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Original Contribution

Circulating 25-Hydroxyvitamin D_3 in Relation to Renal Cell Carcinoma Incidence and Survival in the EPIC Cohort

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Normal renal function is essential for vitamin D metabolism, but it is unclear whether circulating vitamin D is associated with risk of renal cell carcinoma (RCC). We assessed whether 25-hydroxyvitamin D_3 ($25(OH)D_3$) was associated with risk of RCC and death after RCC diagnosis in the European Prospective Investigation into Cancer and Nutrition (EPIC). EPIC recruited 385,747 participants with blood samples between 1992 and 2000. The current study included 560 RCC cases, 557 individually matched controls, and 553 additional controls. Circulating $25(OH)D_3$ was assessed by mass spectrometry. Conditional and unconditional logistic regression models were used to calculate odds ratios and 95% confidence intervals. Death after RCC diagnosis was assessed using Cox proportional hazards models and flexible parametric survival models. A doubling of $25(OH)D_3$ was associated with 28% lower odds of RCC after adjustment for season of and age at blood collection, sex, and country of recruitment (odds ratio = 0.72, 95% confidence interval: 0.60, 0.86; $P = 0.0004$). This estimate was attenuated somewhat after additional adjustment for smoking status at baseline, circulating cotinine, body mass index (weight (kg)/height $(m)^2$), and alcohol intake (odds ratio = 0.82, 95% confidence interval: 0.68, 0.99; $P = 0.038$). There was also some indication that both low and high $25(OH)D_3$ levels were associated with higher risk of death from any cause among RCC cases.

nested case-control study; prospective study; renal cell carcinoma; vitamin D

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; $25(OH)D_2$, 25-hydroxyvitamin D_2; $25(OH)D_3$, 25-hydroxyvitamin D_3; BMI, body mass index; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; OR, odds ratio; RCC, renal cell carcinoma; UVB, ultraviolet B.

Vitamin D is essential for the efficient absorption of dietary calcium. Beyond its role in bone health, vitamin D has been implicated in the etiology of several cancers, most notably colorectal cancer (1, 2). Vitamin D is produced in the skin after exposure to ultraviolet B (UVB) radiation from sunlight, or it is ingested in the diet or through dietary supplements (3). After ingestion or endogenous synthesis, vitamin D is hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D), the major circulating metabolite of vitamin D, which is subsequently converted into its active form

(1,25-dihydroxyvitamin D), primarily in the kidneys. Despite the critical role of the kidneys in vitamin D metabolism, it remains unclear whether vitamin D is relevant to the etiology of kidney cancer.

In 2008, the age-standardized incidence rate of kidney cancer was 3.9 cases per 100,000 people worldwide, but there is notable unexplained variation in incidence from country to country (4). A link between vitamin D and the most prevalent form of kidney cancer, renal cell carcinoma (RCC), was initially suggested on the basis of ecological evidence. For instance, ecological studies have suggested that RCC incidence is inversely associated with exposure to UVB radiation (5, 6). Similarly, vitamin D deficiency is highly prevalent among blacks (7), and rates of RCC are higher among blacks than whites in the United States (8).

To date, 2 prospective studies have assessed prediagnostic circulating 25(OH)D and the risk of RCC, with conflicting results (9, 10). Our aim was to investigate whether circulating 25(OH)D is related to the incidence of RCC and post-RCC survival using a prospective nested case-control sample from a large European cohort.

METHODS

Study cohort

The recruitment and baseline assessment of the European Prospective Investigation into Cancer and Nutrition (EPIC) are described in detail elsewhere (11). Between 1992 and 2000, 521,330 individuals from 10 countries were recruited, 385,747 of whom donated blood samples. Blood fractions were aliquoted into 0.5-mL straws, which were heat sealed and stored in liquid nitrogen tanks at −196° Celsius, except in Umeå, Sweden, where samples were stored in 1.8-mL plastic tubes in freezers at −80° Celsius and in Denmark, where samples were stored in 1-mL plastic tubes in liquid nitrogen vapor at −150° Celsius. Participants completed self-administered questionnaires on lifestyle factors, medical history, and diet, and their heights and weights were measured using standard protocols. All participants gave written informed consent. The study was approved by the ethics committee at the International Agency for Research on Cancer (Lyon, France) and the local ethics committees of the study centers.

Case ascertainment and follow-up

Incident cancer cases were identified via linkage to population-based cancer registries (in Denmark, Italy (except Naples), the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or by active follow-up (in France, Germany, Greece, and Naples, Italy), which involved a combination of methods, including review of health insurance records and cancer and pathology registries, as well as direct contact with participants and their next-of-kin.

Mortality data were obtained from death registries at the regional or national level. Participants were followed up from study entry until cancer diagnosis (except nonmelanoma skin cancer), death, emigration, or the end of follow-up. The end of follow-up was defined as the latest date of complete follow-up for both cancer incidence and vital status and varied by study center from December 2004 to June 2010. Vital status at follow-up is more than 98% complete.

Selection of cases and controls

We identified 905 participants who were diagnosed with RCC (with *International Classification of Diseases for Oncology, Second Edition*, code C64.9). We excluded prevalent cases and cases with a history of another cancer (excluding nonmelanoma skin cancer, $n = 85$); cases who did not donate a blood sample ($n = 153$); cases who had no questionnaire information available ($n = 6$); cases whose cancers were not histologically confirmed ($n = 27$); and cases from the Malmö center in Sweden, which did not participate in this study ($n = 64$), leaving 570 eligible RCC cases. For each case, 1 control was chosen randomly from risk sets consisting of all cohort members who were alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Matching criteria were country, sex, date of blood collection (±1 month, which was relaxed to ±5 months for 27% of sets without available controls), and date of birth (±1 year, which was relaxed to ±5 years for 1% of sets without available controls). Additionally, we included 553 controls (henceforth referred to as "unmatched controls") that were individually matched to cases from another cancer site in a parallel ongoing study using identical matching criteria. These unmatched controls were included to increase the precision of the estimates.

Biochemical analyses

Plasma samples were sent on dry ice to the Bevital AS laboratory (Bergen, Norway). Liquid chromatography coupled with tandem mass spectrometry was used to separately analyze 25-hydroxyvitamin D_2 ($25(OH)D_2$) and 25-hydroxyvitamin D_3 ($25(OH)D_3$) (12). The limit of detection was 3.3 nmol/L, and within-day and between-day coefficients of variation were 4.4%–8.2%. We found that $25(OH)D_2$ was undetectable in the majority of samples, so our analyses focus on $25(OH)D_3$. Sensitivity analyses were conducted using the sum of $25(OH)D_2$ and $25(OH)D_3$ (setting undetectable levels of $25(OH)D_2$ to 0), which yielded essentially identical results. Creatinine and cotinine were also assessed with liquid chromatography coupled with tandem mass spectrometry. For cotinine, the limit of detection was 1 nmol/L, and the within-day and between-day coefficients of variation were 2%–6%; for creatinine, the limit of detection was 0.25 μmol/L, and the within-day and between-day coefficients of variation were 2%–6%. The laboratory is Vitamin D External Quality Assessment Scheme–certified (DEQAS, London, United Kingdom).

Statistical analysis

We used conditional logistic regression to calculate odds ratios and 95% confidence intervals for $25(OH)D_3$ as a continuous variable, conditioning on matched case set. Concentrations were logarithmically transformed (base-2) prior to modeling, so odds ratios correspond to the expected change in odds for a doubling in concentration. We also used unconditional

logistic regression (adjusted for age at blood collection (in years), sex, and country of recruitment) to compare cases with matched controls and to the pooled group of the matched and unmatched controls. To establish whether known risk factors for RCC could explain any association, we fitted models adjusted for tobacco smoking (status at baseline of never, former, or current smoker; and quartiles of circulating cotinine determined by the distribution among current smokers), alcohol intake at recruitment (in g/day), and body mass index (BMI) (weight (kg)/height $(m)^2$). As a sensitivity analysis, we fitted models that were additionally adjusted for systolic blood pressure (in mm Hg), circulating creatinine (a marker of renal function, in μmol/L,), and self-reported prevalent diabetes, all of which may be on the causal pathway. We investigated potential effect modification by fitting interactions with $\log_2$ 25(OH)D_3. Hazard ratios for all-cause mortality after RCC diagnosis were calculated using a Cox proportional hazards model with time since diagnosis as the time scale. We modeled 25(OH)D_3 using restricted cubic splines with knots at its 10th, 33rd, 67th, and 90th percentiles. We included the same covariates as those in the unconditional logistic models, with the addition of age at diagnosis (in years). Visual inspection of smoothed, scaled Schoenfeld residuals revealed no notable departure from proportional hazards. To estimate the survival function at given concentrations of 25(OH)D_3, we fitted a flexible parametric survival model (13), modeling the baseline cumulative hazard with restricted cubic splines (knots at the 0th, 33rd, 67th, and 100th percentiles of the distribution of failure times).

Because 25(OH)D_3 is strongly affected by exposure to UVB radiation, all unconditional logistic models and survival models were explicitly adjusted for day of blood collection. We modeled seasonality by including 2 pairs of sine and cosine functions of day of blood collection in the models. The use of trigonometric functions adjusts for periodic variation in 25(OH)D_3 and produces smooth predictions with no artificial discontinuities from season to season or year to year. We included 2 pairs of sine and cosine functions in the models, because the inclusion of additional terms did not improve model fit, nor did it substantially affect parameter estimates for 25(OH)D_3.

We present 95% confidence intervals to depict the statistical uncertainty in the estimates from the risk and survival models. We also present simulation-based estimates of statistical uncertainty, which we derived by sampling from the asymptotic distribution of the regression coefficients (the multivariate normal distribution with location and scale given by the maximum likelihood estimates and their variance-covariance matrix, respectively). We drew 1,000 samples for each model and used them to generate plausible predicted odds ratios and hazard ratios. We plotted predictions that fell within the 95% confidence interval to provide a visual impression of the 95% highest posterior density for the estimates under uniform prior distributions. *P* values were calculated using the likelihood-ratio test. The data were nearly complete for all covariates, so, where necessary, we excluded the few records with missing data. All statistical analyses were conducted using R, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) (14). Conditional logistic regression models were fitted using the Epi package, version 1.1.49, in R (15).

RESULTS

Baseline and demographic characteristics

Of the 570 matched cases and controls, 10 cases and 13 controls were missing data on 25(OH)D_3 and were excluded from the analyses. Demographic and baseline characteristics for the remaining 560 cases, 557 matched controls, and 553 unmatched controls are presented in Table 1. The median age at diagnosis for cases was 64 years (5th and 95th percentiles, 49 and 75 years), and the median time from blood collection to diagnosis was 6.7 years (5th and 95th percentiles, 0.7 and 11.9). The distributions of established risk factors for RCC showed the expected differences between cases and controls. A higher proportion of cases than controls were current smokers at baseline, and a higher proportion of cases had BMI values of 30 or higher. The unmatched controls had similar characteristics to the matched controls, albeit with a higher proportion of men and a lower proportion of participants from Sweden, Denmark, and Norway. The distribution of 25(OH)D_3 did not vary greatly by country of recruitment (Appendix Table 1).

Seasonal variation in plasma 25(OH)D_3 concentration

There was substantial variation in plasma concentrations of 25(OH)D_3 by date of blood collection. Figure 1 shows the observed concentrations, along with the predicted geometric mean from a linear regression model of log concentration on 2 pairs of sine and cosine functions of the day of blood collection. On average, concentrations were highest among those who had their blood drawn during or near the month of August and lowest among those who had their blood drawn during or near the months of February and March. Despite this seasonal variation, there remained substantial variability in concentration on any given day of blood collection.

Plasma 25(OH)D_3 concentration and risk of RCC

Estimated odds ratios and 95% confidence intervals for a doubling of 25(OH)D_3 are presented in Table 2. Minimally adjusted models suggested an inverse association. Estimates from the conditional logistic model of the matched case sets were similar to those from the unconditional model (conditional odds ratio (OR) = 0.75, 95% confidence interval (CI): 0.61, 0.91, $P = 0.0043$; unconditional OR = 0.73, 95% CI: 0.59, 0.89, $P = 0.002$), and the inclusion of extra unmatched controls did not substantially affect the estimated association (OR = 0.72, 95% CI: 0.60, 0.86, $P = 0.0004$). Further adjustment for smoking status at baseline, circulating cotinine, alcohol intake at recruitment, and BMI attenuated the estimates somewhat, with odds ratios of 0.82 (95% CI: 0.68, 0.99, $P = 0.038$) from the model including all controls and 0.81 (95% CI: 0.65, 1.00, $P = 0.051$) from the model including only matched controls. Among participants whose blood pressure was assessed at baseline (458 cases and 881 controls), further adjustment by continuous systolic blood pressure did not affect the estimates for 25(OH)D_3 (OR = 0.81, 95% CI: 0.65, 0.99). Similarly, additional adjustment for prevalent diabetes did not affect the results (OR = 0.81,

Table 1. Baseline and Demographic Characteristics of EPIC Participants, Recruited 1992–2000

Characteristic	Cases ($n = 560$)				Matched Controls ($n = 557$)				Unmatched Controls ($n = 553$)			
	No.	%	Median	Percentiles (5th, 95th)	No.	%	Median	Percentiles (5th, 95th)	No.	%	Median	Percentiles (5th, 95th)
Sex												
Male	311	56			309	55			374	68		
Female	249	44			248	45			179	32		
Age at recruitment, years												
<55	229	41			224	40			246	44		
55–64.9	285	51			288	52			231	42		
≥65	46	8			45	8			76	14		
Country												
Denmark	114	20			114	20			0	0		
France	13	2			13	2			7	1		
Germany	126	22			124	22			104	19		
Greece	17	3			17	3			22	4		
Italy	88	16			88	16			70	13		
Netherlands	46	8			46	8			77	14		
Norway	4	1			4	1			2	0		
Spain	53	9			52	9			100	18		
Sweden	32	6			32	6			41	7		
United Kingdom	67	12			67	12			130	24		
Smoking status at baseline												
Never	227	41			246	44			230	42		
Former	162	29			179	32			199	36		
Current	166	30			129	23			110	20		
Missing	5	1			3	1			14	3		
Educational attainment												
Primary school or less	233	42			206	37			222	40		
Technical/professional school	124	22			138	25			141	25		
Secondary school	77	14			66	12			70	13		
Higher education	110	20			133	24			99	18		
Missing	16	3			14	3			21	4		
Body mass index[a]												
<25	182	32			223	40			220	40		
25–29.9	248	44			242	43			258	47		
≥30	130	23			92	17			75	14		
Alcohol intake at recruitment, g/day												
<6	271	48			240	43			246	44		
6–17.9	125	22			142	25			148	27		
18–29.9	66	12			75	13			64	12		
≥30	98	18			100	18			95	17		
Age at RCC diagnosis, years			63.8	48.7, 75.1								
Years from blood collection to diagnosis			6.7	0.7, 11.9								
Circulating 25(OH)D_3, nmol/L			43.2	17.6, 79.0			45.8	19.7, 83.2			48.6	19.5, 80.2
Circulating cotinine, nmol/L			3	0, 1,703			2.6	0.0, 1,451.5			3.0	0.0, 1,500.7

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; EPIC, European Prospective Investigation into Cancer and Nutrition; RCC, renal cell carcinoma.

[a] Weight (kg)/height $(m)^2$.

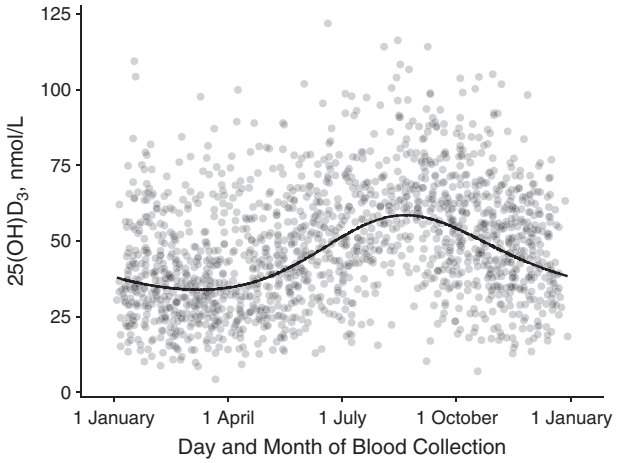

Figure 1. Seasonal variation of 25-hydroxyvitamin D_3 ($25(OH)D_3$) concentrations in plasma. Scattered points show the measured values. The solid line represents the predicted geometric mean concentration given day of blood collection, which was modeled as a linear combination of sine and cosine functions. See the text of the Methods section for further details. Estimates and data are from a renal cell carcinoma case-control sample nested within the European Prospective Investigation into Cancer and Nutrition (EPIC), which recruited participants between 1992 and 2000.

Table 2. Odds Ratios For a Doubling in Concentration of $25(OH)D_3$ and the Risk of Renal Cell Carcinoma Among a Nested Case-Control Sample From the EPIC Cohort, Recruited 1992–2000

Model	No. of Controls	No. of Cases	OR	95% CI	*P* Value[a]
Minimally adjusted[b]					
Conditional	555	555	0.75	0.61, 0.91	0.0043
Unconditional (matched controls)	557	560	0.73	0.59, 0.89	0.002
Unconditional (combined controls)	1,110	560	0.72	0.60, 0.86	0.0004
Fully adjusted[c]					
Conditional	547	547	0.86	0.69, 1.06	0.16
Unconditional (matched controls)	553	555	0.81	0.65, 1.00	0.051
Unconditional (combined controls)	1,092	555	0.82	0.68, 0.99	0.038

Abbreviations: $25(OH)D_3$, 25-hydroxyvitamin D_3; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; OR, odds ratio.

[a] *P* values from likelihood ratio tests of $\log_2 25(OH)D_3$.

[b] Conditional minimally adjusted models were conditioned on matched case set. Unconditional models were adjusted for age at baseline, country, seasonality, and sex.

[c] Fully adjusted models were additionally adjusted for alcohol intake at recruitment (in g/day), body mass index (weight (kg)/height $(m)^2$), cotinine quartiles (based on the distribution among smokers), and smoking status at baseline (never, former, or current smoker).

95% CI: 0.66, 0.98). The estimates were also unaffected by further adjustment for circulating creatinine (OR = 0.81, 95% CI: 0.66, 0.99).

Figure 2 shows the inverse association between concentration and risk by plotting odds ratio estimates from the minimally adjusted (Figure 2A) and adjusted unconditional (Figure 2B) models across the range of observed $25(OH)D_3$ concentrations. Relative to participants with a concentration of 50 nmol/L, participants with less than 25 nmol/L had approximately 20% higher odds of RCC. Correspondingly, participants with concentrations greater than 100 nmol/L had 20% lower odds of RCC relative to those with a concentration of 50 nmol/L, but very few participants had concentrations as high as 100 nmol/L.

To assess potential effect modification, we fitted models that included interaction terms between $\log_2 25(OH)D_3$ and various covariates. Estimates from these models are presented in Figure 3. The association with risk of RCC did not vary substantially by age at baseline, sex, country, level of education, time since blood collection, circulating concentration of creatinine, smoking status, alcohol intake at baseline, or BMI value, though there was a suggestion that the association might be slightly stronger for people with BMI values of 30 or more.

Plasma $25(OH)D_3$ concentration and survival after RCC diagnosis

Of the 560 RCC cases, eight were diagnosed after the end of follow-up for vital status and were thus excluded from the survival analysis. Among the remaining 552 RCC cases, we identified 205 deaths from any cause during a median follow-up of 3.24 years (2,397 person-years were observed in total). We found that $25(OH)D_3$ was nonlinearly associated with risk of death (likelihood ratio test of $25(OH)D_3$ terms $P = 0.01$). Low concentrations of prediagnostic $25(OH)D_3$ were associated with increased hazards of all-cause mortality (Figure 4A). The hazard of death was 1.73 times higher (95% CI: 1.19, 2.51) for participants with concentrations of 25 nmol/L compared to those with concentrations of 50 nmol/L. There was an indication that high concentrations might also be associated with increased hazards of death, but there were very few participants with concentrations greater than 75 nmol/L. Model-based estimates of the survival function evaluated at 25, 50, and 75 nmol/L are presented in Figure 4B. The expected survival probabilities at 5 years after diagnosis were 0.56 (95% CI: 0.49, 0.62) for participants with a concentration of 25 nmol/L, 0.70 for those with a concentration of 50 nmol/L, and 0.66 (95% CI: 0.58, 0.73) for those with a concentration of 75 nmol/L.

The higher hazards of death for low concentrations of $25(OH)D_3$ was apparent regardless of the time between blood collection and diagnosis; the hazard ratios for those with 25 versus 50 nmol/L were 1.75 (95% CI: 1.02, 2.99) for those diagnosed within 5 years of blood collection and 1.67 (95% CI: 1.02, 2.71) for those diagnosed 5 years or more after blood collection. In contrast, the increased hazards of death for

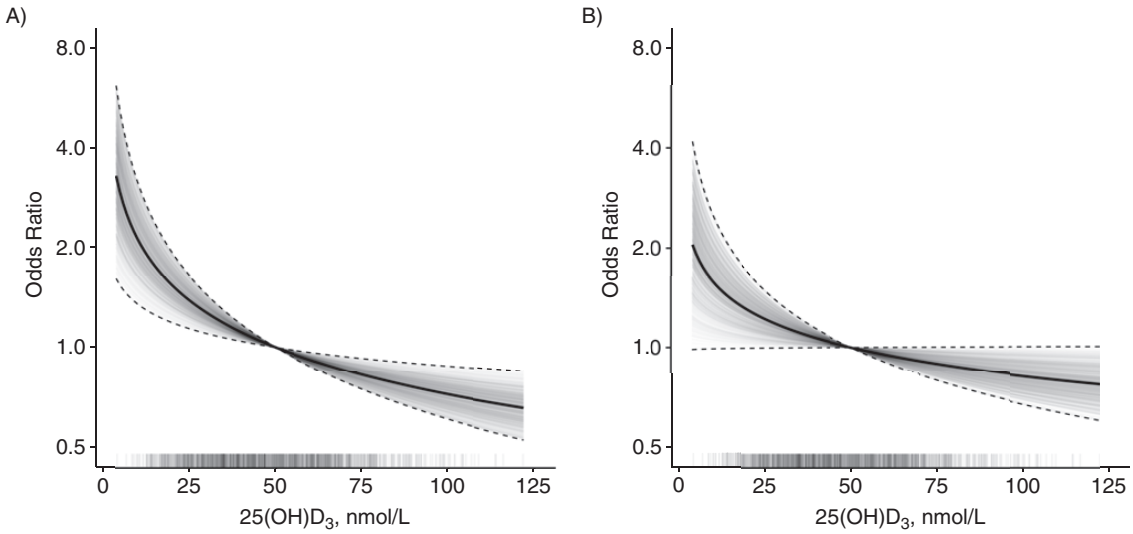

Figure 2. Odds ratios for renal cell carcinoma as a function of circulating concentration of 25-hydroxyvitamin D_3 ($25(OH)D_3$), relative to a concentration of 50 nmol/L. Log-base-2 $25(OH)D_3$ was modeled as a continuous covariate. Solid and dashed lines represent the maximum likelihood estimates and 95% confidence intervals, respectively. The translucent lines are 1,000 draws from the multivariate normal distribution defined by the maximum likelihood estimates and their variance-covariance matrix; they thus give an indication of the posterior density for the odds ratio under a uniform prior on the regression coefficients. The "rug plot" under each panel shows the observed distribution of 25-hydroxyvitamin D_3. Estimates and data are from a nested case-control sample within the European Prospective Investigation into Cancer and Nutrition (EPIC), which recruited participants between 1992 and 2000. A) Estimates adjusted for age at baseline, sex, country, and seasonality (sine and cosine functions of day of blood collection). B) Estimates after additional adjustment for smoking status at baseline (never/former/current smoker), circulating cotinine (quartiles defined among the controls), alcohol intake at recruitment (in g/day), and body mass index (weight (kg)/height $(m)^2$).

higher concentrations was apparent only among those diagnosed within 5 years of blood collection, with hazard ratios for 75 versus 50 nmol/L of 2.22 (95% CI: 1.49, 3.28) for those diagnosed within 5 years of blood collection and 0.62 (95% CI: 0.33, 1.15) for those diagnosed more than 5 years after blood collection (likelihood ratio test of interaction terms $P = 0.001$). Estimated hazard ratios did not vary by sex, age, country, educational level, alcohol intake, smoking status, or circulating creatinine (data not shown).

DISCUSSION

We found suggestive evidence that circulating concentrations of $25(OH)D_3$ are inversely associated with the risk of RCC, such that participants with concentrations of less than 25 nmol/L had approximately 20% greater risk than those with concentrations of 50 nmol/L. We also found that lower concentrations of $25(OH)D_3$ were nonlinearly associated with the risk of all-cause mortality after RCC diagnosis. Among the majority of participants, an inverse association was apparent, whereas higher concentrations of $25(OH)D_3$ also appeared to be associated with higher risk of death.

Previous reports of prospectively measured circulating 25(OH)D and the risk of kidney cancer have produced conflicting results. In accordance with our results, the Copenhagen City Heart Study, a cohort of 9,791 people, including 55 incident kidney cancer cases, reported that a 50% reduction in 25(OH)D was associated with higher risk (hazard ratio = 1.34, 95% CI: 1.04, 1.73) (9). In contrast, the Vitamin D Pooling Project found no association in an analysis of 775 case-control pairs nested within 8 prospective cohorts (10). These discrepant results are not readily explicable. One difference between the Vitamin D Pooling Project and the present study is the method of adjustment for season. The Vitamin D Pooling Project used conditional logistic regression models adjusted for season of blood collection (summer vs. winter), with sensitivity analyses adjusted for seasonality by using the residuals from a local polynomial regression (16). In contrast, we directly modeled seasonality using smooth trigonometric functions. Another difference between the studies is that both the Vitamin D Pooling Project and the Copenhagen City Heart Study used a chemiluminescence immunoassay measuring both $25(OH)D_2$ and $25(OH)D_3$, whereas in the present study, we used liquid chromatography coupled with tandem mass spectrometry to quantify $25(OH)D_3$ specifically. That said, these methodological differences would seem unlikely to fully account for the discrepant results, which remain unexplained.

Although few studies have directly assessed vitamin D status, some investigators have taken a different approach, creating a predicted vitamin D score on the basis of established determinants of vitamin D concentrations (17, 18). Joh et al. (19) investigated predicted 25(OH)D concentrations (calculated on the basis of race, UVB flux, physical activity, BMI value, vitamin D intake, alcohol consumption, and postmenopausal hormone use) and risk of RCC among participants of the Nurses' Health Study and the Health Professionals

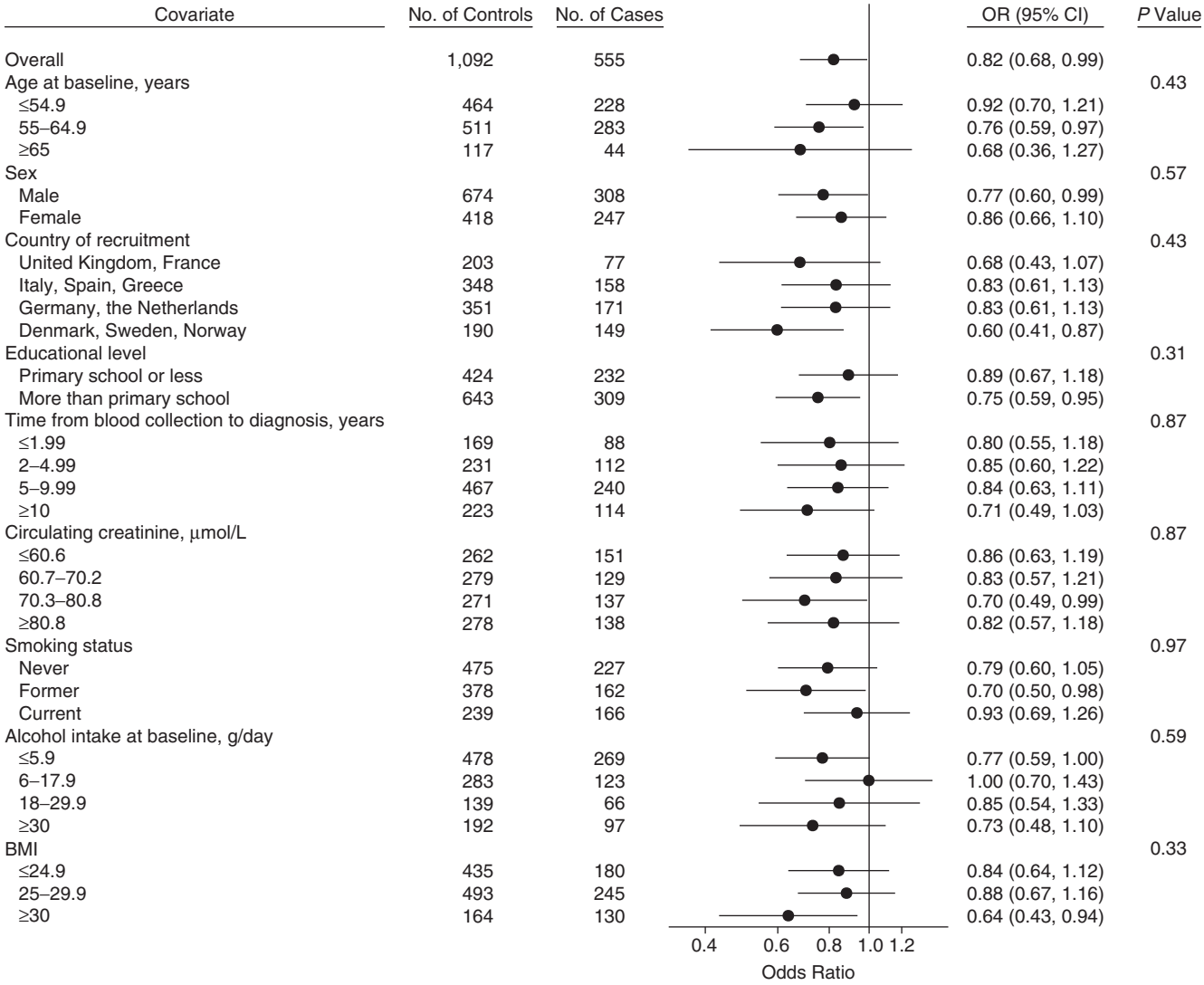

Figure 3. Stratified odds ratios (ORs) and 95% confidence intervals (CIs) for renal cell carcinoma for a doubling in concentration of 25-hydroxyvitamin D_3. Estimates are adjusted for age at baseline, sex, country, seasonality (sine and cosine functions of day of blood collection), smoking status at baseline (never/former/current smoker), circulating cotinine (quartiles defined among the controls), alcohol intake at recruitment (in g/day), and body mass index (BMI) (weight (kg)/height $(m)^2$). *P* values are from likelihood ratio tests of interaction terms. Estimates are from a nested case-control sample within the European Prospective Investigation into Cancer and Nutrition (EPIC), which recruited participants between 1992 and 2000. Bars, 95% CIs.

Follow-up Study. They found a strong inverse association between predicted 25(OH)D and risk, such that a 10-ng/mL (approximately 25-nmol/L) increment in predicted score was associated with a 44% lower hazard of RCC. Although the magnitude of the estimated association is greater, this result is broadly consistent with our observation that incrementing $25(OH)D_3$ from 25 to 50 nmol/L is associated with approximately 20% lower risk. In contrast, studies of dietary sources of vitamin D alone have largely yielded null results (20–22), possibly because dietary sources do not contribute greatly to circulating vitamin D concentrations (17).

There are several plausible mechanisms that might underpin an association between vitamin D and RCC (23). For instance, it is possible that vitamin D modifies the effects of risk factors such as obesity, hypertension, or diabetes. Although we observed an indication that the association between $25(OH)D_3$ and RCC risk might be slightly stronger among those with BMI values of 30 of higher, statistical adjustment for systolic blood pressure or prevalent diabetes did not affect the estimates, suggesting a potential role of vitamin D beyond that of established risk factors. This is consistent with studies of human RCC cell lines and murine RCC, which have shown that vitamin D species can inhibit tumor cell proliferation, angiogenesis, and metastasis (24, 25). Conversely, given the critical role of the kidneys in vitamin D metabolism, it is possible that the observed association is driven

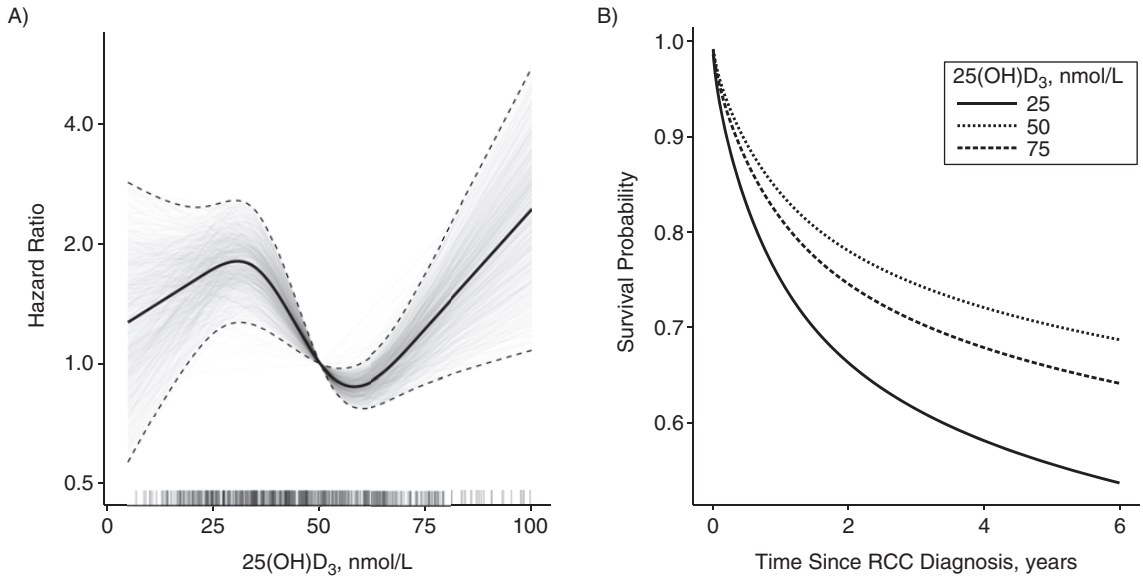

Figure 4. Post–renal cell carcinoma (RCC) survival. Estimates from a nested case-control sample within the European Prospective Investigation into Cancer and Nutrition (EPIC), which recruited participants between 1992 and 2000. A) Hazard ratios from a Cox model for all-cause mortality after RCC diagnosis as a function of circulating concentration of 25-hydroxyvitamin D_3 ($25(OH)D_3$), relative to a concentration of 50 nmol/L. We modeled $25(OH)D_3$ using restricted cubic splines with knots at the 10th, 33rd, 67th, and 90th percentiles of its distribution. The model was adjusted for age at baseline, sex, country, and seasonality (sine and cosine functions of day of blood collection), smoking status at baseline (never/former/current smoker), circulating cotinine (quartiles defined among the controls), alcohol intake at recruitment (in g/day), and body mass index (weight (kg)/height $(m)^2$). Solid and dashed lines represent the maximum likelihood estimates and 95% confidence intervals, respectively. The translucent lines are 1,000 draws from the multivariate normal distribution defined by the maximum likelihood estimates and their variance-covariance matrix; they thus give an indication of the posterior density for the hazard ratio under a uniform prior on the regression coefficients. The "rug plot" shows the observed distribution of $25(OH)D_3$. B) Survival function after RCC diagnosis evaluated at given concentrations of $25(OH)D_3$, derived from a flexible parametric survival model. Restricted cubic splines with knots at the 0th, 33rd, 67th, and 100th percentiles of the distribution of uncensored survival times were used to model the baseline hazard. We modeled $25(OH)D_3$ using restricted cubic splines with knots at the 10th, 33rd, 67th, and 90th percentiles of its distribution.

by perturbed vitamin D metabolism as a consequence of early kidney dysfunction. Although the association remained consistent throughout follow-up and was not affected by adjustment for circulating creatinine, we cannot completely rule out the possibility that early renal dysfunction was the cause, rather than the result, of the observed distribution of circulating vitamin D.

Many researchers have investigated circulating vitamin D and all-cause mortality in general populations. Consistent with our observation, many studies have reported higher risk of death for people with low vitamin D concentrations (26–33). This suggests that the association observed in our study may not be specific to RCC survival, but rather a reflection of a general phenomenon. Our observation that high levels of $25(OH)D_3$ might be associated with higher risk of death is consistent with results from the Uppsala Longitudinal Study of Adult Men, which also suggest a U-shaped association (34). Despite this accord, further studies are required to investigate the intriguing possibility that both low and high concentrations are associated with all-cause mortality.

The principal limitation of our study is that $25(OH)D_3$ was measured using a single blood sample drawn in adulthood. Although individual vitamin D measurements are reasonably reproducible, intraindividual variation may still be important (35). Further, it is possible that a single measurement in adulthood does not capture exposure to vitamin D in an etiologically relevant period.

Our study has several strengths. Importantly, our sample included participants from 10 European countries from different geographical latitudes and with a wide range of $25(OH)D_3$ concentrations. Biospecimen handling was standardized, and quantification of circulating $25(OH)D_3$ took place in a single laboratory, thus minimizing systematic interlaboratory variation. The prospective design of our study, in which $25(OH)D_3$ concentrations were assessed using blood collected prior to diagnosis, minimizes the chance that any differences between cases and controls are caused by existing tumors. Further, the availability of detailed information on potential confounders—particularly the inclusion of circulating cotinine as a biomarker of current smoking intensity and creatinine as a marker of renal function—affords additional confidence that the observed associations were not caused by residual confounding.

In conclusion, we found that low concentrations of $25(OH)D_3$ were associated with higher risk of RCC as well as lower all-cause mortality among RCC cases. High concentrations of $25(OH)D_3$ might also be associated with increased risk of all-cause mortality among RCC cases.

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(Appendix follows)

Appendix Table 1. Distribution of 25(OH)D_3 by Country of Recruitment Among Renal Cell Carcinoma Cases and Controls Nested Within EPIC, Recruitment 1992–2000

Country	No.	Percentile of 25(OH)D_3, nmol/L		
		5%	50%	95%
Denmark	228	18.19	50.68	89.11
France	33	19.30	45.63	86.47
Germany	354	18.01	41.72	78.00
Greece	56	18.72	42.16	65.90
Italy	246	14.55	40.08	77.00
Netherlands	169	22.54	45.79	78.91
Norway	10	25.06	52.42	71.83
Spain	205	22.37	43.97	77.84
Sweden	105	33.92	57.07	85.34
United Kingdom	264	24.25	51.26	82.73
Overall	1,670	18.90	45.69	81.31

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; EPIC, European Prospective Investigation into Cancer and Nutrition.