Insulin-Like Growth Factor (IGF)-1, IGF-Binding Protein-3, and Pancreatic Cancer in Male Smokers

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Abstract

To investigate whether insulin-like growth factor (IGF)-1 and IGF-binding protein-3 (IGFBP-3) are prospectively associated with exocrine pancreatic cancer, we conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort of 29,133 male Finnish smokers, aged 50–69 years. To avoid the potential influence of subclinical cancer on IGF-1 and IGFBP-3, all subjects in this study were alive without clinical evidence of cancer during their 5th year of the cohort follow-up. Four hundred randomly selected cohort controls and 93 incident pancreatic adenocarcinoma cases that occurred between their 5th follow-up year through 1997 (*i.e.*, up to 12.7 years of follow-up) were included in this study. Concentrations of IGF-1 and IGFBP-3 were measured in serum samples obtained at baseline using ELISA. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression models, adjusted for confounders. Neither IGF-1, IGFBP-3, nor the IGF-1:IGFBP-3 molar ratio was significantly associated with pancreatic cancer: highest compared to lowest tertile, OR = 0.67, 95% CI 0.37–1.21, *P* trend = 0.17; OR = 0.70, 95% CI 0.38–1.27, *P* trend = 0.12; and OR = 0.85, 95% CI 0.50–1.46, *P* trend = 0.54, respectively. Our results do not support the hypothesis that serum IGF-1 and IGFBP-3 concentrations are associated with pancreatic cancer risk among male smokers. Further studies are necessary to evaluate these associations in other populations.

Introduction

Insulin-like growth factors (IGFs) are endocrine mediators of growth hormone and also act in paracrine and autocrine fashion to regulate cell growth, differentiation, apoptosis, and transformation (1) in many tissues including the exocrine pancreas (2, 3). The IGF axis is complex and is comprised of growth factors (IGF-1, IGF-2, and insulin), cell surface receptors (IGF-1R and IGF-2R), six binding proteins (IGFBP-1 to IGFBP-6), IGFBP proteases, as well as other IGFBP-interacting molecules that regulate the IGF axis' actions. Imbalance of these processes may support uncontrolled cell proliferation and promote carcinogenesis (1).

Recent epidemiological studies have associated increase in IGF-1 concentrations with risk of a number of malignancies, including breast, prostate, colorectal, and lung cancer (1). Although most tissues produce IGF-1, circulating IGF-1 is secreted primarily by the liver in response to growth hormone and is found in two states: free or bound by one of the six proteins. Over 90% of IGF-1 in the blood is bound to IGF binding protein-3 (IGFBP-3) (1), which inhibits IGF-1 action by making it unable to bind to cell surface IGF-1 receptors. Less then 1% of the IGF-1 circulating is in an unassociated form. The molar ratio of IGF-1 to IGFBP-3 (IGF-1:IGFBP-3), a marker of bioactive IGF-1 (4), has additionally been examined in some epidemiological studies. The epidemiological studies with the most provocative cancer associations have shown that high IGF-1, low IGFBP-3, and/or high IGF-1:IGFBP-3 positively predict cancer risk.

There is evidence to suggest that IGF-1 enhances the growth and survival of human pancreatic cancer cells both through autocrine and paracrine mechanisms (2, 3, 5–11). However, data that examine serum concentrations of IGF-1 in subjects who develop pancreatic cancer are currently limited. IGF-1 is expressed in low concentrations in the stromal elements of normal pancreas tissue (2, 3), while in human pancreatic cancer tissue, IGF-1 mRNA is overexpressed in the cancer cells as well as in surrounding connective tissue, with an increased IGF-IR mRNA expression in approximately half of the tumors (3). In addition, IGF-1 enhances, while antagonists to the IGF-1 receptors inhibit growth of cultured pancreatic

cancer cell lines (3). Yet two small case-control studies have found no significant differences in serum IGF-1 or IGFBP-3 concentrations among pancreatic cancer cases compared to controls (12, 13). Finally, a few studies have suggested that patients with acromegaly, a rare disease caused by a growth hormone-secreting pituitary adenoma that induces both elevated circulating and paracrine IGF-1 and IGFBP-3 concentrations, have an increased risk for gastrointestinal cancers including pancreatic cancer (14, 15).

To further investigate whether IGF-1 and IGFBP-3 are prospectively associated with exocrine pancreatic cancer, we conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study cohort of 29,133 male Finnish smokers, aged 50–69 years. To avoid the potential influence of subclinical cancer on IGF-1 and IGFBP-3 concentrations, all subjects in this study were alive and had not been diagnosed with cancer at least 5 years after their serum was collected.

Methods

Study Population. The ATBC Study was a double-blinded, placebo-controlled, 2 × 2 factorial design primary prevention trial that tested whether α-tocopherol or β-carotene could reduce the incidence of cancer among male smokers. Study rationale, design, and methods have been described previously (16). Between 1985 and 1988, 29,133 eligible men in southwestern Finland, aged 50–69, who smoked at least five cigarettes/day were randomized to receive active supplements (α-tocopherol 50 mg/day, β-carotene 20 mg/day, or both) 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 or used supplements containing vitamin E (>20 mg/day), vitamin A (>20,000 IU/day), or β-carotene (>6 mg/day). All study participants provided written informed consent before the study's 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 (16). Trained study staff measured height and weight 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 (17).

Biomarkers. At their prerandomization visit, study participants had a venipuncture for serum after a 12-h fast and serum was stored in the dark at −70°C. The stored serum samples were sent on dry ice to Dr. Michael Pollak's laboratory at the Jewish General Hospital and McGill University, Montreal, ON, Canada, for IGF-1 and IGFBP-3 measurement. Laboratory personnel were blinded to case and control sample status. Serum concentrations of IGF-I and IGFBP-3 were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX). The QC intra- and inter-batch coefficients of variation were 5.23% and 4.57% for IGF-1 and 4.18% and 6.17% for IGFBP-3, respectively. We also calculated the IGF-1 to IGFBP-3 molar ratio as an indicator of the bioactive IGF-1 using the following equivalents for conversion: 1 ng/ml IGF-1 = 0.130 nM IGF-1, and 1 ng/ml IGFBP-3 = 0.036 nM IGFBP-3.

Selection of Cases and Controls. To avoid the potential influence of subclinical cancer on IGF-1 and IGFBP-3 concentrations, all subjects in this study were alive at the beginning of their 5th year of cohort follow-up. Cases of pancreatic cancer occurring from their 5th year of cohort follow-up through December 1997 were identified from the Finnish Cancer Registry, which provides almost 100% case ascertainment in Finland (18). Two study physicians independently reviewed all relevant medical records for reported pancreatic cancer cases (16). Only cases confirmed by the study physicians as incident primary cancer of the exocrine pancreas [International Classification of Diseases (ICD) 9-157] were used for this analysis. Because their etiology may be different from the exocrine tumors, islet cell carcinomas (ICD9-157.4) were excluded. During the follow-up period, there were 93 confirmed exocrine pancreatic cancer cases. We selected a random sample of 400 subjects among all eligible cohort members alive without a cancer diagnosis as of 5 years of follow-up as the comparison group. This sub-cohort sample was the control group. The interval between serum collection and follow-up was up to 12.7 years (median follow-up time for diagnosis at 8.1 years).

Statistical Analysis. Variables used in analyses included baseline serum IGF-1, IGFBP-3, IGF-1:IGFBP-3 ratio; age at randomization; height, weight, and body mass index (BMI, kg/m^2); smoking history (years smoked, cigarettes smoked/day, and pack-years); urban living; education; medical history of gallstones, pancreatitis, peptic and duodenal ulcers, and diabetes mellitus; occupational and leisure activity; intervention group; and dietary energy, energy-adjusted carbohydrate, fat, saturated fat, carbohydrate, starch, free sugars, sucrose, protein (total, animal, milk, and vegetable), zinc, calcium and folate intake; and alcohol consumption. Case *versus* control comparisons for continuous variables were performed using the Wilcoxon rank sum test. Categorical variables were contrasted using asymptotic χ^2 tests. Generalized linear models adjusted for age as a continuous variable were used to estimate means and 95% confidence intervals (CIs) of the cohort characteristics among the controls by quintile of IGF-1 for continuous variables to help identify potential confounders. Because disease history variables were proportions, logistic regression was used to estimate age-adjusted proportions and 95% CIs by quintile of IGF-1. Quintiles were used instead of tertiles for these descriptive analyses to better describe the population. A test for trend was performed for each characteristic across the IGF-1 quintiles using contrast coefficients (*e.g.*, the five categories used −2, −1, 0, 1, 2 contrast coefficients).

We estimated odds ratios (ORs) and 95% CI using logistic regression models. Variables were categorized based on the distribution of the controls for the analysis. Risk estimates for the serum factors were based on tertiles because this approach gave the most stable estimates. Trends for the categorical variables were tested by calculating a score variable based on the median values of each category. Multivariable models were developed for serum IGF-1, IGFBP-3, and IGF-1:IGFBP-3 molar ratio by individually adding covariates into the models. All multivariable models included continuous variables for age and years smoked and other variables were included in the final model if they were associated with both the risk factor and the disease, changed the risk estimate greater than or equal to 10%, had a P value less than or equal to 0.20 in the full model, and/or increased the precision of the risk estimate. We also examined effect modification of IGF-1, IGFBP-3, and IGF-1:IGFBP-3 ratio by BMI, height, weight, smoking dose (number of cigarettes smoked/day, years smoked, and pack-years), age, energy, energy-adjusted protein, and alcohol intake. Effect modification was examined and tested through cross product terms using categorical trend variables in multivariable models and stratified analyses.

Statistical Analysis Systems (SAS) software (Cary, NC) was used for analyses. All statistical tests were two-tail and considered statistically significant at a P value = 0.05.

Results

Table 1 shows selected baseline characteristics of the pancreatic cancer cases and the sub-cohort controls. Compared to the latter, cases were older, had smoked longer and a greater cumulative smoking dose (pack-years), were more likely to live in a city and have a history of bronchial asthma, and had greater energy-adjusted saturated fat intake.

Table 2 shows the age-adjusted means and 95% CI of selected baseline characteristics of the sub-cohort control subjects according to quintile of IGF-1. Across IGF-1 quintiles, those in the highest quintile of IGF-1 tended to be younger, have higher IGFBP-3 concentrations, IGF-1:IGFBP-3 ratio, height, and weight; have more education; have greater energy adjusted starch and protein (total, animal, milk and vegetable protein) intake; less alcohol intake; and less heavy physical activity (P value ≤0.05).

Serum IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 ratio were not associated with pancreatic cancer (Table 3). These associations were not altered by inclusion of weight; BMI (kg/m^2); cigarettes smoked/day; pack-years; medical history of gallstones, pancreatitis, peptic and duodenal ulcers, diabetes mellitus, or bronchial asthma; occupational and leisure activity; and dietary energy, energy-adjusted fat, saturated fat, carbohydrate,

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

Characteristics	Cases (n = 93)	Non-cases (n = 400)	Wilcoxon P value
Age, yr	58 (54–61)	56 (52–60)	0.01
IGF-1, ng/ml	133.6 (107.5–159.7)	138.5 (113.4–174.5)	0.17
IGFBP-3, ng/ml	2267.9 (1949.1–2616.4)	2334.3 (1955.5–2827.1)	0.13
IGF-1:IGFBP-3 molar ratio	0.21 (0.19–0.25)	0.22 (0.19–0.25)	0.67
Height, cm	174 (171–179)	174 (170–178)	0.42
Weight, kg	79.1 (69.9–86.7)	79.5 (70.9–87.8)	0.53
BMI, kg/m^2	25.4 (23.7–27.7)	26.2 (23.9–28.9)	0.15
Smoking history			
Total cigarettes per day	20 (15–25)	20 (15–25)	0.38
Years smoked, yrs	40 (32–43)	35 (30–40)	0.006
Pack-years	40 (30–50)	35 (25–45)	0.02
Primary school education or less, n (%)	68 (73.1)	314 (78.5)	0.26
Living in city, n (%)	53 (57.0)	183 (45.8)	0.05[a]
Medical history			
Diabetes, n (%)	7 (7.5)	18 (4.6)	0.23[a]
Pancreatitis, n (%)	2 (2.2)	5 (1.3)	0.62[a]
Bronchial asthma	7 (7.5)	9 (2.3)	0.01[a]
Dietary intake per day			
Energy, kcal	2666 (2314–3032)	2721 (2314–3256)	0.31
Fat, g	119 (111–133)	119 (107–131)	0.26
Saturated fat, g	62 (54–70)	59 (49–68)	0.04
Carbohydrate, g	292 (266–320)	292 (263–330)	0.83
Protein, g	103.2 (94.3–110.1)	103.2 (94.3–110.2)	0.57
Alcohol	8.9 (3.7–29.6)	11.4 (2.2–26.9)	0.93
Physical activity			
Occupational			
Sedentary, n (%)	15 (16.1)	54 (13.5)	
Light, n (%)	16 (17.2)	81 (20.3)	
Moderate, n (%)	17 (18.3)	80 (20.0)	
Heavy, n (%)	3 (3.2)	36 (9.0)	
Nonworking, n (%)	42 (45.2)	149 (37.2)	0.25[a]
Leisure			
Sedentary, n (%)	39 (42.4)	172 (43.0)	
Light, moderate, n (%)	48 (52.2)	204 (51.0)	
Exercise to keep fit, n (%)	5 (5.4)	24 (6.0)	0.97[a]

[a] χ^2 or Fisher's exact test P value.

Table 2. Selected age-adjusted characteristics of sub-cohort control subjects by quintile of IGF-1, means, and 95% CI

Characteristics	IGF-1, ng/ml					P trend
	Q1	Q2	Q3	Q4	Q5	
	84.2 (80.3−88.2)	117.5 (115.3−119.6)	137.0 (134.3−139.7)	164.8 (161.1−168.6)	223.7 (215.2−232.2)	
Age, yrs	57.3 (56.2−58.5)	56.4 (55.4−57.5)	56.4 (55.4−57.5)	56.4 (55.3−57.5)	55.3 (54.3−56.4)	0.02
IGF-BP3, ng/ml	1789 (1696−1893)	2204 (2102−2308)	2355 (2252−2459)	2612 (2508−2715)	3032 (2929−3136)	<0.0001
IGF-1:IGFBP-3 molar ratio	0.18 (0.17−0.19)	0.20 (0.19−0.21)	0.22 (0.21−0.23)	0.24 (0.23−0.25)	0.27 (0.26−0.28)	<0.0001
Height, cm	172.36 (171.06−173.65)	173.52 (172.23−174.81)	173.67 (172.38−174.96)	173.81 (172.52−175.11)	175.71 (174.42−177.01)	0.0009
Weight, kg	76.71 (73.84−79.57)	81.43 (78.57−84.28)	79.91 (77.05−82.76)	80.52 (77.67−83.38)	83.70 (80.83−86.57)	0.005
BMI, kg/m^2	25.80 (24.94−26.67)	27.02 (26.16−27.89)	26.44 (25.58−27.31)	26.62 (25.75−27.48)	27.09 (26.23−27.96)	0.12
Smoking history						
Total cigarettes per day	20.8 (19.0−22.6)	21.5 (19.7−23.3)	21.0 (19.2−22.8)	19.5 (17.7−21.3)	19.7 (17.9−21.5)	0.14
Years smoked, yrs	36.2 (34.7−37.7)	34.3 (32.8−35.9)	34.7 (36.2−33.1)	33.5 (32.0−35.0)	35.4 (33.9−37.0)	0.34
Pack-years	37.6 (33.9−41.4)	37.0 (33.2−40.7)	36.7 (33.0−40.4)	33.3 (29.6−37.1)	33.9 (30.2−37.7)	0.07
Medical history						
Diabetes %	7.3 (3.3−15.4)	5.0 (1.9−12.5)	5.0 (1.9−12.5)	2.5 (0.6−9.4)	2.6 (0.6−9.6)	0.11
Pancreatitis %	1.3 (0.2−8.5)	1.2 (0.2−8.3)	2.5 (0.6−9.4)	0	1.2 (0.2−8.1)	0.71
Bronchial asthma %	3.6 (1.1−10.7)	0 (0−1.0)	3.7 (1.2−10.8)	2.5 (0.6−9.3)	1.3 (0.2−8.4)	0.67
Primary school education or less, %	83.5 (73.6−90.2)	81.3 (71.2−88.4)	80.0 (69.8−87.4)	78.9 (68.5−86.4)	69.3 (58.3−78.4)	0.03
Living in city, %	46.6 (35.9−57.6)	41.3 (31.0−52.3)	51.3 (40.4−62.0)	42.5 (32.2−53.5)	47.1 (36.4−58.1)	0.90
Dietary intake, per day[a]						
Energy, kcal	2852 (2668−3038)	2862 (2677−3046)	2773 (2585−2962)	2899 (2714−3084)	2787 (2603−2972)	0.75
Fat, g	124 (120−128)	126 (122−130)	122 (117−126)	123 (119−127)	122 (118−126)	0.32
Saturated fat, g	63 (59−66)	63 (60−67)	61 (57−64)	62 (59−65)	60 (57−64)	0.29
Carbohydrate, g	298 (288−308)	301 (290−311)	308 (298−319)	308 (298−318)	308 (297−318)	0.10
Starch, g	137.4 (129.8−145.0)	144.2 (136.7−151.8)	148.5 (140.8−156.2)	147.5 (140.0−155.1)	147.7 (140.2−155.3)	0.05
Free sugars, g	67.5 (60.5−74.5)	65.4 (58.4−72.4)	72.0 (64.9−79.2)	71.5 (64.5−78.5)	65.7 (58.7−72.7)	0.82
Sucrose, g	60.9 (54.2−67.6)	59.8 (53.2−66.5)	60.9 (54.1−67.8)	62.4 (55.7−69.0)	56.5 (49.8−63.1)	0.56
Protein, g	100 (97.3−103.5)	102.8 (99.7−105.9)	104.3 (101.1−107.5)	102.7 (99.6−105.9)	108.6 (105.3−111.6)	0.002
Animal protein, g	70.2 (66.6−73.8)	71.9 (68.3−75.5)	72.6 (69.0−76.2)	70.8 (67.2−79.9)	76.4 (72.8−79.9)	0.05
Milk protein, g	30.1 (27.0−33.1)	33.4 (30.3−36.5)	34.6 (31.5−37.8)	32.4 (29.3−35.5)	36.5 (33.4−39.6)	0.02
Vegetable protein, g	30.2 (28.6−31.8)	30.6 (29.0−32.1)	31.7 (30.1−33.3)	32.0 (30.4−33.5)	32.1 (30.5−33.7)	0.04
Alcohol intake, g	25.0 (19.8−30.2)	19.1 (13.9 24.3)	18.3 (13.1−23.6)	19.1 (13.9−24.2)	16.6 (11.4−21.8)	0.04
Physical activity						
Occupational %						
Nonworking, %	34.3 (23.3−47.2)	30.2 (20.1−42.6)	44.0 (32.0−56.7)	26.0 (16.8−38.8)	37.2 (25.4−50.4)	0.96
Sedentary, %	13.5 (0.08−23.1)	5.6 (2.3−13.1)	13.9 (7.9−23.3)	11.3 (6.0−20.2)	16.9 (10.2−26.8)	0.24
Light, %	13.6 (7.6−23.1)	21.3 (13.7−31.7)	16.3 (9.7−26.1)	16.1 (9.6−25.8)	26.4 (17.8−37.3)	0.13
Moderate, %	17.4 (10.4−27.6)	22.4 (14.5−33.0)	16.2 (9.6−26.0)	27.2 (18.4−38.2)	10.2 (5.3−18.4)	0.40
Heavy, %	12.3 (6.6−21.7)	12.7 (7.0−21.9)	4.5 (1.6−11.5)	8.9 (4.4−17.3)	3.0 (0.9−9.1)	0.02
Leisure						
Sedentary, %	50.1 (39.3−60.9)	44.5 (34.0−55.4)	45.7 (35.2−56.6)	41.9 (31.7−52.9)	36.2 (26.5−47.2)	0.09
Light, moderate, %	46.4 (35.8−57.3)	45.6 (35.1−56.5)	48.1 (37.5−58.9)	53.1 (42.2−63.7)	58.6 (47.6−68.8)	0.07
Exercise to keep fit, %	3.5 (1.1−10.4)	9.7 (4.9−18.3)	6.1 (2.5−13.8)	4.9 (1.8−12.3)	5.1 (1.9−12.7)	0.85

[a]Dietary variables energy adjusted except alcohol intake.

Table 3. Age and multivariable adjusted ORs and 95% CI of pancreatic cancer by baseline serum IGF-1, IGFBP-3, and IGF-1:IGFBP-3 molar ratio tertile among 93 cases and 400 sub-cohort control subjects

	Tertiles			P trend
	1	2	3	
IGF-1 (ng/ml)	<121.8	121.8–159.1	>159.1	
Case/control, n	35/133	34/134	24/133	
Age-adjusted OR (95% CI)	1.00 (referent)	1.00 (0.59–1.70)	0.73 (0.41–1.30)	0.26
Multivariable adjusted OR (95% CI)[a]	1.00 (referent)	0.97 (0.57–1.67)	0.67 (0.37–1.21)	0.17
Additionally adjusted for IGFBP-3 (95% CI)	1.00 (referent)	1.08 (0.60–1.94)	0.83 (0.40–1.73)	0.37
IGFBP-3 (ng/ml)	<2073	2073–2625	>2625	
Case/control, n	37/133	33/134	23/133	
Age-adjusted OR (95% CI)	1.00 (referent)	0.93 (0.55–1.59)	0.74 (0.41–1.33)	0.21
Multivariable adjusted OR (95% CI)[b]	1.00 (referent)	0.90 (0.52–1.54)	0.70 (0.38–1.27)	0.12
Additionally adjusted for IGF-1 (95% CI)	1.00 (referent)	1.02 (0.57–1.83)	0.78 (0.41–1.47)	0.51
IGF-1:IGFBP-3 molar ratio	<0.20	0.20–0.24	>0.24	
Case/control, n	37/133	23/134	33/133	
Age-adjusted OR (95% CI)	1.00 (referent)	0.62 (0.35–1.10)	0.86 (0.51–1.46)	0.57
Multivariable adjusted OR (95% CI)[c]	1.00 (referent)	0.60 (0.33–1.07)	0.85 (0.50–1.46)	0.54

[a]Adjusted for continuous age, years smoked, and height, and categorical education, and living in a city.
[b]Adjusted for continuous age, years smoked, and height, and categorical education.
[c]Adjusted for continuous age, years smoked, and height.

starch, free sugars, sucrose, and protein (total, animal, milk, and vegetable) intake; and alcohol consumption. Alternative categorization of serum IGF-1, IGFBP-3, and IGF-1:IGFBP-3 exposure as quartiles or quintiles showed similar nonsignificant associations for pancreatic cancer. With the exception of a positive nonsignificant association for pancreatic cancer with higher IGF-1:IGFBP-3 ratio among those who smoked ≤35 years (third tertile compared to first tertile, age- and height-adjusted OR = 1.72, 95% CI 0.72–4.12, P trend = 0.22) and an inverse association among those who smoked >35 years (third tertile compared to first tertile, age- and height-adjusted OR = 0.55, 95% CI 0.27–1.10, P trend = 0.08, P interaction = 0.04), there was no statistically significant effect modification of IGF-1, IGFBP-3, or IGF-1:IGFBP-3 ratio by BMI, height, weight, smoking dose (number of cigarettes smoked/day, years smoked, and pack-years), age, energy, energy-adjusted protein, and alcohol intake.

Discussion

To our knowledge, this is the first study to examine the relationship between pancreatic cancer and IGF-1 and IGFBP-3 measured in prospectively collected serum. We did not observe any significant associations between IGF-1, IGF-BP-3, or the IGF-1:IGFBP-3 ratio and exocrine pancreatic cancer. In addition, the associations for IGF-1 were not altered after adjusting for IGFBP-3 or *vice versa*.

An important strength of our study is its prospective nature with the blood samples from which the biochemical analytes were measured and other exposure data collected up to 12.7 years before the development of pancreatic cancer. Furthermore, to avoid the potential influence of subclinical cancer on IGF-1 and IGFBP-3, we excluded all cases diagnosed within the first 5 years of follow-up after their serum samples were collected. Our study has internal validity, as cases and controls were from the same study cohort, eliminating control selection bias. Although our population's overall IGF-1 concentrations tended to be slightly lower than that of nonsmoking populations, possibly in part owing to our

cohort's older age (50–69 years), our data showed relationships between IGF-1 and other exposures that are similar to those of other studies, for example, age and alcohol intake were inversely associated while IGFBP-3, height and dietary protein intake were positively associated with IGF-1 blood concentrations (Table 2), lending support to the external validity of our observations as well (19–25). These strengths give legitimacy to our observed lack of an association between IGF-1, IGFBP-3, and their molar ratio and pancreatic cancer.

Our study is consistent with the two previous small case-control studies that did not demonstrate differences in serum concentrations of IGF-1 or IGFBP-3 among pancreatic cancer cases as compared to controls. In the first study by Evans *et al.* (12), IGF-1 or IGFBP-3 concentrations in 20 pancreatic cancer cases were compared to those of 20 age-matched healthy hospital controls presenting for minor surgical procedures and found similar IGFBP-3 concentrations (2.3 *versus* 2.3 nM). The second study by Meggiato *et al.* (13) compared IGF-1 concentrations in 35 pancreatic cancer cases to that of 15 subjects with chronic pancreatitis, 15 with benign hepatobiliary diseases, 23 with benign or malignant gastrointestinal diseases, and 22 healthy control subjects. In both studies, pancreatic cancer cases had nonsignificantly lower mean serum IGF-1 concentrations than healthy non-cases (case *versus* non-case IGF-1 concentrations 13.0 *versus* 17 mM and 93.8 *versus* 103.3 g/l, for each respective study) (12, 13). In addition, the IGF-1 concentrations were not associated with disease stage in either study (12, 13) or tumor size in the latter study (13). As pancreatic cancer often presents with weight loss, malnutrition, and cachexia, conditions which are known to decrease IGF-1 levels (26), the disease status could have potentially influenced the observed associations in these prior retrospective studies limited by their case-control design and use of blood samples collected after the diagnosis.

Our observed lack of an association could possibly be related to the fact that all of our study subjects were middle-aged long-term smokers. The overwhelming effects of cigarette smoking on serum IGF axis protein concentrations could mask cancer associations and our

serum measures may not reflect chronic IGF-1 concentrations in the pancreatic tissue. Although the association is not consistent (27, 28), some have reported that current smokers have lower serum IGF-1 concentrations (19, 22, 29) and we observed a weak nonsignificant inverse association between smoking dose (cigarettes/day and pack-years) and serum IGF-1 concentrations within our smoker population (Table 2). Years of smoking appeared to modify the association for IGF-1:IGFBP-3 molar ratio. As the other smoking variables did not modify IGF-1:IGFBP-3 nor did any of the smoking variables modify IGF-1 or IGFBP-3 *per se*, this interaction may be a chance finding. Spitz *et al.* (25) noted that prospective associations between IGF-1 and cancer have only been reported for cancers that tobacco use is not a major predisposing factor. Because cigarette smoking is the most consistent risk factor for pancreatic cancer, with attribution for approximately 25% of the cancer overall (30), our lack of an association may be because IGF-1 and IGFBP-3 exposures are not important risk factors for pancreatic cancer among smokers. Alternatively, our serum IGF-1 and IGFBP-3 concentrations in middle-aged men may have not been measured during the physiologically relevant period for carcinogenesis.

IGFs and IGFBPs are secreted by several tumors and contribute to their malignant behavior (31). It has been suggested that serum IGF-1 and IGFBPs might represent tumor markers potentially useful for the early detection of malignancies including the exocrine pancreas (31, 32). A recent study in the ATBC smoker population suggested that serum IGF-1 may be a marker for prostate cancer rather than an etiological factor (33). As the latency of most cancers is unknown but likely to be several years for pancreatic cancer, care needs to be taken in the design, analysis, and interpretation of future studies to discriminate between etiological and tumor marker observations.

In conclusion, our data do not support the hypothesis that circulating IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 ratio are associated with exocrine pancreatic cancer in middle-aged male smokers. Our findings may not be generalizable to nonsmoking populations and caution is justified in the interpretation of these results. Further studies are necessary to evaluate these associations in both smoking, as well as nonsmoking populations with extended prospective follow-up and with specific consideration of the impact of follow-up time to diagnosis on the observed IGF-1 and IGFBP-3 associations.

Acknowledgments

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