A Prospective Nested Case-Control Study of Vitamin D Status and Pancreatic Cancer Risk in Male Smokers

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Abstract

Sun exposure is associated with lower death rates for pancreatic cancer in some ecological studies. Skin exposure to UVB light induces cutaneous production of precursors to 25-hydroxyvitamin D [25(OH)D]. Pancreatic islet and duct cells express 25(OH)D_3-1α-hydroxylase that generates the biologically active 1,25$(OH)_2$ vitamin D form. Thus, 25(OH)D concentrations could affect pancreatic function and possibly pancreatic cancer etiology. We conducted a prospective nested case-control study in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention cohort of male Finnish smokers, ages 50 to 69 years at baseline, to test whether more adequate vitamin D status, as determined by prediagnostic serum 25(OH)D concentrations, was associated with lower pancreatic cancer risk. Two hundred incident exocrine pancreatic cancer cases that occurred between 1985 and 2001 (up to 16.7 years of follow-up) were matched by age and date of blood draw to 400 controls who were alive and free of cancer at the time the case was diagnosed. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using conditional logistic regression. Higher vitamin D concentrations were associated with a 3-fold increased risk for pancreatic cancer (highest versus lowest quintile, >65.5 versus <32.0 nmol/L: OR, 2.92; 95% CI, 1.56-5.48, P_{trend} = 0.001) that remained after excluding cases diagnosed early during follow-up. Contrary to expectations, subjects with higher prediagnostic vitamin D status had an increased pancreatic cancer risk compared with those with lower status. Our findings need to be replicated in other populations and caution is warranted in their interpretation and implication. Our results are intriguing and may provide clues that further the understanding of the etiology of this highly fatal cancer. (Cancer Res 2006; 66(20): 10213-9)

Introduction

Sun exposure has been associated in ecologic studies with lower death rates for breast, colorectal, non-Hodgkin's lymphoma, and prostate, as well as pancreatic cancer (1, 2). A suggested explanation for these associations is exposure of the skin to solar UVB light (280-320 nm) inducing cutaneous production of precursors to vitamin D (2). Certain risk factors for pancreatic cancer, such as age, obesity, and African American ethnicity, have been associated with reduced vitamin D status (1), and a recent prospective analysis showed that a higher predicted vitamin D status score calculated from six determinants of 25-hydroxyvitamin D [25(OH)D; dietary and supplementary vitamin D, skin pigmentation, adiposity, geographic residence, and leisure activity] was associated with lower total cancer incidence and mortality including pancreatic cancer (3). In addition to vitamin D synthesized endogenously, dietary sources of vitamin D include cholecalciferol (D_3) that occurs naturally in some animal foods (i.e., fatty saltwater fish, liver, and egg), ergocalciferol (D_2) from plants, used in pharmaceutical preparations, and fortified foods such as milk and margarine (D_2 and D_3; refs. 4, 5).

Extrarenal synthesis of hormonally active 1,25-α$(OH)_2$D has been shown to be involved in autocrine and paracrine regulation of cell differentiation, proliferation, and apoptosis, processes involved in carcinogenesis (6). The pancreatic islet cells possess vitamin D receptors and express 25(OH)D_3-1α-hydroxylase (7), the enzyme that catalyzes the synthesis of the active 1,25$(OH)_2$D form, which has led to postulation that vitamin D status may be linked to endocrine pancreatic function (8). Expression of 25(OH)D_3-1α-hydroxylase has been observed in pancreatic duct cells and normal and adenocarcinomatous tissue. Pancreatic cancer cell line growth is inhibited by 25(OH)D_3 (9, 10). 1,25-Vitamin D analogues inhibit pancreatic cancer cell proliferation, induce differentiation, promote apoptosis *in vitro* (11, 12), and inhibit pancreatic tumor growth of BxPC-3 xenographs in athymic mice (13), supporting a potential role for vitamin D in pancreatic cancer etiology.

We conducted and here reported a nested case-control study to examine whether vitamin D status, as determined by serum 25(OH)D concentrations, was prospectively associated with pancreatic cancer incidence. 25(OH)D is the major circulating vitamin D metabolite and is also considered the best indicator of vitamin D status as determined by the sun and diet.

Materials and Methods

Study population. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was a double-blind, placebo-controlled, 2 × 2 factorial design primary prevention trial that tested whether α-tocopherol or β-carotene reduced the cancer incidence in male smokers. Study rationale, design, and methods have been published (14). Between 1985 and 1988, 29,133 eligible men in southwestern Finland, ages 50 to 69 years, who smoked at least 5 cigarettes per day, were randomized to receive active supplements or placebo. Men were excluded from the study if they had a history of malignancy other than nonmelanoma cancer of the skin or carcinoma *in situ*, severe angina on exertion, chronic renal insufficiency, liver cirrhosis, chronic alcoholism, or other medical conditions that might limit long-term participation, if they were receiving anticoagulant therapy

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or used supplements containing vitamin E (>20 mg/d), vitamin A (>20,000 IU/d), or β-carotene (>6 mg/d). All study participants provided written informed consent before randomization and the study was approved by the institutional review boards of both the National Public Health Institute in Finland and the U.S. National Cancer Institute.

Participants completed questionnaires on general background characteristics including medical, smoking, dietary, and physical activity history during their prerandomization baseline visit (14). Trained study staff measured height, weight, and blood pressure at baseline using standard methods. Body mass was calculated from measured weight and height (kg/m^2). Diet was assessed with a validated self-administered dietary history questionnaire, which determined the frequency of consumption and usual portion size of 276 food items during the past year, using a color picture booklet as a guide for portion size (15). Occupational activity was assessed by asking how much exercise and physical burden was received at work during the past year and ranged from not working or sedentary to heavy physical work. Leisure time activity was assessed by asking the average activity level during the past year and ranged from sedentary to moderate (walking, fishing, hunting, or gardening regularly) or heavy exercising (running, jogging, or skiing regularly). During the trial (1984-1993), subjects attended three annual visits to their local field center, during which they were queried about changes in smoking habits since the last visit (14). We defined "smoking cessation during the trial" as reporting to have quit during >3 consecutive follow-up visits (>1 year).

Ascertainment of cases and control selection. All cases of pancreatic cancer diagnosed between January 1985 and December 2001 were identified through the Finnish Cancer Registry, which provides complete case ascertainment in Finland (16). As the etiology of islet cell carcinomas (ICD9-157.4, ICD10-C254) may be different from the exocrine tumors, only exocrine tumors (ICD9-157, ICD10-C25) were included as the cancer outcome of interest. During the follow-up period, 200 exocrine pancreatic cancer cases were confirmed. The interval between serum collection and diagnosis was up to 16 years (median follow-up time, 11.8 years).

Controls were selected from among Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study participants who were alive at the time the case was diagnosed and free from cancer (except nonmelanoma skin cancer) as of December 2001. Two controls were matched to each case by age (±5 years) and month of baseline blood draw (±30 days) to control for age and seasonal vitamin D variation.

Biomarkers. At their prerandomization visit, a blood sample was obtained from study participants after an overnight fast and serum was stored at −70°C (14). Frozen baseline serum samples were sent to Dr. Reinhold Veith's laboratory at Mount Sinai Hospital, Toronto, Ontario, Canada and analyzed by RIA (DiaSorin, Inc., Stillwater, MN) for 25(OH)D measure of vitamin D status including vitamins D_2 and D_3 from plant and animal foods, as well as that synthesized endogenously. In addition, 25(OH)D is stable with long-term storage (17). Case and control specimens were handled in the same standard manner and the laboratory was blinded to case-control status. Matched serum case and control samples were analyzed consecutively as triplets within batches and blinded replicate "phantom" samples from two pooled samples were placed in triplicate towards the beginning and end of each batch and comprised 10% of each batch. Using a nested components of variance analysis, with logarithmically transformed quality control measurements across all batches (18), the estimated overall (intrabatch and interbatch) coefficient of variation percent of the 25(OH)D assay was 16.5%.

Only the hormonally active $1,25(OH)_2D_3$ form binds the nuclear vitamin D receptor. This complex then binds the retinoic X receptor to form a heterodimer complex that interacts with DNA sequences, called vitamin D responsive elements, which influence transcription of vitamin D responsive genes (5). High retinol intake or status may antagonize the action of vitamin D by competing with $1,25(OH)_2D$ for the retinoic X receptor. Therefore, we used previously measured baseline serum retinol and β-carotene to evaluate for confounding and interaction of the vitamin D association (14).

Statistical analysis. We compared the distribution of selected characteristics of the cases and controls using the Wilcoxon rank-sum test for the continuous variables and χ^2 tests for categorical variables. Generalized linear models for continuous and the Cochran-Armitage test for dichotomous factors were used to calculate means, proportions, and trends of characteristics among the controls across 25(OH)D quintiles to help identify potential confounders. Variables examined in analyses and as potential confounders in risk models were age; smoking history (number of cigarettes smoked per day, years smoked, frequency of inhaling, reporting to have quit >3 consecutive visits during the trial); education; living in a city; height, weight, body mass index; measured systolic and diastolic blood pressure; history of diabetes, peptic or duodenal ulcer, pancreatitis, cholelithiasis, bronchial asthma, and renal disease; dietary nutrients from foods (energy, carbohydrate, fat, saturated fat, ω-3 fatty acids, saturated fat, calcium, vitamin D, retinol, and folate) and supplements (vitamin D and calcium); vitamin D–containing food (fish, liver, milk, eggs, and margarine), butter, cream, and alcohol intake; serum nutrients (α-tocopherol, β-carotene, retinol, and cholesterol); occupational and leisure physical activity; and season.

Dietary nutrients and foods highly correlated with energy were energy adjusted using the residual method described by Willett and Stampfer (19). As cutaneous synthesis of pre-vitamin D_3 is affected by exposure to sunlight and thus varies by season, a season variable was created. Blood collected in May, June, August, September, October, and November had average $25(OH)_2D$ concentrations of >50 nmol/L each month and was categorized as reflecting the sunny season, whereas blood collected in December, January, February, March, and April had average concentrations of <50 nmol/L each month and was categorized as reflecting the darker season.

Conditional logistic regression was used to estimate odds ratios (OR) for pancreatic cancer with subjects in the lowest quintile as the reference category. Multivariable models were developed by individually adding covariates to the model and were included if they were associated with both the disease and the risk factor, had a P value of <0.20 in the full model, or changed the risk estimate by >10%. Although smoking intensity and duration did not influence the risk estimates, they were included in the model because they are putative risk factors for pancreatic cancer. The final multivariable models included smoking intensity and duration, reporting to have quit smoking >3 consecutive visits during the trial, education, occupational activity, and serum retinol. Effect modification of 25(OH)D status by season, smoking intensity, duration, reporting to have quit smoking during the trial, serum retinol and β-carotene, and trial intervention group was evaluated with cross product terms of vitamin D status quintile trend and dichotomized variables (yes, no, or median split) in multivariable models and stratified analyses. For evaluation of effect modification by factors other than season, we broke the case-control match and used logistic regression models that additionally adjusted for age and month of blood draw. For analyses stratified by season, quintiles for vitamin D status were examined based on (*a*) the distribution of all the controls, (*b*) the distribution of controls within each season strata, and (*c*) merging subjects in the season-specific vitamin D quintile categories together with dummy variables. We also estimated the adjusted ORs that excluded cases that occurred during the first 5 and 10 years. All statistical analyses were done with Statistical Analytic Systems (SAS) software and statistical tests were two tailed. Because cases and controls were matched, their median values, proportions, and all risk estimates should be interpreted as adjusted for the matching factors (age and season).

Results

Compared with the controls, cases had higher 25(OH)D concentrations (P = 0.03) and less often had vitamin D inadequacy (<40 nmol/L, P = 0.03; ref. 4); were taller (P = 0.005); and distribution of occupational activity differed (P = 0.02; Table 1). Smoking duration and smoking intensity did not significantly differ between our age-matched cases and controls; however, smoking intensity was nonsignificantly positively associated with pancreatic cancer (compared with <15 and >25 cigarettes/d; OR, 1.31). This likely reflects the fact that our cohort is composed of all smokers,

Table 1. Selected baseline characteristics of case and control subjects (median and interquartile range or proportion)

Characteristics	Cases (n = 200)	Controls (n = 400)	P*
Age, y	58.0 (54.0-61.5)	58.0 (55.0-62.0)	0.88
Serum 25(OH)vitamin D (D2 and D3), nmol/L	49.1 (38.4-68.0)	46.3 (26.6-61.6)	0.03
Vitamin D inadequacy (<40 nmol/L†), %	28.0	37.2	0.03
Serum retinol, μg/L	552 (476.5-639.5)	574 (498-662)	0.07
Height, cm	174 (170-179)	173 (168-177)	0.005
Body mass index, kg/m^2	26.0 (23.7-28.3)	26.3 (23.8-28.7)	0.42
Systolic blood pressure, mm Hg	140.0 (129.5-152.0)	144 (132-158)	0.01
Diastolic blood pressure, mm Hg	87.5 (79.0-94.0)	90.0 (81.5-98.0)	0.003
Medical history			
Diabetes mellitus, %	9.5	6.5	0.19
Bronchial asthma, %	4.5	2.8	0.26
Smoking history			
Total cigarettes per day	20.0 (15.0-25.0)	20.0 (15.0-25.0)	0.34
Years smoked	39.0 (33.0-43.0)	39.0 (34.0-43.0)	0.80
Inhalation, always or often, %	91.5	89.8	0.49
Quit smoking during trial,‡ %	16.0	11.0	0.08
Primary school education or less, %	76.8	81.7	0.13
Living in city, %	47.5	43.3	0.32
Dietary intake per day§			
Energy, kcal	2,731 (2,314-3,200)	2,754 (2,230-3,197)	0.90
Fish, g	32.6 (20.3-45.6)	34.9 (21.1-54.3)	0.10
Eggs, g	46.6 (31.5-64.5)	47.2 (31.5-69.7)	0.65
Milk, g	763 (559-943)	735 (500-973)	0.13
Alcohol, g	8.9 (2.0-24.5)	11.4 (3.0-27.6)	0.32
Vitamin D			
Food, μg	4.74 (3.47-6.60)	5.09 (3.38-7.02)	0.45
Supplemental, %yes	8.0	6.0	0.36
Ω-3 fatty acids, g	2.02 (1.57-2.54)	2.03 (1.54-2.54)	0.93
Saturated fat, g	53.9 (45.7-62.8)	52.5 (43.1-61.9)	0.24
Folate, μg	330 (293-364)	334 (296-373)	0.20
Physical activity			
Occupational			
Sedentary, %	17.0	9.5	
Moderate, %	29.5	26.5	
Heavy, %	6.5	8.5	
Nonworking, %	47.0	55.5	0.02
Leisure‖			
Sedentary, %	43.2	45.5	
Light, moderate, %	50.3	48.0	
Exercise to keep fit, %	6.5	6.5	0.86

*P values for categorical variables based on χ^2 or Fisher's exact test and P values for continuous variables based on Wilcoxon rank-sum test.
†7.0% of the cases and 8.5% of the controls had deficient vitamin D concentrations, 25(OH)vitamin D2 and D3 <25 nmol/L; P = 0.52 (χ^2 test).
‡Reporting to have quit smoking for >3 consecutive visits (>1 year) during the trial (1985-1993).
§Dietary intake analysis based on n = 196 cases and n = 370 controls. All foods and nutrients were energy adjusted except supplements and alcohol.
‖Leisure activity variables based on n = 199 cases and n = 400 controls.

our controls are matched to cases by age, and age is significantly correlated to smoking duration. The proportion of subjects with vitamin D deficiency [25(OH)D, <25 nmol/L; ref. 4] did not differ between cases and controls (7.0% versus 8.5%, respectively; P = 0.52).

Table 2 shows the means, proportions, and trends of selected baseline characteristics among the controls according to quintile of 25(OH)D concentrations. Across increasing quintiles of vitamin D, serum retinol, fish and vitamin D intake from foods, and the proportion of subjects having blood collected in the sunny season, less education, light/moderate and exercising to keep fit leisure activity increased, whereas diastolic blood pressure and the proportion of subjects reporting a history of bronchial asthma and sedentary leisure activity decreased (P < 0.05). Similar characteristic distributions were present among the cases, except diastolic blood pressure was not related to vitamin D concentrations.

Higher concentrations of 25(OH)D were positively and significantly associated in a dose-response manner with pancreatic cancer in both crude and adjusted conditional logistic regression models (Table 3). Adding serum retinol to the model increased the magnitude of the positive 25(OH)D-pancreatic cancer association.

Table 2. Selected characteristics of control subjects by quintile of fasting 25(OH)vitamin D (D2 and D3) means and proportions

Characteristics	Quintile serum 25(OH)vitamin D (D2 and D3), nmol/L*					*P*
	Q1	Q2	Q3	Q4	Q5	
	<32	>32 and <41.1	>41.1 and <51.1	>51.1 and <65.5	>65.5	
Age, y	58.5	57.3	58.0	58.8	58.8	0.17
Serum 25(OH)vitamin D, nmol/L	25.6	37.1	46.3	57.7	83.2	<0.0001
Season of blood draw, sunny,† %	27.5	27.5	40.0	46.9	63.3	<0.0001
Serum retinol, μg/L	556	557	599	593	613	0.007
Height, cm	171.9	172.9	173.5	172.6	172.5	0.50
Body mass index, kg/m^2	26.2	27.0	26.7	27.1	25.9	0.30
Systolic blood pressure, mm Hg	149.6	144.7	144.8	142.0	146.6	0.17
Diastolic Blood pressure, mm Hg	92.5	90.4	87.4	88.0	89.3	0.03
Medical history						
Diabetes mellitus, %	8.8	3.8	12.5	4.9	2.5	0.14
Bronchial asthma. %	6.3	1.25	3.75	2.5	0	0.04
Smoking history						
Total cigarettes per day	19.1	19.9	19.9	21.6	20.5	0.36
Years smoked	36.9	37.9	35.9	37.2	38.0	0.47
Inhalation, always or often, %	92.5	87.5	90.0	87.7	91.1	0.90
Quit smoking during the trial,‡ %	10	7.5	13.8	9.9	13.9	0.34
Primary school education or less, %	72.5	85.0	82.5	80.3	88.6	0.04
Living in city, %	19.7	19.7	20.2	16.2	24.3	0.28
Dietary intake per day§						
Energy, kcal	2,772	2,759	3,004	2,694	2,762	0.15
Fish, g	30.5	35.4	40.0	50.6	53.2	<0.0001
Eggs, g	53.8	60.3	52.8	59.7	53.0	0.65
Milk, g	816	705	746	790	666	0.13
Margarine, g	56.0	61.5	59.9	61.4	62.0	0.39
Ω-3 fatty acids, g	1.96	2.06	2.09	2.17	2.31	0.05
Saturated fat, g	52.0	52.7	53.8	54.0	49.5	0.28
Folate, μg	336	335	335	335	348	0.64
Alcohol, g	20.0	17.2	19.7	17.4	21.2	0.78
Vitamin D						
Food, μg	4.56	5.22	5.44	6.34	6.86	<0.0001
Supplemental, %yes	3.8	1.3	8.8	9.9	6.3	0.18
Physical activity						
Occupational						
Sedentary, %	10.0	12.5	10.0	6.2	8.9	0.48
Moderate (walking), %	28.8	28.8	30.0	27.2	17.7	0.09
Heavy, %	7.5	5.0	11.3	8.6	10.1	0.40
Nonworking, %	53.8	53.8	48.8	58.0	63.3	0.14
Leisure						
Sedentary, %	56.3	51.3	46.3	44.4	29.1	0.003
Light, moderate, %	41.3	43.8	45.0	51.9	59.2	0.02
Exercise to keep fit, %	2.5	5.0	8.75	3.7	12.7	0.02

*Vitamin D quintiles based on distribution of all controls (n = 400).

†Sunny season based on blood drawn during May, June, August, September, October, and November versus darker season based on blood drawn during December, January, February, March, and April.

‡Reporting to have quit smoking for >3 consecutive visits (>1 year) during the trial (1985-1993).

§All dietary variables were adjusted for energy except supplements and alcohol. Dietary intake analysis based on n = 370 control subjects.

Serum retinol tended to be inversely associated with pancreatic cancer. A four-knot spline was used to model a nonlinear relationship between continuous 25(OH)D concentrations and pancreatic cancer. The test for nonlinearity did not reach statistical significance (χ^2 = 2.76, P > 0.05), which implies the relationship is close to linear. The association was not significantly modified by season of blood collection ($P_{interaction}$ = 0.50); however, the association tended to be stronger among subjects who had their blood drawn during the darker months regardless of whether the quintile cut points were based on the distribution of all the control subjects or season-specific strata. There was no significant interaction of the association by smoking habits, serum retinol,

or trial supplementation intervention group. The adjusted ORs for each respective quintile compared with the first, after the exclusion of the cases that occurred during the first 5 years, were 1.14. 1.87, 1.36, and 3.00 [95% confidence interval (95% CI), 1.53-5.88; P_{trend} = 0.001], and after the exclusion of cases that occurred during the first 10 years were 1.66, 3.02, 1.41, and 5.02 (95% CI, 1.68-14.94; P_{trend} = 0.007).

Discussion

To our knowledge, this is the first study to examine pancreatic cancer in association with prediagnostic vitamin D status, as assessed with 25(OH)D concentrations. Contrary to our expectations, we observed a 3-fold increased risk for pancreatic cancer among subjects with more adequate vitamin D status compared with those with lower status. The positive association occurred in a dose-response manner and remained after the exclusion of cases that occurred early in follow-up (e.g., 5 and 10 years). In addition, the increased pancreatic cancer risk was apparent among subjects independent of their season of blood collection.

Mechanisms that may explain the observed vitamin D-pancreatic cancer association are highly speculative and are made less clear because the effects of vitamin D on molecular mechanisms underlying pancreatic carcinogenesis are not well understood. The active form of vitamin D functions as a powerful hormone, with the vitamin D receptor-1,25$(OH)_2D_3$ complex binding to its responsive elements on target genes, activating gene transcription, and modulating gene expression. Although speculative, vitamin D inadequacy could plausibly protect against pancreatic cancer development by its influence on the synthesis and regulation of growth factors, particularly insulin. *In vitro* evidence supports the involvement of vitamin D in the regulation of insulin synthesis, binding, and responsiveness (20–22). Vitamin D deficiency has been reported to impair pancreatic insulin synthesis and secretion

Table 3. Age and multivariable adjusted OR and 95% CI of baseline fasting 25(OH)vitamin D (D2 and D3) status and pancreatic cancer among 200 cases and 400 matched control subjects

	Quintiles fasting vitamin D (D2 and D3), nmol/L*					*P*
	1	2	3	4	5	
Fasting vitamin D (D2 and D3), nmol/L quintile cut points*						
	<32	>32 and <41.1	>41.1 and <51.1	>51.1 and <65.5	>65.5	
Case/controls, *n*	27/80	34/80	47/80	35/81	57/79	
Crude OR (95% CI)	1.00 (reference)	1.28 (0.71-2.31)	1.91 (1.06-3.42)	1.34 (0.74-2.41)	2.43 (1.34-4.38)	0.006
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.30 (0.70-2.40)	2.12 (1.15-3.90)	1.50 (0.81-2.76)	2.92 (1.56-5.48)	0.001
Winter season*, ‡						
Case/controls, *n*	22/59	24/55	24/47	23/44	25/24	
Crude OR (95% CI)	1.00 (reference)	1.12 (0.56-2.22)	1.45 (0.71-2.94)	1.41 (0.71-2.81)	2.46 (1.15-5.30)	0.02
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.19 (0.59-2.46)	1.95 (0.92-4.14)	1.84 (0.88-3.84)	3.37 (1.47-7.77)	0.003
Spring, summer, and fall season*, †						
Case/controls, *n*	5/21	10/25	23/33	12/37	32/55	
Crude OR (95% CI)	1.00 (reference)	1.99 (0.59-6.69)	3.30 (1.06-10.27)	1.38 (0.42-4.49)	2.86 (0.98-8.33)	0.15
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.47 (0.43-5.78)	2.18 (0.66-7.18)	0.93 (0.27-3.24)	2.13 (0.68-6.60)	0.29
Fasting vitamin D (D2 and D3), nmol/L quintile cut points based on each season§						
Winter season †, ‖	<29.7	>29.7 and <38.2	>38.2 and <45.8	>45.8 and <56.7	>56.7	
Case/controls, *n*	18/48	19/47	21/47	24/47	36/47	
Crude OR (95% CI)	1.00 (reference)	1.08 (0.50-2.34)	1.23 (0.58-2.59)	1.41 (0.68-2.92)	2.12 (1.04-4.34)	0.02
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.12 (0.50-2.50)	1.56 (0.70-3.45)	1.91 (0.88-4.14)	2.88 (1.33-6.27)	0.003
Spring, summer, and fall season †, ‖	<38.1	>38.1 and <48.8	>48.8 and <60.1	>60.1 and <75.8	>75.8	
Case/controls, *n*	11/33	23/33	11/34	16/32	21/32	
Crude OR (95% CI)	1.00 (reference)	2.17 (0.88-5.36)	0.97 (0.37-2.59)	1.50 (0.62-3.64)	2.00 (0.80-4.91)	0.26
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.67 (0.65-4.33)	0.75 (0.26-2.14)	1.28 (0.50-3.29)	1.67 (0.64-4.41)	0.41
Fasting vitamin D (D2 and D3) for combined quintile seasonal cut points (above)§, ‖						
Case/controls, *n*	29/81	42/80	32/81	40/79	57/79	
Crude OR (95% CI)	1.00 (reference)	1.48 (0.83-2.64)	1.11 (0.61-2.01)	1.47 (0.84-2.57)	2.07 (1.18-3.62)	0.01
Multivariable adjusted OR (95% CI) †	1.00 (reference)	1.42 (0.79-2.57)	1.17 (0.63-2.16)	1.72 (0.96-3.09)	2.41 (1.33-4.36)	0.002

NOTE: All ORs should be considered adjusted for the matching factors age and month of blood draw.

*Vitamin D quintiles based on distribution of all controls.

†Adjusted for years smoked, number of cigarettes smoked per day, reporting to have quit smoking >3 consecutive visits (>1 year) during the trial (1985-1993), occupational physical activity, education, and serum retinol.

‡Sunny season based on blood drawn during May, June, August, September, October, and November (n = 118 cases) versus darker season based on blood drawn during December, January, February, March, and April (n = 82).

§Quintiles based on merging subjects within quintiles of each season strata.

‖Vitamin D quintiles based on distribution of controls within each season strata.

in both animal models and humans (23–28). In addition, vitamin D response elements have been identified in the human insulin receptor gene promoter (22). In a previous study in this same population studied here, we observed a significant association between insulin and pancreatic cancer, although the insulin concentrations that were associated with the increased risk were lower than those corresponding to hyperinsulinemia (>14 μU/mL; ref. 29). This concept may be indirectly supported by positive associations that have been observed between height and pancreatic cancer by others (30, 31), as well as in this nested-case control study. Vitamin D receptor polymorphisms have been associated with adult stature (32–34) and adult height may be a marker for exposure to growth factors during childhood and adolescence. Alternatively, vitamin D status could be correlated to unmeasured exposures that may increase pancreatic cancer risk. In particular, organochlorine compounds have been associated with pancreatic cancer (35, 36) and are potential contaminants of vitamin D–rich fish consumed in the Finnish diet (37). Although fish intake was a strong predictor of vitamin D status in the present study, it did not, however, attenuate the vitamin D-pancreatic cancer association (5th versus 1st quintile vitamin D; HR, 3.62; 95% CI, 1.88-6.97; P_{trend} = 0.0002).

The strength of our study is its prospective nature with vitamin D status being assessed up to 16 years before cancer diagnosis, thereby reducing the influence of reverse causality. Our study also has internal validity, as both the cases and controls were derived from the same cohort, eliminating selection bias. Our measurement of serum 25(OH)D concentrations reflects internal dose and status, which encompasses cutaneous production of the vitamin and is considered superior to vitamin D intake alone. High vitamin D dietary sources (i.e., fish and vitamin D intake) and variables related to sun exposure (i.e., season and leisure activity) were associated with vitamin D status in the expected manner. In addition, in an earlier nested case-control study conducted in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention cohort, 25(OH)D was inversely associated with colorectal cancer, particularly distal colorectal cancer (38), a direction similar to that of other studies (39). These observations lend external validity to association studies of 25(OH)D in our cohort. Finally, because our cases and controls were matched by month of blood draw, misclassification of vitamin D status due to seasonal variation in exposure to sunlight should have been minimized.

Our findings, however, may not be generalizable to populations that include nonsmokers or populations that are vitamin D adequate. Residual confounding by cigarette smoking dose is unlikely in our study because the smoking exposures were not confounders, the positive association between vitamin D and pancreatic cancer was not modified by cigarette smoking dose ($P_{interaction}$ = 0.36), and analyses restricted to men who reported exactly 20 cigarettes daily (n = 53 cases and 133 controls) yielded similar positive results (5th quintile; OR, 2.92). A single measurement of 25(OH)D may not reflect long-term vitamin D status. The biochemical marker has a half-life of 3 weeks; however, in a steady state, it represents the past several weeks to several months of exposure, and is known to display seasonal variability (40). This variability would likely contribute to attenuation of the true association such as is evident among our subjects who had their blood collected during the sunny months (Table 3). Finally, our population had lower vitamin D status compared with other populations (41), which likely reflects Finland's northern latitude with less solar UVB photon exposure and less cutaneous vitamin D synthesis. Approximately 40% of the controls in our study were in the range of inadequacy (4). The association between vitamin D and pancreatic cancer could therefore differ in populations with more adequate or higher vitamin D status.

In conclusion, contrary to expectation, subjects with higher 25(OH)D concentrations were at greater risk for pancreatic cancer compared with those with lower concentrations in our prospective study with long-term follow-up. Caution is warranted in the interpretation and implication of our findings, however, as vitamin D inadequacy is an important public health problem and adequate status is desirable to prevent bone and other diseases (5). Our results, however, are intriguing and may provide clues that further the understanding of the etiology of this highly fatal cancer.

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