Vitamin D–binding protein and pancreatic cancer: a nested case-control study[1–5]

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ABSTRACT

Background: Vitamin D–binding protein (DBP) is the primary carrier of 25-hydroxyvitamin D [25(OH)D] in the circulation. One prospective study in male smokers found a protective association between DBP and pancreatic cancer, particularly among men with higher 25(OH)D concentrations.

Objective: The objective was to examine the association between DBP and pancreatic cancer risk in an American population.

Design: We conducted a nested case-control study in the Prostate, Lung, Colorectal, and Ovarian Cancer screening trial cohort of men and women aged 55–74 y at baseline. Between 1993 and 2010, 295 incident pancreatic adenocarcinoma cases were reported (follow-up to 15.1 y). Two controls (n = 590) were matched to each case by age, race, sex, and month of blood draw. We calculated smoking- and diabetes-adjusted ORs and 95% CIs with the use of conditional logistic regression.

Results: DBP concentration was not significantly associated with pancreatic cancer overall [highest (≥7149.4 nmol/L) vs. lowest (<3670.4 nmol/L) quintile; OR: 1.75; 95% CI: 0.91, 3.37; P-trend = 0.25]. For serum 25(OH)D compared with the referent (50 to <75 nmol/L), individuals in the highest group had a significantly higher risk (≥100 nmol/L; OR: 3.23; 95% CI: 1.24, 8.44), whereas those in the lowest group had no significant association (<25 nmol/L; OR: 2.50; 95% CI: 0.92, 6.81). Further adjustment for DBP did not alter this association.

Conclusion: Our results do not support the hypothesis that serum DBP or 25(OH)D plays a protective role in pancreatic cancer. This trial was registered at clinicaltrials.gov as NCT00339495. *Am J Clin Nutr* 2015;101:1206–15.

Keywords: 25-hydroxyvitamin D, nested case-control, pancreatic cancer, vitamin D-binding protein, prediagnostic status, prospective study

INTRODUCTION

Pancreatic cancer is among the few cancers for which the incidence is increasing (1) and is the fourth leading cause of cancer death in both men and women in the United States (2). There is no effective treatment of the disease, which contributes to its relative 5-y survival rate of 6% (2). Therefore, the identification of potentially modifiable risk factors for pancreatic cancer is important.

The blood concentration of 25-hydroxyvitamin D [25(OH)D],[6] the precursor to the bioactive form of vitamin D (1,25-dihydroxyvitamin D [1,25$(OH)_2$D]), is considered the best biomarker of vitamin D status and reflects both vitamin D from the diet and that synthesized from sun exposure. To date, 4 prospective nested case-control studies have examined the association between prediagnostic blood concentrations of 25(OH)D and pancreatic cancer, with inconsistent associations. Two studies showed significant positive associations, one a significant inverse association, and one no overall association (3–6). Researchers have examined the role of vitamin D–binding protein (DBP) in the relation between 25(OH)D and pancreatic cancer risk in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study population (7). DBP is an estrogen-dependent enzyme synthesized in the liver that acts as the primary carrier of both 25(OH)D and 1,25$(OH)_2$D in the bloodstream (8). DBP serves several other functions in the body, including macrophage activation, actin scavenging, chemotaxis, and fatty acid transport (8). The recent nested case-control study within the ATBC

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[4] Supplemental Table 1 is available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; DBP, vitamin D–binding protein; DBP-maf, vitamin D–binding protein macrophage activating factor; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer; R-B, Robertson-Berger; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

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prospective cohort of male smokers showed a protective association between DBP and pancreatic cancer, particularly among men with higher 25(OH)D concentrations (7). The researchers concluded that higher concentrations of DBP may keep 25(OH)D out of circulation, reducing the possibly carcinogenic effects of 25(OH)D. These results, however, may not be generalizable to women or to nonsmokers.

Therefore, we examined the association between serum DBP and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial cohort, which includes both of these additional populations. In light of an increase in case numbers since the previous PLCO vitamin D analysis (n = 111 additional cases; 295 in total), we reexamined the association between serum 25(OH)D and pancreatic cancer, as well as the joint effects of serum 25(OH)D and DBP. We hypothesized that DBP would be inversely associated with pancreatic cancer risk, particularly among those with higher serum 25(OH)D concentrations.

METHODS

Study design and population

The PLCO screening trial was a randomized intervention that examined the association between screening and cancer mortality and was described previously (6, 9). Briefly, participants totaled 152,810 men and women between the ages of 55 and 74 y at baseline (1993–2001) who were recruited at 10 different clinical sites in the United States (Denver, CO; Washington, DC; Honolulu, HI; Detroit, MI; Minneapolis, MN; St. Louis, MO; Pittsburgh, PA; Salt Lake City, UT; Marshfield, WI; and Birmingham, AL). Individuals with a previous history of prostate, lung, colorectal, or ovarian cancer were excluded, as were those currently undergoing treatment of any form of cancer other than nonmelanoma skin cancer. Individuals were also excluded if they had received screening for prostate or colorectal cancer within the past 3 y.

Institutional review board approval was obtained from the institutions associated with each of the 10 study centers as well as from the National Cancer Institute (9). All participants gave informed consent. The trial was registered at clinicaltrials.gov as NCT00339495.

Case and control selection

Cases were identified by annual mailed follow-up questionnaires, which were then linked to population-based registries (where applicable) and the National Death Index. Appropriate medical and pathologic records were obtained when possible (9). In this study, there were 295 participants with confirmed incident exocrine pancreas adenocarcinoma during the initial study randomization period from 1993 to May 2010 (up to 15.1 y; median: 7.8 y).

Two controls who were free from pancreatic cancer were incidence-density sampled and matched to each case at the time of diagnosis on the basis of age (±5 y), race, sex, and month of blood draw (2-mo blocks; n = 590).

Biomarkers and data collection

Nonfasting blood samples were collected at baseline and stored at −70°C (10). Serum DBP was measured by using the Quantikine Human Vitamin D Binding Protein Immunoassay Kit (R&D Systems Inc.) at the Leidos Biomedical Research/Frederick National Laboratory for Cancer Research according to the kit instructions. Pooled quality-control samples were analyzed for each batch of DBP. Serum 25(OH)D was measured by using chemiluminescence immunoassay [DiaSorin Liaison 25(OH)D Total Assay] at Heartland Assays, according to kit instructions. Two pooled quality-control samples made up ∼10% of each batch of 25(OH)D that was analyzed. By using nested components of variance analysis, with logarithmically transformed quality-control 25(OH)D and DBP measurements across all batches, the overall average (intra- and interbatch) (11) CV percentages were 10.4% for DBP and 2.3% for 25(OH)D.

Information on dietary intake was assessed by using a grid-format food-frequency questionnaire, which contained questions regarding 137 food items consumed over the past year; there were 77 questions about usual portion sizes (12). Supplement use was assessed, with questions on number of years taken as well as current use and use 2 and 5 y ago. Vitamin D intake from supplements was extracted from questions about multivitamins (one-a-day and therapeutic or high-dose type) and vitamin D supplements (with or without calcium). A second self-administered questionnaire contained questions pertaining to demographic information, physical activity, height, weight, tobacco use, medical history, family history of cancer, certain medication use (including hormones such as oral contraceptives), previous screenings, and reproductive history.

Statistical analysis

The distribution of baseline characteristics of cases and controls was compared by using Wilcoxon's rank-sum test for continuous variables and chi-square test for categorical variables. We calculated means (SDs) and proportions of the baseline characteristics for the controls across quintiles of DBP on the basis of the distribution of all controls. Significant differences across quintiles were determined by using generalized linear models for continuous variables and chi-square test for categorical variables. Fisher's exact test was used for categorical variables with small cell counts. Variables examined in our analyses included age, sex, season of blood draw (high sun vs. low sun), residential sun exposure at study entry [determined by Robertson-Berger (R-B) units (meters) for each of the 10 study sites] (13), race-ethnicity, height, BMI (in kg/m^2; continuous and WHO cutoffs: <25.0, 25.0 to <30.0, and ≥30.0), smoking status (never, current, or former; cigarettes per day; number of years smoked), self-reported diabetes mellitus and gallbladder disease, family history of pancreatic cancer, educational level, regular nonsteroidal anti-inflammatory drug use, usual nutrient intake from food (energy, total fat, saturated fat, carbohydrate, fructose, vitamin D, calcium, vitamin A, and folate), usual intake of vitamin D–rich foods (omega-3 fatty acid fish, eggs, and milk), usual intake of red meat, usual intake of alcohol, supplement (vitamin D and calcium) use, and physical activity. Dietary components (except for alcohol and supplement use) were adjusted for total energy intake by using the residual method (14).

Conditional logistic regression was used to calculate ORs and 95% CIs. Because the distribution of DBP varied slightly by sex, we also assessed the association between DBP and pancreatic

cancer with the use of sex-specific quintiles as well as quintiles based on the distribution of all of the controls, with the lowest quintile of DBP as the referent group. There did not appear to be a difference between the results of the 2 models; therefore, we show the all-controls quintiles (quintile 1, <3670.4; quintile 2, 3670.4 to <4818.6; quintile 3, 4818.6 to <5804.7; quintile 4, 5804.7 to <7149.4; and quintile 5, ≥7149.4 nmol/L). The significance of the trends was determined by using a score variable based on the median value of each quintile. We used clinically defined cutoffs for serum 25(OH)D (<25, 25 to <37.5, 37.5 to <50, 50 to <75, 75 to <100, and ≥100 nmol/L) with 50.0 to <75.0 nmol/L as the referent group, as opposed to cohort-specific quantiles, to allow for comparisons to other studies (15). The crude models are considered adjusted for the matching factors (age, sex, race-ethnicity, and month of blood draw). The multivariable models were built by adding potential confounders, which were included in the final model if they changed the risk estimates by >10% or were putative risk factors. The final models for DBP and 25(OH)D included smoking status and diabetes. Other variables did not significantly alter the models. A lag analysis, excluding cases diagnosed during the first 5 y of follow-up, was performed for both the DBP and 25(OH)D associations to evaluate potential reverse causality. This analysis included 214 cases and 428 controls.

We evaluated the interaction of the DBP and 25(OH)D associations by season of blood draw, smoking (never, former, or current; nonsmoker, current; and never or ever), sex, and residential sun exposure in stratified analyses and tested statistical significance by using a multiplicative interaction term and Wald test. The stratified analysis by sex was performed by using conditional logistic regression; the remaining analyses were performed by using unconditional logistic regression additionally adjusting for the matching factors. The high-sun season was defined as June through October [mean monthly 25(OH)D concentration >65 nmol/L], and the low-sun season was defined as November through May [mean monthly 25(OH)D concentration <65 nmol/L]. Residential sun exposure at study entry was defined on the basis of the R-B units for UV radiation (13) for each of the 10 study centers and then classified into the following 3 groups: low sun (≤105 R-B units; Detroit, MI; Minneapolis, MN; and Marshfield, WI), moderate sun (113–134 R-B units; Pittsburgh, PA; St. Louis, MO; Denver, CO; Washington, DC; and Salt Lake City, UT), and high sun (≥154 R-B units; Birmingham, AL, and Honolulu, HI). Season was stratified as high vs. low sun; smoking was stratified as never, former, and current; and residential sun exposure was stratified as low, moderate, and high in these analyses.

We also evaluated the interaction of the DBP and 25(OH)D in joint analyses. Serum DBP was stratified by median value (5358.19 nmol/L), and serum 25(OH)D was categorized by collapsed clinical cutoffs (<50, 50 to <75, and ≥75 nmol/L). The joint analysis was performed by using conditional logistic regression. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute).

RESULTS

Cases and controls did not differ significantly for most baseline characteristics (**Table 1**). Cases were significantly more likely than controls to be current smokers ($P < 0.0001$) and to have diabetes mellitus ($P = 0.03$) and less likely to exercise vigorously ≥4 times/wk ($P = 0.03$). No significant differences in dietary intake of any foods or nutrients were observed, although cases had a slightly lower calcium intake than did controls ($P = 0.08$).

Means and proportions of selected baseline characteristics according to the distribution of serum DBP concentrations among controls are presented in **Table 2**. Male sex, high residential sun exposure at study entry, and history of diabetes mellitus were associated with lower DBP, whereas supplemental calcium intake was positively correlated with higher DBP status. Asian/Pacific Islander and black, non-Hispanic race-ethnicity tended to be correlated with lower DBP concentrations ($P < 0.05$), although there were small numbers of controls in each of these categories.

Higher concentrations of serum DBP were not significantly associated with pancreatic cancer (**Table 3**; highest vs. lowest quintile OR: 1.75; 95% CI: 0.91, 3.37; P-trend = 0.25). The exclusion of cases diagnosed during the first 5 y of follow-up strengthened the association between serum DBP concentrations and risk of pancreatic cancer, with a significant 2-fold higher risk for the highest quintile of serum DBP (highest vs. lowest quintile OR: 2.12; 95% CI: 1.01, 4.43), but the trend was not significant (P-trend = 0.13). A threshold analysis (quintile 1 vs. quintiles 2–5 combined) for serum DBP concentrations using the lowest quintile as the reference group compared with quintiles 2–5 was not significant (adjusted OR: 1.50; 95% CI: 0.89, 2.52). There were no significant interactions by smoking status, sex, or residential sun exposure at study enrollment.

Individuals in the highest (≥100 nmol/L) category of vitamin D status had an elevated risk of pancreatic cancer (**Table 4**). Before adjustment for smoking and diabetes, compared with those with 25(OH)D concentrations of 50.0 to <75.0 nmol/L, the risk was significantly higher for both the lowest and highest 25(OH)D categories [OR: 3.00; 95% CI: 1.13, 7.99 (for <25.0 nmol/L); and OR: 3.10; 95% CI: 1.23, 7.85 (for ≥100 nmol/L)]. In the adjusted model, only the highest category maintained significance (OR: 3.23; 95% CI: 1.24, 8.44), with the lowest category showing a nonsignificant positive association (OR: 2.50; 95% CI: 0.92, 6.81). The test for a quadratic effect was not significant ($P > 0.05$). A sensitivity analysis was performed by using season-specific quintiles, and similar patterns of associations were observed (**Supplemental Table 1**). Compared with the second quintile, both the highest quintile (OR: 2.64; 95% CI: 1.35, 5.17) and the lowest quintile (OR: 2.46; 95% CI: 1.23, 4.90) showed significant elevated risks in the adjusted models. The lag analysis for cases diagnosed after ≥5 y of follow-up showed a significantly higher risk of pancreatic cancer among the lowest (<25.0-nmol/L OR: 3.18; 95% CI: 1.06, 9.54) but not among the highest (≥100-nmol/L OR: 2.57; 95% CI: 0.77, 8.60) category. There was no significant interaction of the association of 25(OH)D and pancreatic cancer by season of blood draw, sex, or residential sun exposure at study entry (P-interaction > 0.05).

Table 5 shows the results for the joint effects of serum 25(OH)D category by DBP. Compared with participants with 25(OH)D concentrations of 50 to <75 nmol/L and DBP below the median (<5358.19 nmol/L), those with 25(OH)D <50 nmol/L and lower DBP had a significantly increased risk of pancreatic cancer (OR: 2.05; 95% CI: 1.06, 3.98), although the interaction was not significant (P-interaction = 0.12). Those with 25(OH)D

TABLE 1
Selected baseline characteristics of case and control subjects[1]

Characteristics	Cases (n = 295)	Controls (n = 587)[2]	P[3]
Age, y	65 (57–71)[4]	65 (57–71)	0.88
Male sex, n (%)	184 (62.4)	365 (62.2)	0.96
Serum DBP, nmol/L	5378.9 (2841.2–8954.5)	5358.2 (2949.8–8828.6)	0.63
Serum 25(OH)D, nmol/L	60.9 (28.2–93.3)	63.2 (35.4–90.7)	0.24
Season of blood draw: sunny season,[5] n (%)	134 (45.4)	268 (45.7)	0.95
Residential sun exposure at study entry,[6] n (%)			0.98
Low sun	138 (46.8)	273 (46.5)	
Moderate sun	132 (44.8)	262 (44.6)	
High sun	25 (8.5)	52 (8.9)	
Race, n (%)			1.00
White, non-Hispanic	266 (90.2)	529 (90.1)	
Black, non-Hispanic	9 (3.1)	18 (3.1)	
Hispanic	4 (1.4)	8 (1.4)	
Asian/Pacific Islander	16 (5.4)	32 (5.5)	
Height, cm			
Male	177.8 (167.6–185.4)	177.8 (170.2–185.4)	0.44
Female	162.6 (152.4–170.2)	162.6 (154.9–170.2)	0.94
BMI, kg/m^2	26.6 (22.4–32.6)	26.6 (21.8–32.5)	0.38
WHO cutoffs, n (%)			0.51
$<$25.0 kg/m^2	98 (33.2)	208 (35.4)	
$\geq$25.0 and $<$30.0 kg/m^2	130 (44.1)	265 (45.1)	
$\geq$30.0 kg/m^2	67 (22.7)	114 (19.4)	
Smoking status, n (%)			$<$0.001
Never	111 (37.6)	292 (49.7)	
Former			
Quit $\geq$15 y	74 (25.1)	170 (29.0)	
Quit $<$15 y	54 (18.3)	83 (14.1)	
Current	56 (19.0)	42 (7.2)	
History of diabetes mellitus, n (%)	38 (12.9)	49 (8.4)	0.03
Family history of pancreatic cancer, n (%)	10 (3.4)	16 (2.7)	0.58
Dietary intake per day[7]			
Red meat, g	74.8 (31.5–138.4)	74.1 (26.7–131.7)	0.46
Alcohol, g	1.5 (0.001–39.6)	1.4 (0.001–33.2)	0.69
Nutrients			
Vitamin D, μg			
Food	5.3 (2.5–8.7)	5.4 (2.8–9.4)	0.53
Supplemental	1.4 (0.00–19.4)	0.00 (0.00–20.0)	0.79
Total	11.9 (3.1–23.2)	10.1 (3.5–24.1)	0.82
Calcium, mg			
Food	929.2 (604.0–1390.9)	966.2 (638.8–1436.1)	0.08
Supplemental	162.0 (0.00–662.0)	100.0 (0.00–800.0)	0.74
Total	1090.1 (673.2–1948.5)	1156.6 (715.8–2060.2)	0.23
Total vitamin A,[8] RE	1587.3 (1042.2–2537.5)	1644.2 (1076.2–2708.9)	0.38
Vigorous physical activity of $\geq$4 h/wk, n (%)[7]	56 (19.0)	151 (25.7)	0.026

[1]DBP, vitamin D–binding protein; RE, retinol equivalents; 25(OH)D, 25-hydroxyvitamin D.

[2]Three controls did not have vitamin D–binding protein data and were thus excluded from the analysis.

[3]P values for categorical and continuous variables were based on chi-square test and Wilcoxon's rank-sum test, respectively.

[4]Median; interdecile range in parentheses (all such values).

[5]Sunny season was defined as June–October; low sun season was defined as November–May.

[6]Residential regions based on ranges of UV radiation levels obtained from annual Robertson-Berger (R-B) meters in states in which screening centers are located: low sun ($\leq$105 R-B meters; Detroit, MI; Minneapolis, MN; and Marshfield, WI), moderate sun (113–134 R-B meters; Pittsburgh, PA; St. Louis, MO; Denver, CO; Washington, DC; and Salt Lake City, UT), and high sun ($\geq$154 R-B meters; Birmingham, AL, and Honolulu, HI) (13).

[7]Based on data for n = 275 cases and n = 554 controls; 20 cases and 33 controls had missing dietary questionnaires, missing data for all of the dietary variables, or missing physical activity data. All foods and nutrients were energy adjusted except for supplements and alcohol.

[8]Total vitamin A includes vitamin A from food and supplements.

TABLE 2
Selected characteristics of 587 control subjects by quintile of serum DBP concentration[1]

Characteristics	Serum DBP quintile					
	1 (<3670.4 nmol/L)	2 (≥3670.4 to <4818.58 nmol/L)	3 (≥4818.58 to <5804.71 nmol/L)	4 (≥5804.71 to <7149.43 nmol/L)	5 (≥7149.43 nmol/L)	P^2
Age, y	64.77 ± 0.44[3]	64.46 ± 0.50	64.43 ± 0.51	64.90 ± 0.44	64.54 ± 0.47	0.94
Male sex, %	69.49	58.26	72.50	60.68	49.57	0.008
Serum DBP, nmol/L	2868.72 ± 54.70	4305.28 ± 33.00	5328.67 ± 27.51	6435.29 ± 35.71	9398.64 ± 181.30	<0.0001
Serum 25(OH)D, nmol/L	60.87 ± 2.31	60.70 ± 1.96	64.56 ± 2.20	66.15 ± 1.79	64.48 ± 1.99	0.23
Season of blood draw: sunny season,[4] %	47.46	41.74	39.17	50.43	49.57	0.38
Residential sun exposure at study entry[5]						<0.0001
Low sun	49.15	49.57	36.67	55.56	41.88	
Moderate sun	30.51	45.22	56.67	39.32	51.28	
High sun	20.34	5.22	6.67	5.13	6.84	
Race, *n* (%)						<0.0001[6]
White, non-Hispanic	71.19	93.04	93.33	96.58	96.58	
Black, non-Hispanic	10.17	2.16	0.00	1.71	0.85	
Hispanic	2.54	0.87	2.50	0.00	0.85	
Asian/Pacific Islander	16.10	3.48	4.17	1.71	1.71	
Height, cm						
Male	177.52 ± 0.92	177.69 ± 0.72	177.48 ± 0.71	179.37 ± 0.67	177.71 ± 0.85	0.39
Female	161.71 ± 1.55	163.46 ± 0.89	161.02 ± 1.00	162.45 ± 0.90	162.22 ± 0.69	0.54
BMI, kg/m^2	26.88 ± 0.42	26.86 ± 0.39	27.31 ± 0.43	26.87 ± 0.36	26.51 ± 0.39	0.73
WHO cutoffs, %						0.85
<25.0 kg/m^2	35.59	32.17	32.50	34.19	42.74	
≥25.0 and <30.0 kg/m^2	44.92	47.83	45.83	46.15	41.03	
≥30.0 kg/m^2	19.49	20.00	21.67	19.66	16.24	
Smoking status, %						0.93
Never	51.69	51.30	49.17	52.99	43.59	
Former						
Quit ≥15 y	27.97	30.43	25.85	26.50	34.19	
Quit <15 y	12.71	11.30	18.33	13.68	14.53	
Current	7.63	6.96	6.67	6.84	7.69	
History of diabetes mellitus, %	11.86	10.43	7.50	6.84	5.13	0.03
Family history of pancreatic cancer, %	4.24	3.48	0.00	2.56	3.42	0.59
Dietary intake per day[7]						
Red meat, g	85.18 ± 7.16	84.77 ± 5.05	73.60 ± 5.28	80.15 ± 4.45	78.00 ± 4.32	0.51
Alcohol, g	12.55 ± 2.62	8.55 ± 1.48	15.06 ± 3.53	8.96 ± 1.48	10.07 ± 2.10	0.26
Nutrients						
Vitamin D, μg						
Food	5.32 ± 0.25	5.72 ± 0.32	5.87 ± 0.30	6.19 ± 0.27	5.73 ± 0.31	0.34
Supplemental	5.87 ± 0.78	7.41 ± 0.92	5.17 ± 0.63	7.00 ± 0.91	7.89 ± 1.00	0.14
Total	11.23 ± 0.85	13.12 ± 0.99	11.08 ± 0.73	13.18 ± 0.91	12.59 ± 1.06	0.15
Calcium, mg						
Food	946.36 ± 32.50	1034.20 ± 38.45	1012.70 ± 38.93	1047.54 ± 34.78	1033.36 ± 37.40	0.31
Supplemental	212.98 ± 31.89	225.85 ± 33.76	214.42 ± 32.77	264.70 ± 33.21	338.37 ± 41.24	0.05
Total	1164.51 ± 46.80	1259.79 ± 52.68	1233.18 ± 52.37	1312.20 ± 48.35	1366.15 ± 54.92	0.06
Total vitamin A,[8] RE	1888.64 ± 86.66	1761.83 ± 63.97	1744.32 ± 60.49	1806.84 ± 75.65	1768.45 ± 58.78	0.61
Vigorous physical activity of ≥4 h/wk, %[7]	27.12	19.13	30.00	29.91	22.22	0.95

[1]DBP, vitamin D–binding protein; RE, retinol equivalents; 25(OH)D, 25-hydroxyvitamin D.

[2]Calculated using generalized linear models for continuous variables and chi-square test for categorical variables, unless otherwise specified

[3]Mean ± SD (all such values).

[4]Sunny season was defined as June–October; low sun season was defined as November–May.

[5]Residential regions based on ranges of UV radiation levels obtained from annual Robertson-Berger (R-B) meters in states in which screening centers are located: low sun (≤105 R-B meters; Detroit, MI; Minneapolis, MN; and Marshfield, WI), moderate sun (113–134 R-B meters; Pittsburgh, PA; St. Louis, MO; Denver, CO; Washington, DC; and Salt Lake City, UT), and high sun (≥154 R-B meters; Birmingham, AL, and Honolulu, HI) (13).

[6]Calculated by using Fisher's exact test due to small cell sizes for non-Hispanic black, Hispanic, and Asian/Pacific Islander study participants.

[7]Based on data for *n* = 554 controls; 33 controls had missing dietary questionnaires, missing data for all the dietary variables, or missing physical activity data. All foods and nutrients were energy adjusted except for supplements and alcohol.

[8]Total vitamin A includes vitamin A from food and supplements.

TABLE 3
Crude and multivariable-adjusted ORs (95% CIs) of baseline serum DBP concentration in association with pancreatic cancer in 295 cases and 587 controls[1]

	Serum DBP quintile					
	1 (<3670.4 nmol/L)	2 (≥3670.4 to <4818.6 nmol/L)	3 (≥4818.6 to <5804.7 nmol/L)	4 (≥5804.7 to <7149.4 nmol/L)	5 (≥7149.4 nmol/L)	*P*-trend
Cases/controls, *n/n*	55/118	58/115	56/120	58/117	68/117	
Crude OR[2] (95% CI)	1.00 (reference)	1.66 (0.89, 3.09)	1.55 (0.85, 2.82)	1.34 (0.73, 2.45)	2.00 (1.07, 3.75)*	0.09
Multivariable-adjusted OR[3] (95% CI)	1.00 (reference)	1.65 (0.88, 3.09)	1.46 (0.79, 2.70)	1.28 (0.69, 2.39)	1.75 (0.91, 3.37)	0.25
Lag analysis for cases diagnosed >5 y after baseline						
Cases/controls (*n* = 214/428), *n/n*	40/93	41/80	36/92	44/80	53/83	
Crude OR[2] (95% CI)	1.00 (reference)	2.00 (0.98, 4.08)	1.49 (0.75, 2.99)	1.64 (0.81, 3.32)	2.39 (1.17, 4.86)*	0.05
Multivariable-adjusted OR[3] (95% CI)	1.00 (reference)	1.98 (0.97, 4.05)	1.44 (0.71, 2.92)	1.58 (0.77, 3.24)	2.12 (1.01, 4.43)*	0.13
DBP stratified by sex[4]						
Men						
Cases/controls (*n* = 184/365), *n/n*	38/82	45/67	31/87	33/71	37/58	
Crude OR[2] (95% CI)	1.00 (reference)	2.23 (1.08, 4.60)*	1.19 (0.59, 2.42)	1.39 (0.66, 2.90)	2.12 (0.96, 4.72)	0.23
Multivariable-adjusted OR[3] (95% CI)	1.00 (reference)	2.28 (1.09, 4.75)*	1.11 (0.54, 2.30)	1.29 (0.61, 2.73)	1.75 (0.76, 4.04)	0.48
Women						
Cases/controls (*n* = 111/222), *n/n*	17/36	13/48	25/33	25/46	31/59	
Crude OR[2] (95% CI)	1.00 (reference)	0.83 (0.22, 3.18)	2.54 (0.74, 8.72)	1.40 (0.44, 4.47)	1.70 (0.54, 5.30)	0.23
Multivariable-adjusted OR[3] (95% CI)	1.00 (reference)	0.73 (0.19, 2.88)	2.32 (0.65, 8.21)	1.42 (0.44, 4.57)	1.49 (0.46, 4.86)	0.29
DBP stratified by smoking status[4]						
Never smokers						
Cases/controls (*n* = 111/292), *n/n*	25/61	19/59	25/59	24/62	18/51	
Crude OR[5] (95% CI)	1.00 (reference)	0.76 (0.38, 1.56)	1.03 (0.53, 2.02)	0.93 (0.47, 1.85)	0.86 (0.41, 1.80)	0.78
Multivariable-adjusted OR[6] (95% CI)	1.00 (reference)	0.83 (0.40, 1.71)	1.10 (0.56, 2.16)	0.98 (0.49, 1.96)	0.94 (0.44, 1.98)	0.93
Former smokers						
Cases/controls (*n* = 128/253), *n/n*	20/48	33/48	22/53	24/47	29/57	
Crude OR[5] (95% CI)	1.00 (reference)	1.72 (0.86, 3.47)	1.06 (0.51, 2.22)	1.27 (0.60, 2.67)	1.24 (0.61, 2.55)	0.98
Multivariable-adjusted OR[6] (95% CI)	1.00 (reference)	1.70 (0.85, 3.44)	1.08 (0.51, 2.26)	1.27 (0.60, 2.67)	1.26 (0.61, 2.58)	0.94
Current smokers						
Cases/controls (*n* = 56/42), *n/n*	10/9	6/8	9/8	10/8	21/9	
Crude OR[5] (95% CI)	1.00 (reference)	0.67 (0.16, 2.87)	1.13 (0.26, 4.81)	1.26 (0.31, 5.21)	2.17 (0.59, 8.05)	0.10
Multivariable-adjusted OR[6] (95% CI)	1.00 (reference)	0.64 (0.15, 2.75)	0.96 (0.22, 4.25)	1.32 (0.32, 5.52)	2.01 (0.54, 7.52)	0.11

[1]A chi-square test of the association between DBP and case status produced a *P* value of 0.85. *Significant. DBP, vitamin D–binding protein.

[2]The crude model was calculated by using conditional logistic regression and conditioned on matched variables (age, race-ethnicity, sex, and date of blood draw based on 2-mo blocks).

[3]The multivariable model was calculated by using conditional logistic regression, conditioned on matched variables (age, race-ethnicity, sex, and date of blood draw based on 2-mo blocks), adjusted for smoking status (never; former, ≥15 y; former, <15 y; current) and self-reported diabetes (yes or no).

[4]*P*-interaction for sex and categorical DBP in the multivariable-adjusted model = 0.47; *P*-interaction for smoking status and categorical DBP in the multivariable-adjusted model = 0.23.

[5]Crude model ORs and 95% CIs were calculated by using unconditional logistic regression and additionally adjusted for matched variables (age, race-ethnicity, sex, and date of blood draw based on 2-mo blocks).

[6]Multivariable model ORs and 95% CIs were calculated by using unconditional logistic regression and additionally adjusted for the matched variables as well as for self-reported diabetes (yes or no).

TABLE 4
Crude and multivariable-adjusted ORs (95% CIs) of baseline serum 25(OH)D concentrations and pancreatic cancer based on clinical cutoffs in 295 cases and 587 controls[1]

	Serum 25(OH)D					
	<25.0 nmol/L	≥25.0 to <37.5 nmol/L	≥37.5 to <50.0 nmol/L	≥50.0 to <75.0 nmol/L	≥75.0 to <100.0 nmol/L	≥100.0 nmol/L
Cases/controls, *n/n*	21/22	32/45	50/99	105/254	67/143	20/24
Crude OR (95% CI)	3.00 (1.13, 7.99)*	1.68 (0.89, 3.19)	1.21 (0.73, 2.01)	1.00 (reference)	1.43 (0.92, 2.24)	3.10 (1.23, 7.85)*
Multivariable-adjusted OR (95% CI)	2.50 (0.92, 6.81)	1.42 (0.74, 2.76)	1.25 (0.74, 2.11)	1.00 (reference)	1.42 (0.90, 2.25)	3.23 (1.24, 8.44)*[2]
Lag analysis for cases diagnosed >5 y after baseline						
Cases/controls (*n* = 199/404), *n/n*	19/16	19/31	36/83	70/176	58/104	12/18
Crude OR (95% CI)	3.75 (1.28, 10.96)*	1.29 (0.59, 2.84)	1.11 (0.62, 1.99)	1.00 (reference)	1.87 (1.11, 3.15)*	2.53 (0.78, 8.20)
Multivariable-adjusted OR (95% CI)	3.18 (1.06, 9.54)*	1.05 (0.47, 2.37)	1.15 (0.64, 2.09)	1.00 (reference)	1.85 (1.09, 3.15)*[2]	2.57 (0.77, 8.60)

[1]A chi-square test of the association between 25(OH)D and case status produced a *P* value of 0.03. The crude model was calculated by using conditional logistic regression and conditioned on matched variables (age, race-ethnicity, sex, and date of blood draw based on 2-mo blocks). The multivariable model was calculated by using conditional logistic regression, conditioned on matched variables (age, race-ethnicity, sex, and date of blood draw based on 2-mo blocks) and adjusted for smoking status (never; former, ≥15 y; former, <15 y; current) and self-reported diabetes (yes or no). *Significant. 25(OH)D, 25-hydroxyvitamin D.
[2]Significant at the Bonferroni-corrected *P* value, 0.05/2 = 0.025.

concentrations ≥75 nmol/L and higher DBP also had a significantly increased risk of pancreatic cancer (OR: 2.08; 95% CI:1.08, 4.00). In the 5-y lag analysis, individuals who were in either the lowest or the highest groups for both 25(OH)D and DBP had a significant 2-fold elevated risk of pancreatic cancer compared with the reference group (*P*-interaction = 0.04). We also examined the association between the serum 25(OH)D:DBP molar ratio, as an estimate of free 25(OH)D, and pancreatic cancer and did not observe an association (highest vs. lowest quintile OR: 0.86; 95% CI: 0.60, 1.23; *P*-trend = 0.34).

The following associations were significant at the Bonferroni-corrected *P* value (0.05/2 < 0.025) for the 2 biomarkers examined: 25(OH)D (Table 4) for overall concentrations ≥100 nmol/L (OR: 3.23; 95% CI: 1.24, 8.44; *P* = 0.017) and for lag analysis concentrations of 75 to <100 nmol/L (OR: 1.85; 95% CI: 1.09, 3.15; *P* = 0.023) and for the lag analysis of the joint effects (Table 5) for DBP concentrations equal to or greater than the median (≥5358.19 nmol/L) and 25(OH)D ≥75 nmol/L (OR: 3.19; 95% CI: 1.44, 7.09; *P* = 0.004).

DISCUSSION

We observed a nonsignificant positive association between prediagnostic DBP and pancreatic cancer that became significant after excluding cases that occurred during the first 5 y of follow-up. This result was not what we hypothesized. We also observed

TABLE 5
ORs (95% CIs) for the joint effect of serum DBP and serum 25(OH)D and risk of pancreatic cancer in 295 cases and 587 controls[1]

	Serum 25(OH)D				
	<50.0 nmol/L	≥50.0 to <75.0 nmol/L	≥75.0 nmol/L	*P*-trend	*P*-interaction[2]
Multivariable-adjusted OR[3] (95% CI) (*n* = 295/587)					0.12
Cases/controls, *n/n*	62/96	45/125	39/72		
DBP less than median	2.05 (1.06, 3.98)*	1.00 (reference)	1.60 (0.82, 3.11)	0.63	
Cases/controls, *n/n*	41/70	60/129	48/95		
DBP of median or greater	1.27 (0.65, 2.47)	1.31 (0.71, 2.39)	2.08 (1.08, 4.00)*	0.11	
Lag analysis for cases diagnosed >5 y after baseline[3] (*n* = 214/428)					0.04
Cases/controls, *n/n*	46/71	26/84	32/64		
DBP less than median	2.28 (1.04, 5.00)*	1.00 (reference)	1.88 (0.86, 4.10)	0.87	
Cases/controls, *n/n*	28/59	44/92	38/58		
DBP of median or greater	1.17 (0.54, 2.55)	1.43 (0.69, 2.96)	3.19 (1.44, 7.09)*[4]	0.02*	

[1]A chi-square test of the association between the joint variable [DBP and 25(OH)D combined status] and case status produced a *P* value of 0.21. Multivariable and lag analysis ORs and 95% CIs were calculated by using conditional logistic regression, conditioned on matched variables (age, race-ethnicity, sex, month of blood draw) and adjusted for smoking status (never; former, ≥15 y; former, <15 y; current) and self-reported diabetes (yes or no). *Significant. DBP, vitamin D–binding protein; 25(OH)D, 25-hydroxyvitamin D.
[2]Based on clinically relevant 25(OH)D cutoffs and median split DBP.
[3]The median DBP is 5358.19 nmol/L.
[4]Significant at the Bonferroni-corrected *P* value, 0.05/2 = 0.025.

a positive association for 25(OH)D and pancreatic cancer, such that compared with those with serum 25(OH)D between 50 and 75 nmol/L, those with serum 25(OH)D concentrations >100 nmol/L had significant elevated risks. Finally, in the joint-effects model overall and in lag analyses, compared with participants with DBP below the median and 25(OH)D between 50 and 75 nmol/L, participants with lower DBP and 25(OH)D <50 nmol/L, as well as those with DBP above the median and 25(OH)D ≥75 nmol/L, had a significantly elevated risk of pancreatic cancer. To the best of our knowledge, this study is the second to examine DBP and pancreatic cancer and the first in a US-based population of both men and women.

Our results contrast with those from one previous epidemiologic study—the ATBC study in male Finnish smokers—that showed a significant inverse association between prediagnostic serum DBP and pancreatic cancer in a threshold pattern for quartiles 2 through 4 (highest vs. lowest quartile OR: 0.66; 95% CI: 0.39, 1.12; *P*-trend = 0.02) (7). In the stratified analysis, the inverse DBP association in the ATBC population was only apparent among men who also had serum 25(OH)D concentrations above the median (highest vs. lowest quartile OR: 0.30; 95% CI: 0.13, 0.68; *P*-trend = 0.001), whereas no DBP association was observed in participants with lower 25(OH)D concentrations (7). We performed a joint-effects analysis of serum DBP and 25(OH)D concentrations and did not observe a protective association for DBP with higher concentrations of 25(OH)D.

We explored possible explanations for the difference between our DBP results and those of the ATBC study, including differences in demographic and behavioral characteristics. The ATBC study included only male smokers, whereas the PLCO study consisted mainly of nonsmokers and included women. Even among men and smokers in the PLCO trial, however, we saw no evidence of a decreased risk of pancreatic cancer with high concentrations of DBP. Although the distributions of DBP appear similar, the primary, nondemographic difference between the 2 study populations is vitamin D status. Control participants in the ATBC study had lower median 25(OH)D concentrations (46.3 nmol/L; IQR: 26.6, 61.6 nmol/L) (4) than did those in the PLCO study (overall: 63.2 nmol/L; IQR: 47.8, 77.7 nmol/L; current smokers only: 49.8 nmol/L; IQR: 30.9, 70.8 nmol/L). Given that only a small minority of our PLCO study controls were current smokers (n = 42), this median value may not be an accurate representation of the true smoker population mean. It is possible that this difference in the serum concentrations of 25(OH)D might explain the conflicting results for the association between DBP, 25(OH)D, and pancreatic cancer. Contrasting results have been reported between the ATBC and PLCO studies for 25(OH)D and colorectal cancer (16, 17) but not for DBP (16–19).

Our PLCO trial 25(OH)D and pancreatic cancer findings are somewhat similar to the results from the Vitamin D Pooling Project pooled analysis of 8 cohorts (952 cases) (3) and a pooled analysis of 5 cohorts (501 cases) (5). Similar to ours, both of these studies used clinically relevant cutoffs of vitamin D status. In the Vitamin D Pooling Project, compared with participants with 25(OH)D concentrations between 50 and 75 nmol/L, those with concentrations >100 nmol/L had a 2-fold elevated risk (OR: 2.12; 95% CI: 1.23, 3.64) (3), whereas those with lower concentrations had a null association. The pooled analysis of 5 cohorts (Health Professionals Follow-Up Study, Nurses' Health Study, Physicians' Health Study, Women's Health Initiative Observational Study, and Women's Health Study) showed a significantly higher risk only for participants with serum 25(OH)D concentrations between 37.5 and <50 nmol/L (OR: 1.41; 95% CI: 1.05, 1.90), whereas those with sufficient or inadequate concentrations did not have either lowered or elevated risks (5). We observed a nonsignificant positive association for participants with 25(OH)D <25 nmol/L that was significant with the exclusion of the cases that occurred during the first 5 y of follow-up. There may be additional factors that explain these associations that we currently do not understand.

The association between DBP, 25(OH)D, and pancreatic cancer is likely complex and may involve additional, yet to be determined mechanisms. Experimental research suggests that the deglycosylated form of DBP, DBP macrophage activating factor (DBP-maf), may inhibit angiogenesis in immune-compromised mice with pancreatic cancer (20). Given that our study did not show a decreased risk of pancreatic cancer at higher concentrations of DBP, the effect in vivo may be primarily through the transport of 25(OH)D and not via anticancer properties of DBP individually. In mice, DBP appears to extend the half-life of 25(OH)D, which may explain the increased risk of pancreatic cancer with high DBP and high 25(OH)D (21). A number of in vitro studies support the hypothesis that high concentrations of vitamin D may prevent pancreatic cancer (22–28), although a study suggests that the vitamin D receptor may interact with mutant p53 and convert $1,25(OH)_2D$ into an antiapoptotic agent (29). Our epidemiologic research along with that of others has not shown convincing support for 25(OH)D playing a strong protective role in pancreatic cancer carcinogenesis.

Our study has several strengths. Most important, our study is prospective, with blood samples and other information collected before cancer diagnosis and therefore is less likely to suffer from reverse causality due to the effects of undiagnosed disease and recall bias. Because the survival time of pancreatic adenocarcinoma is relatively short (30), information collected before diagnosis is also beneficial because there is no need to rely on next of kin for exposure data. The participants in our study were selected from the same cohort population, and controls were incidence-density sampled. Our study is therefore not subject to selection bias of either cases or controls and has internal validity. Our study included 111 more pancreatic cancer cases than the previous PLCO study and includes women, nonsmokers, and individuals living at a wide range of latitudes. Our results may be more generalizable to the US population than previous research with the ATBC study population. The wide latitude range broadens the range of serum 25(OH)D concentrations, increasing the likelihood of detecting an association if one exists.

Our study also has limitations. First, because our study population is predominantly composed of nonsmokers, we lack adequate power to evaluate the associations among current smokers. Second, the PLCO study participants are not representative of the larger US population in several respects. Our study is predominantly white, non-Hispanic (90.14%), which limits our ability to evaluate risk differences by racial-ethnic group. A more racially diverse population may therefore result in a different association between 25(OH)D and risk of pancreatic cancer. In addition, participants who enrolled in the PLCO trial may be different from those who did not, such that they may be more health conscious and proactive. Third, our study population lacks adequate power to assess the interaction by DBP genetic

variants, particularly those identified in genomewide association studies (31, 32). Genetic data might help elucidate the biological mechanism underlying the associations that we observed. Finally, we do not have tissue-specific concentrations of 25(OH)D. Pancreatic ductal adenocarcinomas express the vitamin D receptor (33–35), and tumor tissue may have a different concentration of 25(OH)D and therefore have a different association compared with that observed with peripheral blood concentrations. Animal and in vitro studies might also help clarify the associations that we observed.

In conclusion, we did not observe a significant association between serum DBP concentrations and pancreatic cancer overall. We did observe a positive association for prediagnostic serum 25(OH)D concentrations, such that compared with normal concentrations, those with a higher vitamin D status had an elevated risk. The joint-effects analysis of both DBP and 25(OH)D status showed that, overall, the risk is highest for those in the lowest group for both biomarkers as well as for those in the highest group for both biomarkers. Given the limitations of this study, we believe future research should focus on study populations with greater diversity in race-ethnicity and smoking status. It would also be useful to examine the concentration of vitamin D in tissues, particularly in pancreatic tissue, compared with the serum concentration.

The authors' responsibilities were as follows—MRP: performed the statistical analyses and wrote the manuscript; DMF and KR: reviewed and revised the manuscript; WK and HR: assayed the vitamin D–binding protein samples; RLH: assayed the 25(OH)D samples; and RZS-S: designed and conducted the nested case-control study and supervised the statistical analysis and writing of the manuscript; and all authors: read and approved the final manuscript. RLH is the president and chief executive officer of Heartland Assays Inc. None of the other authors declared a conflict of interest.

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