Vitamin D-binding protein, circulating vitamin D and risk of renal cell carcinoma

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Cell culture experiments suggest that vitamin D may inhibit renal carcinogenesis, but human studies of circulating 25-hydroxyvitamin D [25(OH)D], the accepted measure of vitamin D status, and kidney cancer have been null. Limited research has examined the role of circulating vitamin D-binding protein (DBP) in the association between 25(OH)D and disease risk, and it is unclear whether free 25(OH)D in circulation is a better measure of effective exposure, or if DBP may independently impact outcomes. We conducted a nested case–control analysis within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study to examine whether circulating DBP concentration was prospectively associated with risk of renal cell carcinoma, and whether it modified the association with 25(OH)D. Renal cell carcinoma cases ($n = 262$) were matched 1:1 to controls on age (± 1 year) and date of blood collection (± 30 days). We estimated odds ratios (ORs) and 95% confidence intervals (CIs) of renal cell carcinoma risk by quartiles of 25(OH)D, DBP and the molar ratio of 25(OH)D:DBP, a proxy for free circulating 25(OH)D. Men with higher DBP concentrations were at significantly decreased risk of kidney cancer (Q4 vs. Q1: OR = 0.17, 95% CI = 0.08–0.33; p-trend < 0.0001), a finding unchanged by adjustment for 25(OH)D. Although we observed no association with total 25(OH)D, we found slightly increased risk with higher levels of estimated free 25(OH)D [Q4 vs. Q1 of the 25(OH)D:DBP ratio, OR = 1.61, 95% CI = 0.95–2.73; p-trend = 0.09]. The strong protective association observed between higher circulating DBP concentration and kidney cancer risk requires replication but suggests a vitamin D-independent influence of DBP.

Kidney cancer is the sixth most common cancer among men in the United States, and is the tenth leading cause of cancer death.[1] The most common histological type of kidney cancer is renal cell carcinoma, and its known risk factors include smoking, obesity and hypertension.[2] These factors do not fully explain its etiology, however. Because the kidney is the major organ responsible for vitamin D metabolism and resorption, there has been considerable interest in whether vitamin D may be related to kidney carcinogenesis. Cell culture experiments suggest that vitamin D may be protective,[3] and a recent epidemiologic study of predicted vitamin D status and risk of renal cell carcinoma found a strong inverse association.[4] To our knowledge, however, only one study has examined the association between measured vitamin D concentrations and risk of renal cell carcinoma in humans. This study, the Vitamin D Pooling Project of Rarer Cancers (VDPP), was a large, pooled analysis of data from ten cohorts, including the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, that showed no association.[5]

Recent studies have suggested a role for circulating vitamin D-binding protein (DBP) concentration in the etiology of several cancers, both directly and by modifying the association between circulating vitamin D and risk of disease.[6–8] Vitamin D status is measured by blood concentration of 25-hydroxyvitamin D [25(OH)D], which is bound in circulation to DBP; very little 25(OH)D circulates in a free state.[9–11] The "free hormone hypothesis" postulates that only free, unbound hormones can have biological effects on target tissues,[10] and recent epidemiologic studies support that hypothesis with respect to 25(OH)D and cancers of both the pancreas and bladder.[6,8] DBP may also directly impact carcinogenesis through its non-vitamin D-related biological functions, including being a member of the extracellular actin scavenger system and by playing a role in chemotaxis, macrophage activation, apoptosis and angiogenesis.[10,11] To our knowledge, no studies have examined circulating DBP concentration in relation to renal cell carcinoma, either directly or as a potential modifier of the relation with 25(OH)D. Therefore, we conducted a nested case–control analysis within the ATBC Study to examine whether circulating DBP concentration was

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What's new?

Circulating vitamin D binding protein (DBP) has been implicated in the etiology of certain cancers, where it may act directly or by modifying circulating vitamin D concentrations and disease risk. In this examination of the association between DBP and renal cell carcinoma (RCC) specifically, a strong protective association was found between elevated circulating DBP concentrations and kidney cancer. The association was unchanged after adjustment for circulating vitamin D. The results suggest that DBP may influence risk of RCC through a biologic mechanism unrelated to vitamin D status.

prospectively associated with risk of renal cell carcinoma, and whether it modified the previously observed null association from this cohort, reported as part of the VDPP, between circulating 25(OH)D and risk of renal cell carcinoma.[5]

Methods

Study population

The ATBC Study was a randomized, double-blind, placebo-controlled, primary prevention trial designed to examine the effects of α-tocopherol and β-carotene supplementation on cancer incidence.[12] From 1985 to 1988, 29,133 men were recruited from southwestern Finland. Participants were between the ages of 50 and 69 years at baseline and smoked at least five cigarettes per day as part of the enrollment criteria. Participants were assigned to one of four groups based on a 2 × 2 factorial design: (*i*) α-tocopherol (dl- α-tocopheryl acetate, 50 mg/day), (*ii*) β-carotene (20 mg/day), (*iii*) both supplements or (*iv*) placebo. Men were supplemented for 5–8 years, until death or until the trial ended on April 30, 1993. Follow-up is ongoing through the Finnish Cancer Registry and the Register of Causes of Death and for this analysis is complete through April 20, 2005. Written informed consent was obtained from all participants; the ATBC Study was approved by institutional review boards at both the Finnish National Public Health Institute and the US National Cancer Institute. At the time of enrollment, participants completed questionnaires providing information on general risk factors, smoking and medical history, as well as a food-frequency questionnaire. Participants were also examined by registered nurses who measured their height and weight, and collected an overnight fasting blood sample.

This analysis was conducted in the same nested case–control sample that was included as part of the VDPP.[5,13] Renal cell carcinoma cases were identified by linkage with the Finnish Cancer Registry, which provides nearly 100% complete incident cancer ascertainment for ATBC Study participants.[14] For those cases diagnosed before May 1999, medical records were reviewed by one or two study physicians to confirm the cancer diagnosis, with subsequent cases based solely on the Finnish Cancer Registry data. All renal cell carcinoma cases (ICD-9 code 189.0) that occurred through April 30, 2005 were selected (*n* = 282). Controls were sampled without replacement from ATBC Study participants who were alive and cancer free at the time the case was diagnosed and were matched 1:1 with cases on age at randomization

(±1 year) and date of blood collection (±30 days). Renal cell carcinoma cases were not eligible to be selected as controls. The present analysis excludes one matched pair where the control had a missing value for serum 25(OH)D and excludes 19 pairs where the case or control (or both) had insufficient residual serum remaining after the earlier 25(OH)D assay for measurement of circulating DBP concentration, leaving 262 matched pairs for analysis.

Laboratory measures

Fasting serum samples collected at baseline were stored at –70°C. The 25(OH)D was measured by Heartland Assays, LLC (Ames, IA) using the DiaSorin Liaison 25(OH)D TOTAL assay[15] for all subjects at one time. Blinded quality control (QC) samples from an ATBC Study pool as well as standard reference material provided by the National Institute of Standards and Technology (NIST) were included in each batch, comprising ~5% of the total sample.[16] The overall interbatch and intrabatch coefficient of variations (CVs) were 7.1 and 10.1%, respectively. The laboratory and QC methods are discussed in detail elsewhere.[13]

Circulating DBP concentration was measured by the Clinical Support Laboratory, SAIC-Frederick, Frederick National Laboratory for Cancer Research (Frederick, MD) using the Quantikine Human Vitamin D Binding Protein Immunoassay Kit (Catalog number DVDBP0, R&D Systems, Minneapolis, MN). Each batch contained blinded QC samples comprising ~10% of the total samples. Our group has measured circulating DBP concentration for nested case–control sets of several different cancer sites with shared controls, and samples were sent to the laboratory at two different times, once in February 2011 and again in December 2011. For our analysis, 234 controls and 13 cases were measured in February 2011, and 28 controls and 249 cases were measured in December 2011. The interbatch and intrabatch CVs for samples assayed in February were 10.8 and 15.2%, respectively, and for samples assayed in December were 14.6 and 8.9%, respectively.

Statistical analysis

Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of renal cell carcinoma by quartiles of 25(OH)D, DBP and the molar ratio of 25(OH)D:DBP, an estimation of free circulating 25(OH)D.[17,18] Because 25(OH)D concentrations are

Table 1. Selected baseline characteristics [medians (interquartile range) or percent] for renal cell carcinoma case and control subjects in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

Characteristic	Controls (*n* = 281)	Cases (*n* = 281)	*p*-value[1]
Age (years)	57.0 (54.0–61.0)	57.0 (54.0–61.0)	Matched
BMI (kg/m^2)	25.8 (23.7–28.1)	26.6 (24.5–28.8)	0.01
Cigarettes per day	20 (15–25)	20 (15–25)	0.03
Years of smoking	37.0 (32.0–42.0)	37.0 (32.0–42.0)	0.93
History of diabetes (%)	5.7	3.6	0.23
History of renal failure (%)	0	0	–
Systolic blood pressure (mmHg)	142 (128–158)	142 (132–158)	0.34
Diastolic blood pressure (mmHg)	90 (82–96)	90 (82–96)	0.55
Measured hypertension (%)	66.9	69.0	0.76
Physically active (%)	19.6	21.0	0.67
Elementary school education (%)	18.9	21.4	0.46
Intake/day			
Total energy (kcal)	2,647 (2,135–3,036)	2,523 (2,142–3,030)	0.65
Dietary vitamin D (IU)	4.6 (3.1–6.6)	4.7 (3.4–7.1)	0.24
Dietary calcium (mg)	1,312 (1,026–1,715)	1,358 (1,002–1,686)	0.99
Alcohol (g)	8.7 (1.2–21.2)	9.1 (1.8–21.6)	0.60
Use of dietary supplements (%)			
Vitamin D	8.9	6.4	0.23
Calcium	11.7	10.7	0.55
Serum cholesterol (mmol/L)	6.2 (5.5–7.0)	6.0 (5.4–6.8)	0.28
Serum alpha-tocopherol (mg/L)	11.5 (10.0–13.7)	11.7 (10.0–13.7)	0.55
Serum beta-carotene (µg/L)	176 (115–278)	171 (109–267)	0.48
Serum retinol (µg/L)	568 (485–653)	579 (506–667)	0.20

[1]Chi-square test for categorical variables and Wilcoxon test for continuous variables.

known to vary by season, quartile cutpoints for 25(OH)D were based on the distribution of the controls in our nested renal cell carcinoma set for each season (sunnier season = May to October, darker season = November to April); quartiles were created separately for each season and then combined into one variable.

To address whether the measurement of circulating DBP concentration for cases and controls at two different times (*i.e.*, sets) in the same laboratory may have biased our findings, we used multiple analytical approaches. First, we created set-specific DBP quartiles based on the distribution of all controls assayed in each of the two sets, not just the controls matched to renal cell carcinoma cases (*i.e.* 1,109 controls in February 2011 set, and 129 controls in December 2011 set), and combined the two sets of quartiles into one variable. Second, we conducted a naïve case–control analysis of quartiles of cases and matched controls based on the distribution among the renal cell carcinoma controls, ignoring the assay sets. Our third approach was an unmatched analysis that compared cases to all controls measured at the same time point as the cases (*e.g.*, *n* = 123 for the December 2011 set), regardless of whether they were originally matched to a renal cell carcinoma case or to a case at another cancer site. Our findings from the three approaches were very similar, suggesting that any bias introduced, if any, was minimal (Supporting Information Table). Therefore, we present findings from the time/set-specific quartiles. Quartile cutpoints for the molar ratio of 25(OH)D:DBP were determined based on the distribution among controls in our nested renal cell carcinoma set. We evaluated the trend across categories by modeling the ordinal categorical variable [for 25(OH)D and DBP] or the median of each category [for the molar ratio of 25(OH)D:DBP] as a continuous variable and by evaluating its statistical significance using the Wald test.

Factors significantly associated ($p < 0.05$) with either risk of renal cell carcinoma or with serum 25(OH)D or DBP in our data, or factors that are known to be associated with renal cell carcinoma were included in the multivariable models. In addition to being conditioned on the matching factors (*i.e.*, age and date of blood collection), our multivariable model was adjusted for cigarettes per day, body mass index (BMI) and hypertension. We also present our results mutually adjusted for 25(OH)D or DBP. Analyses were conducted stratifying DBP by 25(OH)D, age, serum total and HDL

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Table 2. Odds ratios and 95% confidence intervals for the association between serum DBP, serum 25(OH)D and 25(OH)D:DBP molar ratio, and renal cell carcinoma risk

	Q1	Q2	Q3	Q4	p-trend
DBP[1]					
# cases/# controls	98/58	62/76	71/53	29/73	
OR[2] (95% CI)	1.0 (ref)	0.42 (0.25–0.71)	0.80 (0.48–1.33)	0.17 (0.09–0.32)	<0.0001
OR[3] (95% CI)	1.0 (ref)	0.41 (0.25–0.70)	0.76 (0.45–1.28)	0.17 (0.09–0.33)	<0.0001
OR[4] (95% CI)	1.0 (ref)	0.40 (0.24–0.68)	0.75 (0.44–1.28)	0.17 (0.08–0.33)	<0.0001
25(OH)D[5]					
# cases/# controls	68/73	64/67	74/74	75/67	
OR[2] (95% CI)	1.0 (ref)	1.03 (0.66–1.60)	1.07 (0.69–1.67)	1.20 (0.76–1.90)	0.62
OR[3] (95% CI)	1.0 (ref)	1.07 (0.68–1.70)	1.07 (0.68–1.68)	1.28 (0.79–2.05)	0.50
OR[4] (95% CI)	1.0 (ref)	0.99 (0.60–1.63)	1.17 (0.71–0.91)	1.45 (0.86–2.44)	0.18
25(OH)D:DBP molar ratio ($\times 10^3$)					
Range	<3.9	3.9–<5.9	5.9–<9.0	≥9.0	
# cases/# controls	60/70	66/65	61/68	73/57	
OR[2](95% CI)	1.0 (ref)	1.18 (0.73–1.92)	1.03 (0.64–1.66)	1.57 (0.94–2.62)	0.12
OR[3] (95% CI)	1.0 (ref)	1.11 (0.67–1.85)	1.04 (0.63–1.72)	1.61 (0.95–2.73)	0.09

[1]February 2011 cutpoints (in nmol/L) = Q1: <4,396, Q2: 4,396–<5,569, Q3: 5,569–<6,999, Q4: ≥6,999; December 2011 cutpoints (in nmol/L) = Q1: <4,896, Q2: 4,896–<6,155, Q3: 6,155–<7,637, Q4: ≥7,637.
[2]Conditioned on age and date of baseline blood collection.
[3]Conditioned on age and date of baseline blood collection and adjusted for hypertension, BMI and cigarettes smoked per day.
[4]Conditioned on age and date of baseline blood collection and adjusted for hypertension, BMI and cigarettes smoked per day. Models are mutually adjusted for DBP and 25(OH)D.
[5]Winter quartile cutpoints (in nmol/L) = Q1: <19, Q2: 19–<29, Q3: 29–<44, Q4: ≥44; summer quartile cutpoints (in nmol/L) = Q1: <29, Q2: 29–<43, Q3: 43–<57, Q4: ≥57.

cholesterol, hypertension, BMI, weight, height, cigarettes per day and time from blood draw to diagnosis with renal cell carcinoma (all continuous variables split at the median). Analyses were also conducted stratifying 25(OH)D by DBP (<median *vs.* ≥median). Stratified analyses were conducted using unconditional logistic regression adjusting for the matching factors. The main model results were unchanged when an unmatched analysis was used instead of conditional logistic regression, making biased estimates unlikely. Statistical interaction was assessed using the likelihood ratio test.

Results
Characteristics of the cases and controls were similar for most of the factors examined, with the exception of BMI being higher among the cases (Table 1). We observed a strong inverse association between circulating DBP concentration and risk of renal cell carcinoma (Q4 *vs.* Q1, OR = 0.17, 95% CI = 0.09–0.32, p-trend < 0.0001, Table 2). This finding was essentially unchanged with multivariable adjustment or with further adjustment for serum 25(OH)D (Table 2). Similar to the observation in the VDPP analysis, we found no statistically significant association between serum 25(OH)D and risk of renal cell carcinoma (season-specific Q4 *vs.* Q1 multivariable-adjusted OR = 1.28, 95% CI = 0.79–2.05, p-trend = 0.50, Table 2). Further adjustment for serum DBP resulted in a stronger positive association,

although it was not statistically significant (Table 2). When we examined risk in relation to the 25(OH)D:DBP molar ratio [a proxy for free 25(OH)D concentration], a borderline statistically significant association was observed (Q4 *vs.* Q1 multivariable-adjusted OR = 1.61, 95% CI = 0.95–2.73, p-trend = 0.09). Although 25(OH)D did not modify the circulating DBP-risk relation, the analyses of 25(OH)D within DBP subgroups suggested that the association differed by DBP (p for interaction = 0.03), but the patterns were inconsistent (Table 3). There were no interactions between serum DBP concentration and any other factor examined, with strong inverse associations between DBP and risk of renal cell carcinoma in all subgroups (Table 4).

Discussion
We found a strong inverse association between circulating DBP concentration and risk of renal cell carcinoma that was independent of 25(OH)D concentration. In addition, although we observed no association between total circulating 25(OH)D and risk of renal cell carcinoma, there was a positive association of borderline statistical significance with our estimate of free circulating 25(OH)D [*i.e.*, the 25(OH)D:DBP molar ratio]. To our knowledge, this is the first study to examine circulating DBP or free 25(OH)D concentrations in relation to risk of renal cell carcinoma.

Table 3. Odds ratios and 95% confidence intervals for the association of serum DBP stratified by 25(OH)D and serum 25(OH)D stratified by DBP with renal cell carcinoma risk

	Q1	Q2	Q3	Q4	*p* for interaction
DBP					
25(OH)D below median					
# cases/# controls	53/29	26/37	34/31	11/31	
OR[1] (95% CI)	1.0 (ref)	0.38 (0.19–0.75)	0.60 (0.31–1.16)	0.19 (0.08–0.43)	
OR[2] (95% CI)	1.0 (ref)	0.37 (0.19–0.75)	0.56 (0.28–1.12)	0.20 (0.09–0.46)	0.74
25(OH)D above median					
# cases/# controls	45/29	36/39	37/22	18/42	
OR[1] (95% CI)	1.0 (ref)	0.59 (0.31–1.13)	1.08 (0.54–2.19)	0.28 (0.13–0.57)	
OR[2] (95% CI)	1.0 (ref)	0.55 (0.28–1.08)	0.96 (0.47–1.99)	0.28 (0.13–0.58)	
25(OH)D					
DBP below median					
# cases/# controls	32/38	47/31	47/36	35/35	
OR[1] (95% CI)	1.0 (ref)	2.00 (1.03–3.89)	1.52 (0.80–2.89)	1.30 (0.66–2.54)	
OR[2] (95% CI)	1.0 (ref)	1.99 (1.01–3.94)	1.52 (0.79–2.94)	1.42 (0.71–2.84)	0.03
DBP above median					
# cases/# controls	30/33	15/35	23/36	35/31	
OR[1] (95% CI)	1.0 (ref)	0.54 (0.24–1.19)	0.66 (0.32–1.39)	1.21 (0.60–2.44)	
OR[2] (95% CI)	1.0 (ref)	0.49 (0.22–1.12)	0.67 (0.32–1.43)	1.23 (0.60–2.52)	

[1]Adjusted for the matching factors: age at baseline (continuous) and date of blood collection (continuous).
[2]Adjusted for the matching factors: age at baseline (continuous) and date of blood collection (continuous). Further adjusted for number of cigarettes per day and years of smoking.

The kidney is a major organ impacting vitamin D status, with 25(OH)D being converted to its active hormonal form, 1-25-dihydroxyvitamin D [1,25(OH)$_2$D], in the proximal tubules. DBP-bound 25(OH)D in the glomerular filtrate is absorbed into proximal tubule cells through endocytic ligand binding and uptake by the plasma membrane megalin–cubilin receptor complex, after which intracellular 25(OH)D is converted to 1,25(OH)$_2$D in mitochondria, and DBP undergoes proteolysis in lysosomes.[19,20] In addition to its canonical role in vitamin D transport in circulation, DBP has other important biological functions that may impact carcinogenesis. DBP is a member of the extracellular actin scavenging system that protects against the harmful effects resulting from release of actin into circulation following tissue injury or cell death.[10,11] DBP may also have an anticarcinogenic effect through its role in macrophage activation: It is deglycosylated by T- and B-cell glycosidases to DBP-macrophage-activating factor, which induces apoptosis through increased proapoptotic activity.[11] It has also been shown to have anti-angiogenic effects.[11] As the protective association we observed for circulating DBP concentration was independent of 25(OH)D concentrations, if this association is causal our findings suggest that DBP may impact renal carcinogenesis through one or more of these non-vitamin D-related mechanisms. Alternatively, that the DBP-25(OH)D complex is reabsorbed in the proximal tubules, and renal cell carcinoma originates in the same epithelium, could support the idea that higher circulating DBP concentration increases intracellular renal 25(OH)D with protective consequences. The relationship of circulating 25(OH)D and DBP with tissue-level availability of 25(OH)D requires study.

We considered the possibility that the inverse DBP-risk association reflects reverse causality; *e.g.*, undiagnosed renal tumors alter megalin receptor function, resulting in lower circulating concentrations of DBP. This appears unlikely, however, as we observed similar DBP–renal cell carcinoma associations for cases diagnosed during both the first and second decades following baseline blood collection (Table 4). Reverse causality would be more consistent with a stronger inverse association early in follow-up.

Despite observing no association for total 25(OH)D, we found borderline statistically significantly increased risk of renal cell carcinoma with higher levels of estimated free 25(OH)D. That free 25(OH)D may be more etiologically relevant than total 25(OH)D supports the "free hormone hypothesis,"[10] and is consistent with findings from this cohort for other cancer sites including bladder and pancreas.[6,8] Although laboratory studies point to a protective role for vitamin D in cancer, including renal cell carcinoma,[3] human studies have suggested that this may not be the case for all cancer sites; *e.g.*, some studies have reported higher circulating 25(OH)D to be associated with increased risks of

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Table 4. Odds ratios and 95% confidence intervals for the association between serum DBP and renal cell carcinoma risk stratified by various factors

Subgroup		Q1	Q2	Q3	Q4	p for interaction
				-Quartile of DBP		
Age (years)						
<58 (median)	# cases/# controls	53/31	37/43	32/25	19/42	0.88
	OR[1] (95% CI)	1.0 (ref)	0.49 (0.26–0.93)	0.69 (0.34–1.40)	0.27 (0.13–0.56)	
≥58 (median)	# cases/# controls	45/27	25/33	39/28	10/31	
	OR[1] (95% CI)	1.0 (ref)	0.42 (0.20–0.86)	0.76 (0.38–1.54)	0.19 (0.08–0.46)	
Total cholesterol (mm/L)						
<median	# cases/# controls	61/35	37/42	39/23	16/36	
	OR[1] (95% CI)	1.0 (ref)	0.49 (0.26–0.90)	0.89 (0.46–1.76)	0.27 (0.13–0.56)	0.88
≥median	# cases/# controls	37/23	25/34	32/30	13/37	
	OR[1] (95% CI)	1.0 (ref)	0.44 (0.21–0.92)	0.61 (0.29–1.27)	0.21 (0.09–0.48)	
HDL cholesterol						
<median	# cases/# controls	54/37	33/47	48/30	14/43	
	OR[1] (95% CI)	1.0 (ref)	0.47 (0.25–0.89)	1.07 (0.57–2.02)	0.23 (0.11–0.48)	0.17
≥median	# cases/# controls	44/21	29/29	23/23	15/30	
	OR[1] (95% CI)	1.0 (ref)	0.44 (0.21–0.92)	0.39 (0.17–0.87)	0.24 (0.10–0.53)	
Hypertension						
No	# cases/# controls	23/21	22/31	24/14	10/22	
	OR[1] (95% CI)	1.0 (ref)	0.68 (0.30–1.54)	1.59 (0.64–3.95)	0.43 (0.16–1.12)	0.21
Yes	# cases/# controls	75/37	40/45	47/39	19/51	
	OR[1] (95% CI)	1.0 (ref)	0.40 (0.22–0.72)	0.52 (0.29–0.96)	0.19 (0.10–0.36)	
BMI (kg/m²)						
<24 (median)	# cases/# controls	15/14	11/16	16/16	9/24	
	OR[1] (95% CI)	1.0 (ref)	0.68 (0.23–2.01)	0.92 (0.33–2.57)	0.35 (0.12–1.02)	0.86
≥24 (median)	# cases/# controls	83/44	51/60	55/37	20/49	
	OR[1] (95% CI)	1.0 (ref)	0.44 (0.26–0.74)	0.76 (0.43–1.34)	0.22 (0.11–0.41)	
Weight						
<median	# cases/# controls	37/32	22/36	27/29	15/41	
	OR[1] (95% CI)	1.0 (ref)	0.52 (0.26–1.08)	0.80 (0.39–1.63)	0.33 (0.15–0.71)	0.75
≥median	# cases/# controls	61/26	40/40	44/24	14/32	
	OR[1] (95% CI)	1.0 (ref)	0.42 (0.22–0.80)	0.73 (0.37–1.47)	0.18 (0.08–0.40)	
Height						
<median	# cases/# controls	34/28	21/42	29/24	11/32	
	OR[1] (95% CI)	1.0 (ref)	0.40 (0.19–0.84)	0.99 (0.47–2.10)	0.29 (0.12–0.68)	0.43
≥median	# cases/# controls	64/30	41/34	42/29	18/41	
	OR[1] (95% CI)	1.0 (ref)	0.56 (0.30–1.07)	0.63 (0.33–1.22)	0.20 (0.10–0.41)	
Cigarettes per day						
<20 (median)	# cases/# controls	35/17	19/34	22/25	9/33	
	OR[1] (95% CI)	1.0 (ref)	0.25 (0.11–0.56)	0.39 (0.17–0.90)	0.13 (0.05–0.33)	0.15
≥20 (median)	# cases/# controls	63/41	43/42	49/28	20/40	
	OR[1] (95% CI)	1.0 (ref)	0.65 (0.36–1.17)	1.08 (0.58–2.02)	0.34 (0.17–0.66)	
Time to diagnosis						
<10 years	# cases/# controls	44/28	31/29	29/20	11/38	
	OR[1] (95% CI)	1.0 (ref)	0.34 (0.18–0.66)	0.60 (0.31–1.18)	0.30 (0.14–0.62)	0.20
≥10 years	# cases/# controls	54/30	31/47	42/33	18/35	
	OR[1] (95% CI)	1.0 (ref)	0.66 (0.33–1.33)	0.91 (0.42–1.95)	0.18 (0.08–0.42)	

[1]Adjusted for age, date of baseline blood collection, hypertension, BMI and cigarettes smoked per day.

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prostate and pancreatic cancers.[21–23] The biologic mechanism through which 25(OH)D might increase cancer risk remains speculative, but one hypothesis is that 25(OH)D may adversely regulate the expression of genes involved in carcinogenesis. It is also not clear whether bound and free 25(OH)D impact cancer-related pathways in target tissues differently. It should be noted, however, that our findings for free 25(OH)D were not formally statistically significant, and could be explained by chance. Further, we did not directly measure free 25(OH)D, although any misclassification due to an imperfect estimate of free 25(OH)D would likely be nondifferential with respect to case status, which would bias our findings toward the null. Therefore, the true positive association between free 25(OH)D and risk of renal cell carcinoma may actually be stronger than that which we observed. Measurement of free 25(OH)D in future studies of renal cell carcinoma would be useful.

Strengths of our investigation include the prospective design, laboratory measurement of circulating 25(OH)D and DBP concentrations in fasting serum and detailed information on (and adjustment for) many potential confounding factors. Although we were able to conduct exploratory analyses of many potential effect modifiers, we had limited statistical power to detect modest differences between strata. Another potential limitation of our study is the measurement of circulating DBP concentrations in cases and controls at two different times. However, the multiple sensitivity analyses we conducted and which we describe in Methods and Supporting Information Table suggest that this design issue does not explain our findings. In fact, the distribution of DBP concentrations among controls measured in December 2011, when most of the cases were measured, was slightly higher than that of controls measured in February 2011, when most of the renal cell carcinoma controls were measured

(Supporting Information Fig.). Thus, we would expect any difference in the measurements and values between the two time points to bias our results toward a positive association with renal cell carcinoma, which is opposite to the direction of the observed finding. DBP exists as several isoforms that have different binding affinities for vitamin D compounds, although their impact on the other biological activities of DBP is less well understood.[24] Two genetic variants encode the isoforms, and we had genetic information for them in a subset of our cohort, but with insufficient power to examine the risk association for each isoform; future studies may examine this. Because our study included only male smokers, any relation between circulating DBP concentration and renal cell carcinoma risk in women and nonsmokers would have to be studied. That smoking intensity and duration did not modify the circulating DBP-risk association makes it unlikely that a markedly different association would exist in nonsmokers, however.

Conclusions

In this prospective analysis, men with higher serum concentrations of DBP experienced lower risk of renal cell carcinoma, whereas the 25(OH)D:DBP molar ratio, a proxy for free circulating 25(OH)D, showed a possible positive risk association. Combined, the two findings suggest that the DBP association may reflect a biological mechanism unrelated to vitamin D status. Our findings require confirmation in additional studies, particularly in populations that include women and nonsmokers. The relationship of circulating bound and free 25(OH)D and DBP with tissue-level availability of 25(OH)D also warrants examination, and may help elucidate some of the conflicting results observed in epidemiologic studies of vitamin D and cancer.

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