident liver cancer and mortality from chronic liver disease, although the association with chronic liver disease mortality was somewhat attenuated after multivariable adjustment. The associations persisted among participants without diabetes at baseline, those who were HBV(−) or HCV(−), and

Table 1. Selected baseline characteristics across 25(OH) vitamin D season-specific quartiles in controls, ATBC Study [presented as medians (IQRs) or percentages]

	Q1 ($n = 106$)	Q2 ($n = 108$)	Q3 ($n = 105$)	Q4 ($n = 108$)	*P* value[a]
% Blood collected in winter (November–April)	62.3	62.0	62.9	62.0	1.0
Serum 25(OH)D, nmol/L					
Winter	16.7 (12.9–18.9)	26.8 (23.8–28.6)	36.6 (34.2–39.9)	55.5 (49.9–64.9)	<0.0001
Summer	25.5 (19.6–27.3)	40.2 (37.5–43.0)	52.3 (49.6–56.9)	71.8 (66.4–81.3)	<0.0001
Age, years	57 (53–60)	58 (55–61)	56 (52–59)	58 (56–64)	0.03
BMI, kg/m^2	26.3 (23.6–29.4)	25.6 (24.1–29.5)	25.9 (24.1–28.3)	25.9 (24.4–28.0)	0.94
% Elementary school education or less	77.4	86.1	77.1	69.4	0.07
% Currently married	82.1	75.0	81.0	83.3	0.57
% History of diabetes	3.8	6.5	2.9	2.8	0.52
Years smoking	37 (31–41)	36 (30–40)	35 (30–40)	34 (30–40)	0.12
No. of cigarettes/day	20 (15–25)	20 (15–25)	20 (12–24)	20 (15–25)	0.32
% Urban residence	54.7	56.5	65.7	66.7	0.03
% Leisure physical activity ≥1/week	41.5	48.6	59.1	54.6	0.02
Daily dietary intake					
Energy intake, kcal/day	2,816 (2,350–3,144)	2,694 (2,219–3,182)	2,710 (2,250–3,131)	2,613 (2,240–3,191)	0.76
Calcium, mg/day	1,412 (1,096–1,848)	1,375 (1,144–1,650)	1,340 (1,065–1,634)	1,290 (947–1,637)	0.12
Vitamin D, μg/day	3.7 (2.6–5.2)	4.7 (3.4–6.6)	4.9 (3.2–7.1)	6.2 (4.0–8.0)	<0.0001
Alcohol, g/day	9.9 (1.7–26.0)	11.0 (2.0–30.1)	10.7 (3.0–27.4)	12.7 (4.4–27.2)	0.90
Coffee, g/day	550 (440–880)	550 (340–750)	550 (330–770)	600 (440–770)	0.94
Supplement use					
% Calcium (yes)	2.8	8.3	6.7	19.4	0.0001
% Vitamin D (yes)	0.9	4.6	4.8	16.7	<0.0001
% HBsAg[b]	0.0	0.0	1.2	1.2	0.48
% Anti-HBc[b]	7.6	2.3	10.3	6.1	0.17
% Anti-HCV[b]	0.0	1.2	0.0	0.0	0.48

[a]Wilcoxon tests for continuous variables; Chi-square tests, or Fisher exact test where appropriate, for categorical variables.
[b]Among 347 controls who had information on HBsAG, anti-HBc, and anti-HCV.

among cancer cases occurring more than 15 years after blood collection.

Our results are consistent with a genetic Mendelian randomization study that observed an inverse association between SNPs associated with higher vitamin D concentrations and liver cancer (17) and the results of two previous cohorts that investigated associations between 25(OH)D concentrations and incident liver cancer, one of which also examined mortality from chronic liver disease. The first of these cohort studies, set in the Nutritional Intervention Trials (NIT) of Linxian, China, included 226 incident cases of liver cancer and 282 deaths from chronic liver disease over 22 years of follow-up (5). This study was marked by very low vitamin D concentrations in study participants, regardless of case–control status, with a median

Table 2. Selected baseline characteristics of cases and controls, ATBC Study [presented as medians (IQRs) or percentages]

	Incident liver cancer			Mortality from chronic liver disease		
	Cases ($n = 202$)	Controls ($n = 202$)	P-value[a]	Cases ($n = 225$)	Controls ($n = 225$)	*P* value[a]
Age, years	58 (55–61)	57 (54–61)	Matched	55 (52–57)	55 (51–57)	Matched
% Blood collected in winter (November-April)	67.8	63.9	0.40	59.6	60.9	0.77
BMI, kg/m^2	27.8 (24.5–30.0)	26.1 (24.1–28.9)	0.009	26.5 (23.9–29.3)	25.8 (23.9–29.0)	0.33
% Elementary school education or less	73.3	77.7	0.30	72.0	77.3	0.19
% Currently married	82.2	85.2	0.42	69.8	76.0	0.14
% History of diabetes	11.4	4.5	0.01	4.0	3.6	0.80
Years smoking	38 (34–43)	36 (30–41)	0.03	35 (30–40)	35 (30–39)	0.88
# cigarettes/day	20 (15–25)	20 (13–20)	0.03	23 (15–25)	20 (15–25)	0.02
% Urban residence	60.9	56.4	0.36	76.0	64.9	0.01
% Leisure physical activity ≥1/week	47.5	54.2	0.18	48.0	50.9	0.92
Daily dietary intake						
Energy intake, kcal/day	2451 (2071–3068)	2683 (2223–3111)	0.04	2546 (2070–3100)	2795 (2262–3200)	0.02
Calcium, mg/day	1221 (883–1606)	1308 (1081–1649)	0.06	1192 (853–1602)	1385 (1081–1693)	0.001
Vitamin D, μg/day	4.7 (3.4–6.5)	4.7 (3.3–6.8)	0.75	4.8 (3.3–7.0)	4.6 (3.2–7.0)	0.86
Alcohol, g/day	13.9 (4.2–29.4)	9.9 (3.4–23.6)	0.001	30.5 (15.3–54.0)	12.8 (2.6–31.1)	<0.0001
Coffee, g/day	450 (300–660)	550 (406–880)	0.06	330 (150–550)	550 (350–770)	<0.0001
Supplement use						
% Calcium (yes)	14.4	9.9	0.17	17.3	8.9	0.008
% Vitamin D (yes)	9.4	7.9	0.60	6.2	5.8	0.84
Serum biomarkers						
25(OH)D, nmol/L	30.4 (21.0–42.6)	35.2 (23.1–50.0)	0.04	31.4 (22.0–45.4)	37.4 (26.4–52.6)	0.002
% HBsAg(+)[b]	1.2	0.6	1.00	0.5	0.5	1.00
% Anti-HBc(+)[b]	15.0	7.7	0.04	6.3	5.8	0.84
% Anti-HCV(+)[b]	4.8	0.6	0.04	2.4	0.0	0.06

[a]Wilcoxon tests for continuous variables; Chi-square tests, or Fisher's exact test where appropriate, for categorical variables.
[b]Among 167 men diagnosed with liver cancer, 208 men who died from chronic liver disease, and 347 controls who had information on HBsAG, anti-HBc, and anti-HCV.

Table 3. Association of serum 25(OH)D with incident liver cancer and chronic liver disease mortality (presented as ORs and 95% CIs from unconditional logistic regression models)

	Incident liver cancer					Chronic liver disease mortality				
Clinical cutpoints of serum 25(OH)D (nmol/L)										
Overall	**<25**	**25–<37.5**	**37.5–<50**	**≥50**	**P_{trend}**	**<25**	**25–<37.5**	**37.5–<50**	**≥50**	**P_{trend}**
No. of cases / controls	71/106	62/117	31/85	38/119		79/106	61/117	36/85	49/119	
Age and date-adjusted	1.98 (1.22–3.20)	1.67 (1.03–2.71)	1.12 (0.64–1.98)	1.00 (ref)	0.003	1.93 (1.23–3.03)	1.28 (0.81–2.02)	1.03 (0.64–2.04)	1.00 (ref)	0.006
Multivariable-adjusted[a]	1.91 (1.16–3.15)	1.57 (0.95–2.59)	1.24 (0.69–2.20)	1.00 (ref)	0.008	1.67 (1.02–2.75)	1.24 (0.75–2.06)	1.07 (0.61–1.88)	1.00 (ref)	0.05
Summer months										
No. of cases / controls	14/19	22/32	9/41	20/69		21/19	23/32	17/41	30/69	
Multivariable-adjusted[a]	2.51 (1.01–6.22)	1.93 (0.87–4.29)	0.80 (0.31–2.04)	1.00 (ref)	0.03	3.04 (1.24–7.45)	1.75 (0.77–3.94)	1.17 (0.51–2.67)	1.00 (ref)	0.02
Winter months										
No. of cases / controls	57/87	40/85	22/44	18/50		58/87	38/85	19/44	19/50	
Multivariable-adjusted[a]	1.55 (0.80–3.03)	1.24 (0.62–2.49)	1.36 (0.62–2.97)	1.00 (ref)	0.23	1.27 (0.63–2.55)	0.97 (0.48–1.97)	0.99 (0.43–2.25)	1.00 (ref)	0.53
Season-specific quartiles of serum 25(OH)D										
Overall	**Q1**	**Q2**	**Q3**	**Q4**	**P_{trend}**	**Q1**	**Q2**	**Q3**	**Q4**	**P_{trend}**
No. of cases / controls	72/106	56/108	41/105	33/108		72/106	63/108	52/105	38/108	
Age and date-adjusted	2.07 (1.26–3.41)	1.76 (1.05–2.94)	1.23 (0.72–2.10)	1.00 (ref)	0.001	2.01 (1.24–3.24)	1.60 (0.98–2.60)	1.42 (0.86–2.34)	1.00 (ref)	0.004
Multivariable-adjusted[a]	1.87 (1.12–3.12)	1.52 (0.90–2.57)	1.12 (0.64–1.96)	1.00 (ref)	0.007	1.69 (1.00–2.88)	1.27 (0.75–2.17)	1.25 (0.73–2.16)	1.00 (ref)	0.06
Summer months										
No. of cases / controls	25/40	20/41	13/39	7/41		35/40	21/41	20/39	15/41	
Multivariable-adjusted[a]	2.86 (1.06–7.77)	2.35 (0.86–6.46)	1.66 (0.57–4.83)	1.00 (ref)	0.03	2.65 (1.12–6.28)	1.17 (0.47–2.91)	1.45 (0.58–3.62)	1.00 (ref)	0.04
Winter months										
No. of cases / controls	47/66	36/67	28/66	26/67		37/66	42/67	32/66	23/67	
Multivariable-adjusted[a]	1.56 (0.84–2.90)	1.21 (0.64–2.31)	1.00 (0.51–1.94)	1.00 (ref)	0.12	1.30 (0.64–2.64)	1.37 (0.69–2.72)	1.19 (0.59–2.39)	1.00 (ref)	0.43
Season-standardized quartiles of serum 25(OH)D (among those whose levels were assayed in January 2008 or February 2013)										
Overall	**Q1**	**Q2**	**Q3**	**Q4**	**P_{trend}**	**Q1**	**Q2**	**Q3**	**Q4**	**P_{trend}**
No. of cases / controls	66/105	61/103	39/102	34/104		71/105	58/103	45/102	40/104	
Age and date-adjusted	1.89 (1.14–3.12)	1.90 (1.14–3.15)	1.22 (0.71–2.10)	1.00 (ref)	0.004	1.76 (1.09–2.84)	1.41(0.86–2.30)	1.09 (0.65–1.81)	1.00 (ref)	0.01
Multivariable-adjusted[a]	1.72 (1.02–2.88)	1.73 (1.03–2.93)	1.15 (0.65–2.01)	1.00 (ref)	0.02	1.40 (0.82–2.38)	1.19 (0.70–2.05)	1.05 (0.60–1.83)	1.00 (ref)	0.18
Summer months										
No. of cases / controls	18/28	23/44	12/47	11/36		30/28	21/44	20/47	15/36	
Multivariable-adjusted[a]	1.90 (0.73–4.93)	1.56 (0.63–3.85)	0.88 (0.33–3.37)	1.00 (ref)	0.09	2.60 (1.03–6.58)	1.11 (0.43–2.84)	1.27 (0.51–3.18)	1.00 (ref)	0.06
Winter months										
No. of cases / controls	48/77	38/59	27/55	23/68		41/77	37/59	25/55	25/68	
Multivariable-adjusted[a]	1.61 (0.86–3.01)	1.80 (0.93–3.47)	1.25 (0.62–2.52)	1.00 (ref)	0.10	1.10 (0.56–2.17)	1.42 (0.71–2.85)	1.03 (0.50–2.12)	1.00 (ref)	0.64

[a]Adjusted for age, date of blood draw, BMI, history of diabetes, number of years smoked, and daily intake of alcohol and coffee.

Table 4. Association of serum 25(OH)D, using season-specific quartiles, with incident liver cancer and chronic liver disease mortality among selected subgroups (presented as ORs and 95% CIs from unconditional logistic regression models)

	Incident liver cancer					Chronic liver disease mortality				
Overall	**Q1**	**Q2**	**Q3**	**Q4**	P_{trend}	**Q1**	**Q2**	**Q3**	**Q4**	P_{trend}
# of cases / controls	72/106	56/108	41/105	33/108		72/106	63/108	52/105	38/108	
Multivariable-adjusted[a]	1.87 (1.12–3.12)	1.52 (0.90–2.57)	1.12 (0.64–1.96)	1.00 (ref)	0.007	1.69 (1.00–2.88)	1.27 (0.75–2.17)	1.25 (0.73–2.16)	1.00 (ref)	0.06
Without diabetes										
# of cases / controls	63/102	49/101	36/102	31/105		69/102	60/101	51/102	36/105	
Multivariable-adjusted[a]	1.81 (1.07–3.07)	1.55 (0.90–2.66)	1.09 (0.62–1.93)	1.00 (ref)	0.01	1.71 (1.00–2.95)	1.30 (0.75–2.25)	1.26 (0.72–2.21)	1.00 (ref)	0.06
Alcohol, <11.3 g/day[b,c]			$P_{interaction} = 0.41$					$P_{interaction} = 0.03$		
# of cases / controls	23/53	25/51	20/53	12/47		13/53	10/51	5/53	14/47	
Multivariable-adjusted[a]	1.49 (0.65–3.41)	1.93 (0.85–4.39)	1.40 (0.60–3.28)	1.00 (ref)	0.29	0.80 (0.32–2.00)	0.61 (0.23–1.64)	0.31 (0.10–0.98)	1.00	0.86
Alcohol, ≥11.3 g/day[b,c]										
# of cases / controls	42/48	29/49	18/48	19/58		51/48	48/49	40/48	21/58	
Multivariable-adjusted[a]	2.13 (1.06–4.28)	1.44 (0.69–3.00)	0.87 (0.39–1.93)	1.00 (ref)	0.01	2.64 (1.32–5.27)	2.07 (1.04–4.11)	2.16 (1.07–4.35)	1.00 (ref)	0.01
Among the subset with information on hepatitis B and C[d]										
# of cases / controls	63/92	47/86	34/87	23/82		65/92	60/86	50/87	33/82	
Multivariable-adjusted[a]	2.06 (1.14–3.73)	1.74 (0.94–3.22)	1.15 (0.60–2.18)	1.00 (ref)	0.005	1.60 (0.89–2.87)	1.38 (0.77–2.47)	1.32 (0.73–2.38)	1.00 (ref)	0.13
HBV- and HCV-[e]										
# of cases / controls	55/85	41/83	27/77	18/77		62/85	52/83	48/77	31/77	
Multivariable-adjusted[a]	2.33 (1.23–4.41)	1.81 (0.93–3.51)	1.25 (0.62–2.54)	1.00 (ref)	0.003	1.75 (0.96–3.20)	1.28 (0.69–2.35)	1.48 (0.80–2.75)	1.00 (ref)	0.12
Lag analyses, ≥5 years										
# of cases / controls	64/104	48/105	36/104	30/104		50/104	50/105	42/104	31/104	
Multivariable-adjusted[a]	1.88 (1.10–3.21)	1.40 (0.81–2.44)	1.06 (0.59–1.89)	1.00 (ref)	0.009	1.53 (0.86–2.73)	1.31 (0.74–2.33)	1.29 (0.72–2.31)	1.00 (ref)	0.17
Lag analyses, ≥10 years										
# of cases / controls	39/90	35/96	29/92	25/100		31/90	34/96	25/92	21/100	
Multivariable-adjusted[a]	1.60 (0.87–2.93)	1.32 (0.72–2.43)	1.22 (0.64–2.30)	1.00 (ref)	0.13	1.58 (0.80–3.12)	1.36 (0.70–2.64)	1.29 (0.64–2.59)	1.00 (ref)	0.20
Lag analyses, ≥15 years										
# of cases / controls	21/70	26/83	19/84	19/94		13/70	22/83	14/84	12/94	
Multivariable-adjusted[a]	1.55 (0.75–3.19)	1.50 (0.76–2.99)	1.17 (0.57–2.44)	1.00 (ref)	0.17	1.44 (0.58–3.57)	1.80 (0.79–4.11)	1.32 (0.55–3.19)	1.00 (ref)	0.29

[a]Adjusted for age, date of blood draw, BMI, history of diabetes, number of years smoked, and daily intake of alcohol and coffee.
[b]Median alcohol intake in controls was 11.3 g/day.
[c]Alcohol information was not available in 14 liver cancer cases, 23 chronic liver disease deaths, and 20 controls.
[d]Hepatitis B and C information was available for 167 liver cancer cases, 208 chronic liver disease deaths, and 347 controls.
[e]147 men diagnosed with incident liver cancer, 193 men who died from chronic liver disease, and 322 controls were negative for both HBV and HCV.

level in the controls of 20.1 nmol/L. Although these concentrations are considered to be clinically deficient according to the US Institute of Medicine and the Endocrine Society, typical concentrations of vitamin D in Asian populations are less clear. Nevertheless, even in a context of very low vitamin D concentrations, this study observed evidence for an inverse association between baseline vitamin D concentrations and occurrence of both endpoints during follow-up (quartile 4 vs. quartile 1, liver cancer incidence: 0.74; 95% CI, 0.47–1.18; $P_{trend} = 0.21$; chronic liver disease mortality: 0.34; 95% CI, 0.21–0.55; $P_{trend} < 0.001$), although we note that the association with incident liver cancer was not statistically significant. In contrast to our case–control study nested within the ATBC cohort, there was very little alcohol intake in this study population. The second cohort study was set in a population with far higher vitamin D concentrations, that of the large multicountry European Prospective Investigation into Cancer and Nutrition (EPIC; ref. 6), in which controls had a median measured vitamin D level of 49.9 nmol/L. This later study included 138 incident cases of hepatocellular carcinoma that occurred over a mean 6 years of follow-up and again observed evidence for an inverse association between baseline vitamin D concentrations and subsequent incidence of liver cancer (tertile 3 vs. tertile 1: 0.51; 95% CI, 0.26–0.99; $P_{trend} = 0.036$).

The relative consistency of our present findings and those of two previous studies, set in populations that differ notably in numerous characteristics such as race and dietary/lifestyle factors and HBV and HCV prevalence, supports the plausibility of an association, as do data from *in vitro* and animal studies. As described previously, vitamin D is a hormone that regulates a wide range of signaling pathways and has been shown to have antiproliferative, antiinflammatory, and antiangiogenic properties in cell lines, including those originally generated from the liver (18–25). Furthermore, vitamin D has been shown to be protective against liver fibrosis and liver cancer in rodent studies (4, 20, 21, 26, 27).

Although these associations are consistent and biologically plausible, noncausal explanations remain possible. One concern is reverse causality. 25(OH)D is formed in the liver and many, although not all, previous studies have observed lower 25(OH)D concentrations among patients with fibrosis and cirrhosis (28). Thus, observed associations with 25(OH)D in our study could reflect undiagnosed liver disease. We tried to address this possibility in several ways. For example, our study excluded patients with a known diagnosis of cirrhosis or alcohol dependence at baseline. Also, our follow-up was long and associations were of a similar direction among cases that occurred more than 15 years after sample collection, although attenuated with wider CIs. Similar patterns were observed in the previously mentioned EPIC study where similar estimates were observed after cases diagnosed in the first 2, 4, or 6 years subsequent to baseline were excluded (6).

It is also possible that low vitamin D concentrations are both a marker of underlying liver disease and an etiologic contributor to liver disease progression and carcinogenesis. For chronic liver disease mortality, associations were particularly observed among participants who were heavier drinkers of alcohol suggesting that vitamin D concentrations could possibly potentiate the effects of alcohol on liver disease. However, to resolve this and other questions, future longitudinal studies of liver disease progression that include serial measurements of vitamin D status are needed.

Additionally, as in all observational studies, confounding by another factor is possible. In our study, associations for liver cancer persisted after adjustment and stratification for the major liver cancer risk factors, that is HBV, HCV, alcohol, diabetes, obesity, and tobacco smoking. Another potential confounder was sun exposure, which triggers vitamin D production in the skin (29) and varies widely by season. We took several different approaches to account for season in our analysis, including matching by date of blood draw (within 30 days) when we selected controls for each case. In addition to using clinically defined cut-points for vitamin D, we examined analyses that were stratified by season, and also used season-specific and season-standardized quartiles. We observed generally similar results across each of these approaches, indicating that season of blood draw, a proxy for sun exposure, was not a confounder.

Our study has several strengths including a prospective design and nearly 25 years of follow-up, allowing assessment of vitamin D status well before cancer diagnosis. We had information on and adjusted for major risk factors for liver cancer in this population, including alcohol, obesity, diabetes, and tobacco smoking as well as HBV and HCV for the majority of the cases and controls. However, our study also had several limitations. We were only able to measure 25(OH)D concentrations in participants at a single point during the lifetime and, as discussed above, lacked information about undiagnosed liver fibrosis or cirrhosis at baseline or during follow-up. Our study also included vitamin D concentrations which were measured on baseline blood samples but at different times. Multiple measurements of vitamin D over the lifetime and repeated assessments of underlying liver disease would allow better classification of vitamin D status and evaluation of reverse causality. Ideally, these types of data will be available in future studies. Set in a population of European male smokers, it is unclear whether our findings should be extrapolated to other populations, such as women, nonsmokers, or those with high HBV or HCV burden, although similar findings were observed in two other cohorts that included very different populations.

In conclusion, men with lower serum 25(OH)D concentrations had higher risk of incident liver cancer and, to a lesser degree, chronic liver disease mortality over a period of nearly 25 years of follow-up in the ATBC cohort. Future studies are needed to replicate these findings, in particular among studies with serial sample collection and comprehensive, longitudinal assessment of underlying liver disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: G.Y. Lai, S.J. Weinstein, D. Albanes, N.D. Freedman
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G.Y. Lai, S.J. Weinstein, R.L. Horst, S. Männistö, D. Albanes, N.D. Freedman
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G.Y. Lai, S.J. Weinstein, N.D. Freedman
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Disclaimer

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Association of 25-Hydroxyvitamin D with Liver Cancer Incidence and Chronic Liver Disease Mortality in Finnish Male Smokers of the ATBC Study

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