Published in final edited form as:
Cancer Res. 2012 March 1; 72(5): 1190–1198. doi:10.1158/0008-5472.CAN-11-2950.

Impact of Circulating Vitamin D Binding Protein Levels on the Association Between 25-Hydroxyvitamin D and Pancreatic Cancer Risk: A Nested Case-Control Study

Stephanie J. Weinstein[1], **Rachael Z. Stolzenberg-Solomon**[1], **William Kopp**[2], **Helen Rager**[2], **Jarmo Virtamo**[3], and **Demetrius Albanes**[1]

[1]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland [2]Clinical Support Laboratory, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland [3]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland

Abstract

High concentrations of circulating 25-hydroxyvitamin D [25(OH)D] have been associated with elevated pancreatic cancer risk. As this is contrary to an expected inverse association between vitamin D status and cancer, we examined whether vitamin D binding protein (DBP), the primary carrier of vitamin D compounds in circulation, plays a role in this relationship. Prediagnostic serum DBP and 25(OH)D were studied in relation to risk of pancreatic cancer in a nested case-control study of 234 pancreatic cancer cases and 234 controls in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study of Finnish men. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression, and statistical tests were two-sided. We found that DBP and 25(OH)D were correlated (r=0.27; p<0.0001), and DBP was inversely associated with pancreatic cancer risk (OR=0.66, 95% CI=0.39–1.12, for the highest vs. lowest quartile; p-trend=0.02). Importantly, this association appeared to have a threshold between quartiles 2–4 and quartile 1, and was primarily evident among men with concurrent high 25(OH)D concentrations (OR=0.33, 95% CI=0.16–0.70 for highest vs. lowest quartile; p-trend=0.002), with no association in men with lower serum 25(OH)D (OR=1.28, 95% CI=0.62–2.61 for highest vs. lowest quartile, p-trend 0.63, p-interaction= 0.01). Men with higher 25(OH)D concentrations and serum DBP below the median showed greatly elevated risk of pancreatic cancer (OR=5.01, 95% CI 2.33–10.78, for highest vs. lowest quartile; p-trend < 0.0001), while risk was weakly inversely associated with serum 25(OH)D when DBP concentrations were higher (p-interaction = 0.001). Taken together, our findings indicate that higher DBP concentrations may sequester more 25(OH)D and reduce free 25(OH)D bioavailability. Simultaneous examination of DBP and 25(OH)D may be important in determining the association of vitamin D with cancer risk.

Keywords

Vitamin D Binding Protein; 25-Hydroxyvitamin D; Pancreatic Cancer; serum biomarkers; prospective study

Correspondence to: Demetrius Albanes, M.D., Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., Suite 320, Bethesda, MD 20982-7242 (phone: 301-594-2869; fax: 301-496:6829; daa@nih.gov).

Potential Conflicts of Interest: None.

Malignancies of the exocrine pancreas are the fourth most common cause of cancer death in the U.S., with an estimated 44,000 new cases and 38,000 deaths in 2011 (1). The uniformly poor prognosis for pancreatic cancer further justifies efforts to identify etiologic factors, particularly those that are modifiable. For example, there is great interest in a possible role for vitamin D in several cancers including pancreas; however, we and others have observed higher serum concentrations of 25-hydroxyvitamin D [25(OH)D], the established biomarker of vitamin D status (2), to be associated with elevated risk of pancreatic cancer, first in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (3), and subsequently within a large multicohort pooling project of men and women (4).

Vitamin D binding protein (DBP) is a 58 kD polypeptide that transports vitamin D compounds in circulation (5,6). Also known as group-specific component, or Gcglobulin, DBP binds approximately 88% of the 25(OH)D and 85% of the active hormonal form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. An additional 12% of 25(OH)D and 15% of 1,25$(OH)_2$D are bound to albumin, leaving very little vitamin D in a free state (5–7). Current laboratory assays of vitamin D do not differentiate between free and bound vitamin D, and it is not known whether vitamin D associations with several cancers and other health outcomes are adequately captured based on total circulating 25(OH)D, the majority of which is bound to DBP, or if the free fraction is more biologically relevant and influential in target organs.

We examined whether circulating DBP, the major vitamin D transport protein, is associated with pancreatic cancer risk both overall and relative to 25(OH)D concentration, and whether it modifies the association between higher 25(OH)D status and elevated pancreatic cancer risk.

Participants and Methods

Study population

The overall design, rationale, and objectives of the ATBC Study have been previously published (8). From 1985 to 1988, the ATBC Study recruited 29,133 Finnish male smokers between the ages of 50 and 69 years to participate in a controlled primary prevention trial. Daily supplementation with α-tocopherol (50 mg dl-α-tocopheryl acetate/day), β-carotene (20 mg/day), both, or placebo continued for 5–8 years (median 6.1 years) until death or trial closure (April, 1993)(8). Participants were followed-up passively during the post-intervention period through linkage with the Finnish Cancer Registry, which provides nearly 100% case ascertainment (9). The study was approved by the institutional review boards of the U.S. National Cancer Institute and the National Public Health Institute of Finland, and written informed consent was obtained from each participant.

Case identification and control selection

Subjects for the present study were drawn from two existing ATBC nested case/control sub-studies, both of which included prior measurement of 25(OH)D. All case/control matched pairs with available residual serum were selected for DBP assay. Of the initial 200 cases and 400 controls in a nested set with serum 25(OH)D assayed in 2005 (3), 53 cases and 164 controls did not have residual serum available for the DBP assay, 24 cases and 60 controls were excluded because their matched pair case or control did not have sufficient serum, and 53 controls were randomly dropped to obtain 1:1 matching, leaving 123 matched sets. Of the initial 115 matched sets with serum 25(OH)D assayed in 2008 as part of the Vitamin D Pooling Project of Rare Cancers (4), 3 cases and 1 control did not have available residual serum, and 1 case and 3 controls were excluded because they no longer had a matched pair, leaving 111 matched sets. All cases (n=234) were identified through the Finnish Cancer Registry, and defined as incident exocrine pancreatic tumors [International Classification of

Diseases (ICD) 9, code 157, excluding islet cell carcinomas (ICD9 code 157.4)] diagnosed through April 2004. Controls were alive and cancer free at the time of case diagnosis and matched to cases (1:1) on age at randomization (+/− 5 years (3) or +/− 1 year (4)) and date of baseline serum collection (+/− 30 days). One case and one control were missing data on 25OHD.

Serum 25(OH)D and vitamin D binding protein assays

Blood samples were drawn from the antecubital vein by specially trained, registered nurses during a clinic visit prior to trial randomization, between 1985 and 1988. Serum was separated after 30–60 minutes by centrifuging for 10 minutes, and thereafter stored at −70°C in 1.5 ml glass vials. Serum samples were transported from Finland to US on dry ice in February–March 1995. All samples were aliquotted from the larger parent tubes prior to assay and were therefore thawed and refrozen at least once.

25(OH)D was previously measured in matched case/control sets using a radioimmunoassay (RIA, DiaSorin, Inc, Stillwater, MN) at Mount Sinai Hospital, Toronto, Canada (3) or a direct, competitive chemiluminescence immunoassay (CLIA, DiaSorin Liaison 25(OH)D TOTAL assay) at Heartland Assays, Inc., Ames, Iowa (4). Matched case/control sets were assayed consecutively within the same batch along with 10% (RIA) or 5% (CLIA) blinded quality control samples. Samples from the same quality control pool were included in both sets of assays, with a median of 47.9 nmol/L (n=30 samples) using RIA and 53.0 nmol/L (n=154) using CLIA. In addition, 17 controls were included in both sub-studies and all but one was higher using the RIA compared with the CLIA, with a mean difference of 13.1 nmol/L between the two measurements. Coefficients of variation were 16.5% overall for the RIA (5.5% interbatch and 15.5% intrabatch) and 12.3% overall for the CLIA (7.1% interbatch and 10.1% intrabatch). Intraclass correlations were 86.7% and 96.0% for the RIA and CLIA, respectively.

Vitamin D binding protein was measured in fasting baseline serum samples using the Quantikine Human Vitamin D Binding Protein Immunoassay kit (Catalog number DVDBP0, R&D Systems, Inc, Minneapolis, MN) at the SAIC NCI-Frederick research facility, Frederick, MD. Each batch contained matched case/control sets and blinded quality control material comprising approximately 10% of the total samples. The overall, interbatch and intrabatch coefficients of variation, calculated using a nested components of variance analysis (10), were 18.7%, 10.8% and 15.2%, respectively and the intraclass correlation was 82.6%.

Statistical analysis

Baseline descriptive data, by quartile of vitamin D binding protein, were calculated as medians (continuous variables) or proportions (categorical variables) among controls. Odds ratios (OR) and 95% confidence intervals (CI) were determined using conditional logistic regression models. Because the unconditional logistic regression results were similar, this approach was used for stratified modeling in order to retain subjects who were not in the same stratum as their matched case or control. Both 25(OH)D and DBP were categorized as quartiles based on the distribution among the controls and entered into the models as indicator variables. Quartiles rather than quintiles were used for greater stability in estimation of the odds ratios, especially for stratified models, but findings for quintiles were similar. Because of the slight differences in the 25(OH)D data from the two laboratories, and because 25(OH)D levels are known to fluctuate throughout the year, the 25(OH)D quartiles are calculated separately for each laboratory by sunnier and less sunny season categories (May–October and November–April) and then merged into one quartile variable. Results were essentially unchanged when 25(OH)D was instead categorized using clinically defined

cutpoints or adjusted for season using the residual method (11). Exclusion of participants reporting a history of diabetes did not materially change the risk estimates, therefore, these subjects were retained.

A molar ratio of 25(OH)D:DBP was created as a proxy for free circulating 25(OH)D (12,13). In addition, we estimated "free 25(OH)D" based on total 25(OH)D and DBP using the affinity constants of albumin and DBP (12,14) both by ignoring albumin (based on its relatively smaller vitamin D transport function) and by assigning a fixed albumin value to every subject (because of limited biospecimen availability, we did not measure albumin). As described previously (12), estimating "free 25(OH)D" without albumin provides a valid approximation that is comparable to the 25(OH)D:DBP molar ratio. We also adjusted 25(OH)D for DBP through the residual method used to adjust for season (11). Tests for linear trend were obtained by assigning to each category an ordinal value (1–4) and treating this parameter as a continuous variable. DBP was also modeled as a continuous variable of 1000 nmol/L increments.

Variables assessed for confounding included those identified in previous publications (3,4) and those associated with DBP as identified in Table 1. These included body mass index, number of cigarettes smoked per day, years of smoking, smoking cessation during the trial period (reporting to have quit smoking > 3 consecutive visits (i.e., >1 year)), history of diabetes, occupational physical activity, education; serum α-tocopherol, retinol, and cholesterol; and intake of fat, vitamin D, calcium, and ethanol. Both forward and backward stepwise regression procedures were used to determine whether the addition or removal of a potential confounder produced >10% change in the 25(OH)D or DBP coefficients. As none of the identified factors met the definition of a confounder, we present a crude model (Model 1) conditioned on the matching factors, and a multivariate-adjusted model (Model 2), which includes known pancreatic cancer risk factors and factors strongly associated with DBP in this analysis (i.e., body mass index, number of cigarettes smoked/day, number of years of smoking, history of diabetes, occupational physical activity, education, ethanol intake, and serum retinol, cholesterol, β-carotene and α-tocopherol). A third model (Model 3) builds on Model 2 and includes mutual adjustment for 25(OH)D or DBP. Additional testing of several medical history variables (history of renal failure, pancreatitis, liver cirrhosis, lung emphysema, chronic bronchitis, stroke, cerebral hemorrhage, cerebral infarction, valvular heart disease, myocardial infarction, coronary heart disease, angina pectoris, or heart failure) did not alter the DBP results.

To evaluate effect modification, we stratified DBP by 25(OH)D, and 25(OH)D and the ratio of 25(OH)D:DBP by DBP. We also examined DBP stratified by age, BMI, number of cigarettes/day, occupational physical activity, season of blood collection, intakes of fat (total, monounsaturated, saturated, and trans fat), folate, vitamin D, calcium, and ethanol, and follow-up time. Effect modification was tested by including the cross-product term of the biomarker quartiles and the effect modifier (split at the median or yes/no), and by stratified analyses conducted using unconditional logistic regression, adjusted for the matching factors only. Statistical analyses were performed using SAS software version 9.1.3 (SAS Institute, Inc., Cary, North Carolina) and all *P*-value were 2-sided.

Results

Serum DBP concentrations in controls ranged from 2973–8544 nmol/L (for 10^{th}–90^{th} percentiles) and did not vary by season of blood draw. Baseline characteristics of controls by quartile of DBP are presented in Table 1. Median DBP values for quartiles 2, 3, and 4 were 50%, 85%, and 161% higher, respectively, compared with those in the first quartile. Men with higher DBP had higher circulating 25(OH)D, α-tocopherol, cholesterol, retinol, and β-

carotene, and lower 25(OH)D:DBP molar ratio and ethanol intake. In addition, men with higher DBP had lower levels of education, were less likely to have a history of diabetes or used vitamin D supplements, and were more likely to have had their blood sampled in the sunnier months. The prevalence of diabetes and vitamin D supplement use in this population was low; therefore, the patterns observed are based on small numbers of subjects and may or may not indicate important biological relationships.

Spearman correlation coefficients (and related p-values) for DBP and selected factors were 0.08 (0.22) for age, −0.08 (0.20) for BMI, −0.08 (0.22) for cigarettes/day, 0.10 (0.13) for years of smoking, 0.14 (0.03) for serum α-tocopherol, 0.11 (0.09) for serum cholesterol, 0.18 (0.005) for serum retinol, 0.06 (0.37) for serum β-carotene, - 0.20 (0.003) for alcohol intake, 0.27 (< 0.0001) for total 25(OH)D, and −0.42 (< 0.0001) for the 25(OH)D:DBP molar ratio. The correlations between total 25(OH)D and both the 25(OH)D:DBP molar ratio and the calculated “free 25(OH)D” were 0.72 (p<0.0001).

Serum DBP concentration was inversely associated with risk of pancreatic cancer (Table 2, Model 1, p-trend = 0.02), with an apparent threshold effect such that risk in quartiles 2–4 were lower than for quartile 1 (OR=0.57, 95% CI = 0.38–0.85 for quartiles 2–4 combined versus quartile 1). Multivariate-adjustment for pancreatic cancer risk factors and factors associated with DBP (Model 2), or further adjustment for 25(OH)D (Model 3) did not alter the risk estimates as compared with the base model, indicating little confounding by these factors. The odds ratio for a 1000 nmol/L increment in DBP was 0.92 (95% CI 0.84–1.00, p=0.04).

Serum 25(OH)D was positively associated with risk of pancreatic cancer (Table 2), although none of the patterns appear monotonic and statistical significance was achieved for the highest quartile only. When the data were examined as quintiles as was done in the earlier study of vitamin D (3), the odds ratio for the highest vs. lowest quintile was 2.01 (95% CI 1.08–3.72, p-trend 0.03). Risk estimates were slightly stronger with multivariate-adjustment or further adjustment for circulating DBP. The association between 25(OH)D and pancreatic cancer was attenuated when 25(OH)D was residually-adjusted for DBP (OR=1.51, 95% CI 0.82–2.78 for highest vs. lowest quartile).

The risk of pancreatic cancer was significantly elevated in the highest quartile of the 25(OH)D:DBP molar ratio [a proxy for free 25(OH)D], with risks similar to those for 25(OH)D alone (Table 2, Model 1), although again, the patterns do not appear monotonic. Multivariate-adjustment did not alter the risks. Using the estimation of “free 25(OH)D” without albumin resulted in the exact same risk estimates as those for the 25(OH)D:DBP molar ratio. Including a constant to represent albumin in the estimation of “free 25(OH)D” yielded similar patterns.

We found strong and statistically significant interactions between circulating DBP and 25(OH)D in relation to pancreatic cancer risk (Table 3). Higher DBP was associated with a significantly reduced risk of pancreatic cancer only among men with 25(OH)D concentrations above the median (OR=0.33, 95% CI 0.16–0.70, for highest vs. lowest quartile; p-trend = 0.002), with no association in men with lower serum 25(OH)D (p-interaction = 0.01). Similarly, men in the highest quartile of 25(OH)D concentrations who also had DBP levels below the median were at substantially elevated pancreatic cancer risk (OR=5.01, 95% CI 2.33–10.78, for highest vs. lowest quartile; p-trend < 0.0001), and risk was inversely associated with serum 25(OH)D when DBP concentrations were above the median (p-interaction = 0.001). These patterns persisted when lab-specific 25(OH)D data were examined separately. Finally, the pattern of interaction for the 25(OH)D:DBP molar ratio mirrored that of 25(OH)D, with elevated risks when DBP was low (OR=3.40, 95% CI

1.53–7.54 for the highest vs. lowest quartile), and reduced risks when DBP was high (OR=0.31, 95% CI 0.11–0.86 for the highest vs lowest quartile)(p-interaction 0.002).

The inverse association between DBP and pancreatic cancer was stronger among older men, those with lower intakes of fat (all types examined), vitamin D, calcium, and ethanol, those who smoked less, and for cases with at least 10 years between blood collection at study entry and diagnosis (data not shown). None of the interaction tests for these factors were significant, however.

Discussion

To our knowledge, this is the first investigation of the role of circulating vitamin D binding protein (DBP) and risk of pancreatic cancer. We found that serum DBP was inversely associated with pancreatic cancer risk, particularly among men with higher serum 25(OH)D concentrations. Serum 25(OH)D concentration was positively associated with risk overall, as previously reported (3), but we observed greatly elevated risk (i.e., OR>5) for men in the highest 25(OH)D quartile whose DBP levels were below the median, possibly representing a stronger impact of higher vitamin D status relative to the plasma binding capacity of DBP. Among men with higher DBP concentrations, 25(OH)D appeared inversely associated with pancreatic cancer risk, although this was not statistically significant. The 25(OH)D:DBP molar ratio, which is a proxy for free 25(OH)D(12,13), displayed risk patterns similar to those of 25(OH)D. There was no evidence of confounding by any of the factors we examined, and inclusion of both serum DBP and 25(OH)D in the regression models did not attenuate the risk associations for either factor.

Two previous studies of prostate cancer risk reported no association with serum DBP concentrations (15) or the 25(OH)D:DBP molar ratio (what the authors referred to as the “free 25-D index”) (16), although an interaction between the vitamin D receptor polymorphism *BsmI* and the free 25-D index was observed in the latter study (17).

Previously within the ATBC Study cohort, we observed a positive association between serum 25(OH)D and pancreatic cancer risk (OR= 2.92, 95% CI 1.56–5.48, for the highest vs. lowest quintile; p-trend 0.0001) (3). In the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), circulating 25(OH)D was not associated with pancreatic cancer risk overall, but elevated risks for higher serum levels were noted among those whose blood was collected in the fall or winter (OR = 3.91, 95% CI 1.19–12.85, for highest vs. lowest quintile; p-trend 0.10), and those residing in areas with low residential sun exposure (OR = 4.03, 95% CI 1.38–11.79; p-trend = 0.02) (18). Within the Vitamin D Pooling Project of Rare Cancers, which included eight cohorts of men and women and 952 pancreatic cancer cases, higher concentrations of circulating 25(OH)D were associated with elevated risk (OR = 2.12, 95% CI 1.23–3.64 for ≥ 100 nmol/L vs. 50-<75 nmol/L), and elevated risk persisted when the previously reported data from ATBC and PLCO were excluded (4).

DBP binds and transports vitamin D compounds in circulation, including, importantly, 25(OH)D and $1,25(OH)_2D$, as well as other molecules (2,6,19). The plasma concentration of DBP is orders of magnitude greater than that of 25(OH)D, its half-life is approximately 2 ½ to 3 days, and its affinity for 25(OH)D is substantially greater than that for $1,25(OH)_2D$ (6,19–21). As a consequence, only 0.4% of $1,25(OH)_2D$ and 0.04% of 25(OH)D is in a free state (6,21), and less than 5% of circulating DBP is associated with vitamin D compounds (19,20). The large excess of DBP compared to vitamin D provides a reservoir for 25(OH)D, prolongs its half-life and protects against short-term vitamin D deficiency; it may also relate to other functions of DBP that are described below (5,20). Circulating DBP concentrations show some diurnal variation with a morning nadir and rapid daytime increase in levels, but

in contrast to vitamin D levels, they do not vary by season (13,22). Fatty acids also bind to DBP, thereby reducing its capacity for vitamin D compounds (5,6); of note in this regard, the inverse pancreatic cancer association for DBP was marginally stronger among those with lower fat intake in our study.

In addition to its role in vitamin D transport, DBP functions as an actin scavenger, as a precursor to a macrophage activating factor, and as a cochemotactic factor (5,6,23,24). Actin is released into circulation following cellular injury or death, and can result in platelet aggregation, vascular obstruction, and organ dysfunction. By binding actin, DBP attenuates these effects (5,6), which may be one reason for very high DBP concentrations compared with vitamin D (5). The DBP-macrophage activating factor, a selectively deglycosylated form of DBP, has anti-angiogenic, anti-tumorigenic, immunemodulatory, and pro-apoptotic properties (5,25,26), and has been shown to inhibit pancreatic (25) and prostate tumor growth (26). DBP also plays a role in inflammation, acting as a co-chemotactic factor by attracting neutrophils to sites of inflammation through its influence on complement-derived peptides (C5a) (5,6,24). Although these other properties of DBP may impact cancer, the strong interaction with 25(OH)D concentrations supports the notion that its role in binding 25(OH)D is important for pancreatic cancer risk.

Although direct measurement of unbound 25(OH)D in circulation is currently limited to small laboratory investigations and has not been undertaken in larger-scale epidemiologic studies, our finding of a much stronger vitamin D-pancreatic cancer risk association among men with lower DBP levels as well as strengthening of the inverse association for DBP in the setting of higher 25(OH)D concentrations may be indicative of higher concentrations of free 25(OH)D available to cells to initiate a range of transcriptional events through conversion to $1,25(OH)_2D$ and binding the nuclear vitamin D receptor in target organs. This would be consistent with the “free hormone theory” that only unbound hormones are able to enter target cells and influence transcriptional and downstream biological events (5,6). Two recent studies support this concept with respect to vitamin D, with one showing calculated “free 25(OH)D” to be more highly correlated with bone mineral density (r=0.41) than was total 25(OH)D alone (r=0.17)(27), and the other finding no difference in plasma total 25(OH)D or $1,25(OH)_2D$ but significantly lower “free” concentrations of these metabolites in men with osteoporosis, compared with controls (12). Although we did not measure serum albumin and therefore could not calculate the concentration of “free 25(OH)D” in the same manner, because ~88% of 25(OH)D is carried on DBP and only ~12% on albumin, our stratification for DBP levels and examination of the 25(OH)D:DBP molar ratio represent reasonable proxies for free versus bound vitamin D in circulation (12).

Cellular uptake of the DBP-bound 25(OH)D is also mediated by the plasma membrane receptor megalin, known also as low-density lipoprotein receptor protein 2, or LRP2 (28). This is particularly important in the renal proximal tubules for clearance of DBP-25(OH)D from the glomerular filtrate and conversion of 25(OH)D to $1,25(OH)_2D$; e.g., megalin knockout mice are unable to resorb the 25(OH)D-DBP complex from urine, resulting in excretion of vitamin D, drastically reduced circulating 25(OH)D and $1,25(OH)_2D$ levels, and rachitic bone disease (23,28). Megalin-mediated, endocytic internalization of vitamin D has also been demonstrated in other epithelial tissues including the small intestine, breast, uterus, epididymis, and brain (23,24,28), but as yet not in the pancreas. It is therefore possible that higher circulating DBP levels promote greater renal tubule DBP-25(OH)D resorption and higher vitamin D status, which, if vitamin D were inversely associated with pancreatic cancer as experimental data support, could explain both the inverse DBP-pancreatic cancer association and the greatly elevated risk for high 25(OH)D in the low DBP stratum we observed. The fact that mutually adjusting for 25(OH)D and DBP does not alter the associations argues against this, however. Establishing whether megalin is expressed in

pancreatic tissue may help elucidate any role cellular absorption of DBP-bound 25(OH)D might play in the interrelations among DBP, vitamin D status, and pancreatic cancer risk.

The present finding of an inverse association between 25(OH)D and pancreatic cancer, which was evident only when DBP concentrations were high, is not easily explained in light of consistent findings of elevated risk for 25(OH)D in several studies. Since 25(OH)D is preferentially bound to DBP (14), in a setting of high DBP, the high 25(OH)D could potentially displace $1,25(OH)_2D$, which has been shown experimentally to have several anti-tumorigenic properties (29,30).

Our study has several strengths and limitations. The prospective evaluation of 25(OH)D and DBP and the long follow-up greatly reduce any effect of pancreatic cancer on these biochemical measures and the possibility of reverse causality. Although the ATBC Study participants were male smokers, our pancreatic cancer-25(OH)D findings have been replicated in other populations that included women and were predominantly nonsmokers (4). It is unclear whether the present findings for DBP are generalizable to women or to non-smoking populations. DBP concentrations were not correlated with our smoking measures, and smoking did not confound any of our observed associations, suggesting that residual confounding from smoking is unlikely to explain our findings. In addition, limited data on participants who reported cessation of smoking during follow-up does not indicate that this behavior change influenced the findings. We found no evidence of confounding by any of the factors examined, including those associated with DBP concentrations; however, we cannot completely rule out residual confounding by smoking, smoking behavior change, or factors associated with circulating DBP. Circulating 25(OH)D is considered the accepted biomarker of vitamin D status that integrates exposure from diet, supplements, and sunlight (31). The vitamin D status within the ATBC Study population was relatively low as a result of the high latitude of Finland, low use of vitamin D supplements in the study population, and few blood collections during the summer months when vitamin D levels tend to peak. Within the Vitamin D Pooling Project of Rare Cancers, however, risk of pancreatic cancer was elevated at 25(OH)D concentrations greater than those in ATBC (≥100 nmol/L)(4). The DBP concentrations we measured were within the range of those reported by others (5,6,19,23). We measured 25(OH)D and DBP at only one point in time, but DBP concentrations appear to be stable during an individual's lifetime (32), and evidence indicates that 25(OH)D in samples collected up to 14 years apart are well-correlated (33–35). The relatively high coefficients of variation for the 25(OH)D and DBP assays indicate variation in the laboratory assays and may have reduced our ability to observe stronger associations. All serum samples were collected in the morning after an overnight fast, thereby reducing the potential impact of diurnal and postprandial variation.

In conclusion, the simultaneous examination of the vitamin D carrier protein, DBP, and 25(OH)D supports previous findings of an adverse relationship between vitamin D and pancreatic cancer. Specifically, circulating levels of the DBP were inversely associated with pancreatic cancer risk, particularly when the concentration of 25(OH)D - which itself was directly associated with risk - was high. This may indicate that higher levels of DBP lead to more bound 25(OH)D and less 25(OH)D freely available to impact target tissues. Studies of vitamin D-related genes, including GC, the gene encoding DBP, and risk of pancreatic and other cancers could provide further evidence to test this hypothesis, and DBP should be examined in other investigations of vitamin D, including particularly women and non-smokers. Simultaneous examination of DBP and 25(OH)D appears warranted in future risk association studies of vitamin D.

Acknowledgments

Financial support: This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health. Additionally, this research was supported by U.S. Public Health Service contracts (N01-CN-45165, N01-RC-45035, N01-RC-37004, HHSN261201000006C, and HHSN261200800001E) from the National Cancer Institute, National Institutes of Health. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Reference List

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011; 61:212–36. [PubMed: 21685461]
2. IOM (Institute of Medicine). Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.
3. Stolzenberg-Solomon RZ, Vieth R, Azad A, Pietinen P, Taylor PR, Virtamo J, et al. A prospective nested case-control study of vitamin D status and pancreatic cancer risk in male smokers. Cancer Res. 2006; 66:10213–9. [PubMed: 17047087]
4. Stolzenberg-Solomon RZ, Jacobs EJ, Arslan AA, Qi D, Patel AV, Helzlsouer KJ, et al. Circulating 25-Hydroxyvitamin D and Risk of Pancreatic Cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol. 2010; 172:81–93. [PubMed: 20562185]
5. Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta. 2006; 372:33–42. [PubMed: 16697362]
6. Cooke, NE.; Haddad, JG. Vitamin D Binding Protein. In: Feldman, D.; Glorieux, FH.; Pike, JW., editors. Vitamin D. San Diego, CA: Academic Press; 1997. p. 87-101.
7. Bikle DD, Gee E. Free, and not total, 1,25-dihydroxyvitamin D regulates 25-hydroxyvitamin D metabolism by keratinocytes. Endocrinology. 1989; 124:649–54. [PubMed: 2463902]
8. The ATBC Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. Ann Epidemiol. 1994; 4:1–10. [PubMed: 8205268]
9. Korhonen P, Malila N, Pukkala E, Teppo L, Albanes D, Virtamo J. The Finnish Cancer Registry as follow-up source of a large trial cohort--accuracy and delay. Acta Oncol. 2002; 41:381–8. [PubMed: 12234031]
10. Fears TR, Ziegler RG, Donaldson JL, Falk RT, Hoover RN, Stanczyk FZ, et al. Reproducibility studies and interlaboratory concordance for androgen assays in female plasma. Cancer Epidemiol Biomarkers Prev. 2000; 9:403–12. [PubMed: 10794485]
11. Gallicchio L, Helzlsouer KJ, Chow WH, Freedman DM, Hankinson SE, Hartge P, et al. Circulating 25-Hydroxyvitamin D and the Risk of Rarer Cancers: Design and Methods of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Am J Epidemiol. 2010; 172:10–20. [PubMed: 20562188]
12. Al-oanzi ZH, Tuck SP, Raj N, Harrop JS, Summers GD, Cook DB, et al. Assessment of vitamin D status in male osteoporosis. Clin Chem. 2006; 52:248–54. [PubMed: 16339300]
13. Bouillon R, Van Assche FA, Van Baelen H, Heyns W, De Moor P. Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration. J Clin Invest. 1981; 67:589–96. [PubMed: 6894152]
14. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab. 1986; 63:954–9. [PubMed: 3745408]
15. Corder EH, Friedman GD, Vogelman JH, Orentreich N. Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. Cancer Epidemiol Biomarkers Prev. 1995; 4:655–9. [PubMed: 8547833]

16. Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, Stampfer MJ. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev. 1996; 5:121–6. [PubMed: 8850273]
17. Ma J, Stampfer MJ, Gann PH, Hough HL, Giovannucci E, Kelsey KT, et al. Vitamin D receptor polymorphisms, circulating vitamin D metabolites, and risk of prostate cancer in United States physicians. Cancer Epidemiol Biomarkers Prev. 1998; 7:385–90. [PubMed: 9610787]
18. Stolzenberg-Solomon RZ, Hayes RB, Horst RL, Anderson KE, Hollis BW, Silverman DT. Serum vitamin D and risk of pancreatic cancer in the prostate, lung, colorectal, and ovarian screening trial. Cancer Res. 2009; 69:1439–47. [PubMed: 19208842]
19. Gomme PT, Bertolini J. Therapeutic potential of vitamin D-binding protein. Trends Biotechnol. 2004; 22:340–5. [PubMed: 15245906]
20. White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab. 2000; 11:320–7. [PubMed: 10996527]
21. Meier U, Gressner O, Lammert F, Gressner AM. Gc-globulin: roles in response to injury. Clin Chem. 2006; 52:1247–53. [PubMed: 16709624]
22. Rejnmark L, Lauridsen AL, Vestergaard P, Heickendorff L, Andreasen F, Mosekilde L. Diurnal rhythm of plasma 1,25-dihydroxyvitamin D and vitamin D-binding protein in postmenopausal women: relationship to plasma parathyroid hormone and calcium and phosphate metabolism. Eur J Endocrinol. 2002; 146:635–42. [PubMed: 11980618]
23. Chun RF, Adams JS, Hewison M. Back to the future: a new look at ‘old’ vitamin D. J Endocrinol. 2008; 198:261–9. [PubMed: 18495944]
24. Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. Thorax. 2010; 65:456–62. [PubMed: 20435872]
25. Kisker O, Onizuka S, Becker CM, Fannon M, Flynn E, D’Amato R, et al. Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. Neoplasia. 2003; 5:32–40. [PubMed: 12659668]
26. Gregory KJ, Zhao B, Bielenberg DR, Dridi S, Wu J, Jiang W, et al. Vitamin D binding protein-macrophage activating factor directly inhibits proliferation, migration, and uPAR expression of prostate cancer cells. PLoS One. 2010; 5:e13428. [PubMed: 20976141]
27. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, et al. Vitamin D binding protein modifies the vitamin D-bone mineral density relationship. J Bone Miner Res. 2011; 26:1609–16. [PubMed: 21416506]
28. Willnow TE, Nykjaer A. Cellular uptake of steroid carrier proteins--mechanisms and implications. Mol Cell Endocrinol. 2010; 316:93–102. [PubMed: 19646505]
29. Bouillon R, Eelen G, Verlinden L, Mathieu C, Carmeliet G, Verstuyf A. Vitamin D and cancer. J Steroid Biochem Mol Biol. 2006; 102:156–62. [PubMed: 17113979]
30. Fleet JC. Molecular actions of vitamin D contributing to cancer prevention. Mol Aspects Med. 2008; 29:388–96. [PubMed: 18755215]
31. IARC. Vitamin D and Cancer. Lyon, France: International Agency for Research on Cancer; 2009.
32. Haddad JG. Plasma vitamin D-binding protein (Gc-globulin): multiple tasks. J Steroid Biochem Mol Biol. 1995; 53:579–82. [PubMed: 7626513]
33. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control. 2004; 15:255–65. [PubMed: 15090720]
34. Hofmann JN, Yu K, Horst RL, Hayes RB, Purdue MP. Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Cancer Epidemiol Biomarkers Prev. 2010; 19:927–31. [PubMed: 20332255]
35. Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. Am J Epidemiol. 2010; 171:903–8. [PubMed: 20219763]

Table 1

Selected baseline characteristics across vitamin D binding protein quartiles in controls, ATBC Study, 1985–2004[a]

	Quartile of serum vitamin D binding protein[b]			
Characteristic	**Q1**	**Q2**	**Q3**	**Q4**
Age, years	58	58	58	59
Height, cm	175	172	172	173
Weight, kg	81.8	76.8	77.7	77.7
Body mass index, kg/m^2	26.4	26.3	26.0	25.6
Education, % > elementary	28.8	22.4	25.4	6.9
Cigarettes/day	20	20	20	20
History of diabetes, %	11.9	1.7	5.1	5.2
Occupational physical activity (% heavy)	6.8	5.2	13.6	8.6
Dietary vitamin D, ug/day	4.7	4.1	5.0	4.6
Vitamin D supplement use, %	10.2	6.9	5.1	1.7
Total vitamin D (diet and supplements, ug/day)	6.0	4.1	5.4	4.7
Calcium intake (mg/day)	1411	1311	1396	1343
Total fat intake, g/day	118	112	115	113
Ethanol intake, g/day	11.4	13.3	6.1	6.1
Season of blood draw, % May–October	20.3	27.6	37.3	36.2
Serum biomarkers				
DBP, nmol/L	3163	4745	5851	8243
25(OH)D, nmol/L	31.5	39.2	45.1	49.9
25(OH)D:DBP molar ratio (x10^3)[c]	10.45	8.29	7.27	6.16
α-tocopherol, mg/L	10.9	11.2	10.8	11.7
β-carotene, ug/L	164	159	159	181
Total cholesterol, mmol/L	6.1	6.2	5.9	6.4
HDL cholesterol, mmol/L	1.08	1.09	1.13	1.09
Retinol, ug/L	514	548	613	568

[a] values are medians or proportions

[b] Quartile cuts for DBP were: Q1: ≤ 4026, Q2: >4026 and ≤ 5329, Q3: > 5329 and ≤ 6721, Q4: >6721 nmol/L

[c] a proxy for free 25(OH)D

Table 2

Odds ratios and 95% confidence intervals for the association between vitamin D binding protein, 25(OH)D, 25(OH)D:DBP molar ratio, and risk of pancreatic cancer, ATBC Study, 1985–2004[a]

	Quartile 1 OR (95% CI)		Quartile 2 OR (95% CI)		Quartile 3 OR (95% CI)		Quartile 4 OR (95% CI)		p-trend
DBP									
Range, nmol/L	≤ 4026		> 4026 & ≤ 5329		> 5329 & ≤ 6721		> 6721		
N[b]	87/59		58/58		31/59		58/58		
Model 1[c]	1.00	reference	0.69	0.42–1.12	0.36	0.21–0.63	0.66	0.39–1.12	0.02
Model 2[d]	1.00	reference	0.74	0.44–1.24	0.35	0.19–0.62	0.70	0.40–1.22	0.03
Model 3[e]	1.00	reference	0.70	0.42–1.19	0.35	0.20–0.64	0.67	0.37–1.19	0.02
25(OH)D									
Range[f]									
N[b]	48/60		61/58		50/59		74/56		
Model 1[c]	1.00	reference	1.33	0.79–2.24	1.14	0.65–1.98	1.78	1.02–3.11	0.08
Model 2[d]	1.00	reference	1.51	0.87–2.62	1.21	0.68–2.17	1.91	1.05–3.46	0.07
Model 3[e]	1.00	reference	1.42	0.80–2.49	1.28	0.71–2.33	1.81	0.97–3.37	0.10
25(OH)D:DBP molar ratio (x10^3)[g]									
Range	≤4.91		> 4.91 & ≤ 8.07		> 8.07 & ≤ 11.03		> 11.03		
N[b]	55/59		52/58		38/58		88/58		
Model 1[c]	1.00	reference	1.09	0.59–2.03	0.81	0.43–1.53	1.89	1.01–3.54	0.04
Model 2[d]	1.00	reference	1.00	0.52–1.92	0.77	0.39–1.50	1.86	0.97–3.56	0.04

[a] All models used conditional logistic regression.

[b] N for cases/controls

[c] Model 1 is conditioned on the matching factors of age and date of blood collection.

[d] Model 2 is conditioned on the matching factors of age and date of blood collection, and adjusted for body mass index (continuous), number of cigarettes smoked/day (continuous), years of smoking (continuous), history of diabetes (yes/no), occupational physical activity (heavy vs. no heavy activity), education (> elementary vs. elementary), ethanol intake (quartiles, with separate categories for non-drinkers and missing), and serum retinol (continuous), cholesterol (continuous), β-carotene (continuous) and α-tocopherol (continuous)

[e] Model 3 is the same as Model 2, with additional adjustment for either 25(OH)D or DBP, respectively (quartiles).

[f] Season-specific/laboratory-specific quartiles for the 25(OH)D RIA data were: Q1: ≤ 31.5, Q2: >31.5 and ≤ 45.1, Q3: >45.1 and ≤ 56.7, Q4: > 56.7 nmol/L for the darker months; Q1: ≤ 40.9, Q2: >40.9 and ≤ 54.7, Q3: >54.7 and ≤ 73.4, Q4: > 73.4 nmol/L for sunnier months. Season-specific/laboratory-specific quartiles for the 25(OH)D CLIA data were: Q1: ≤ 18.4, Q2: >18.4 and ≤ 26.8, Q3: >26.8 and ≤ 40.3, Q4: > 40.3 nmol/L for the darker months; Q1: ≤ 29.0, Q2: >29.0 and ≤ 41.5, Q3: >41.5 and ≤ 61.8, Q4: > 61.8 nmol/L for sunnier months.

[g] a proxy for free 25(OH)D

Table 3

Odds ratios and 95% confidence intervals for the association between serum vitamin D binding protein and 25(OH)D and risk of pancreatic cancer – stratified models, ATBC Study, 1985–2004[a]

	Quartile 1 OR (95% CI)		Quartile 2 OR (95% CI)		Quartile 3 OR (95% CI)		Quartile 4 OR (95% CI)		p-trend	p-interaction
DBP, nmol/L	≤ 4026		> 4026 & ≤ 5329		> 5329 & ≤ 6721		> 6721			
25(OH)D below median										
N[b]	44/41		21/33		13/23		28/20			
Model 1[c]	1.00	reference	0.59	0.30–1.19	0.51	0.23–1.14	1.28	0.62–2.61	0.63	0.01
Model 2[d]	1.00	reference	0.61	0.29–1.26	0.56	0.23–1.34	1.42	0.67–3.05	0.59	
Model 3[e]	1.00	reference	0.63	0.30–1.33	0.60	0.25–1.45	1.41	0.65–3.05	0.59	
25(OH)D above median										
N[b]	43/18		37/25		17/36		30/37			
Model 1[c]	1.00	reference	0.62	0.29–1.31	0.20	0.09–0.40	0.33	0.16–0.70	0.002	
Model 2[d]	1.00	reference	0.59	0.26–1.33	0.17	0.07–0.41	0.32	0.14–0.71	0.001	
Model 3[e]	1.00	reference	0.64	0.28–1.44	0.18	0.08–0.43	0.30	0.13–0.68	0.001	
25(OH)D, nmol/L[f]										
DBP below median										
N[b]	28/40		40/37		28/26		49/14			
Model 1[c]	1.00	reference	1.55	0.81–3.00	1.53	0.74–3.15	5.01	2.33–10.78	<0.0001	0.001
Model 2[d]	1.00	reference	1.62	0.79–3.34	1.54	0.70–3.41	5.72	2.46–13.30	0.0001	
Model 3[e]	1.00	reference	1.57	0.77–3.25	1.62	0.73–3.60	5.76	2.46–13.48	0.0001	
DBP above median										
N[b]	20/20		21/21		22/33		25/42			
Model 1[c]	1.00	reference	0.96	0.40–2.31	0.65	0.29–1.49	0.59	0.27–1.31	0.12	
Model 2[d]	1.00	reference	1.01	0.39–2.57	0.71	0.29–1.74	0.50	0.21–1.21	0.08	
Model 3[e]	1.00	reference	0.90	0.34–2.36	0.79	0.31–1.99	0.42	0.17–1.04	0.05	

[a] All models used unconditional logistic regression.

[b] N for cases/controls

[c] Model 1 is adjusted for the matching factors of age (continuous) and date of blood collection (continuous).

[d] Model 2 is adjusted for the matching factors of age (continuous) and date of blood collection (continuous), as well as body mass index (continuous), number of cigarettes smoked/day (continuous), years of smoking (continuous), history of diabetes (yes/no), occupational physical activity(heavy vs. no heavy activity), education (> elementary vs. elementary), ethanol intake (quartiles, with separate categories for non-drinkers and missing), and serum retinol (continuous), cholesterol (continuous), β-carotene (continuous) and α-tocopherol (continuous)

[e] Model 3 is the same as Model 2, with additional adjustment for either 25(OH)D or DBP, respectively (quartiles).

[f] Season-specific/laboratory-specific quartiles for the 25(OH)D RIA data were: Q1: ≤ 31.5, Q2: >31.5 and ≤ 45.1, Q3: >45.1 and ≤ 56.7, Q4: > 56.7 nmol/L for the darker months; Q1: ≤ 40.9, Q2: >40.9 and ≤ 54.7, Q3: >54.7 and ≤ 73.4, Q4: > 73.4 nmol/L for sunnier months. Season-specific/laboratory-specific quartiles for the 25(OH)D CLIA data were: Q1: ≤ 18.4, Q2: >18.4 and ≤ 26.8, Q3: >26.8 and ≤ 40.3, Q4: > 40.3 nmol/L for the darker months; Q1: ≤ 29.0, Q2: >29.0 and ≤ 41.5, Q3: >41.5 and ≤ 61.8, Q4: > 61.8 nmol/L for sunnier months.