Prediagnostic Plasma C-Peptide and Pancreatic Cancer Risk in Men and Women

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Abstract

Background: **Hyperinsulinemia and insulin resistance have been proposed as underlying mechanisms for the increase in pancreatic cancer among long-standing diabetics and obese individuals. An association between serum insulin levels and pancreatic cancer risk was reported in a recent study, but the population was composed of heavy smokers and their findings may not be generalizable to nonsmokers.**

Methods: **Pancreatic cancer cases and matched controls were obtained from four large-scale prospective cohorts to examine the association between prediagnostic plasma levels of C-peptide and insulin and pancreatic cancer. One hundred ninety-seven pancreatic cancer cases were diagnosed during a maximum of 20 years of follow-up, after excluding cases diagnosed within 2 years of blood collection or with baseline diabetes. We estimated OR and confidence intervals (CI) using conditional logistic regression with adjustment for pancreatic cancer risk factors.**

Results: **Prediagnostic plasma C-peptide was positively associated with pancreatic cancer risk (OR, 1.52; 95% CI, 0.87-2.64, highest compared with the lowest quartile, P_{trend} = 0.005). The association was not modified by body mass index or physical activity but seemed to be slightly stronger among never smokers than ever smokers. Fasting C-peptide and insulin were not related to pancreatic cancer; however, we observed a strong linear association for nonfasting C-peptide and pancreatic cancer (OR, 4.24; 95% CI, 1.30-13.8, highest versus lowest quartile, P_{trend} < 0.001).**

Conclusions: **Based on our finding of a strong positive association with nonfasting C-peptide levels, we propose that insulin levels in the postprandial state may be the relevant exposure for pancreatic carcinogenesis; however, other studies will need to examine this possibility.**

Introduction

With a 5-year survival rate of <5%, pancreatic cancer is one of the most rapidly fatal malignancies (1). Because pancreatic cancer is typically detected at a late stage, prevention could play an important role in reducing mortality from this disease. In recent years, type 2 diabetes has become a more widely accepted risk factor for pancreatic cancer; numerous studies noted that long-standing diabetes is associated with a significant increase in the subsequent risk of pancreatic cancer (2, 3). In addition, a growing number of studies link obesity to risk of pancreatic cancer (4-8), lending support to a role for obesity in the etiology of pancreatic cancer.

Hyperinsulinemia and insulin resistance have been proposed as underlying mechanisms through which diabetes and obesity may be linked to pancreatic cancer risk. Insulin and insulin resistance seem to play a key role in colon carcinogenesis (9), and animal studies suggest that these pathways may be important in pancreatic cancer as well (10, 11). In a recently published prospective study of Finnish male smokers, a 2-fold increased risk was observed for men in the highest quartile of fasting serum insulin compared with the lowest quartile [relative risk (RR), 2.01; 95% confidence interval (95% CI), 1.03-3.93; ref. 12].

Proinsulin is synthesized in pancreatic β cells and is enzymatically cleaved to create insulin and C-peptide, which are secreted into the portal circulation in equimolar amounts when insulin is required. The half-life of C-peptide in the circulation is between two and five times longer than that of the more rapidly changing levels of insulin, and due to its relative metabolic inertness, is a more stable biomarker for β-cell secretory activity. C-peptide measurements are preferable to insulin measurements because C-peptide is not removed by the liver, has slower metabolic clearance rate, and lacks cross-reactivity with antibodies to insulin (13).

To date, no study has examined plasma C-peptide levels in relation to pancreatic cancer risk. Moreover, the role of insulin and insulin resistance has not been

examined in women or nonsmokers. Therefore, we prospectively examined the association between plasma C-peptide, insulin, and pancreatic cancer risk in four large U.S. cohorts of healthy men and women in which plasma samples were collected at baseline, before any diagnosis of cancer.

Materials and Methods

Populations. To obtain sufficient numbers of prediagnostic bloods for this analysis, we drew on four large prospective studies: the Health Professionals Follow-up Study (HPFS), the Nurses' Health Study (NHS), the Physicians' Health Study (PHS), and the Women's Health Initiative Observational Study (WHI-OS). The HPFS was initiated in 1986 when 51,529 U.S. men ages 40 to 75 years responded to a mailed questionnaire. Participants of the HPFS are dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists. The NHS was established in 1976 when 121,700 female registered nurses ages 30 to 55 years responded to a mailed questionnaire. The PHS was a randomized, double-blind trial of aspirin and β-carotene among 22,071 male physicians, predominantly Caucasian, 40 to 84 years of age in 1982. The aspirin component of the trial was terminated in 1988 (14), the β-carotene component was terminated in 1995, and all study participants are actively followed as an observational cohort. The WHI-OS consists of 93,676 postmenopausal women of ages 50 to 79 years who were enrolled between 1994 and 1998. The health of these participants was tracked over an average of 8 years via annual health forms and a clinic visit 3 years after enrollment. A brief description of each cohort is summarized in Table 1.

For each study, individual characteristics and habits, including weight, height, smoking status, physical activity, and history of diabetes, were either obtained on the baseline questionnaires, or on subsequent questionnaires. Weight and height were measured at the baseline clinic visit in the WHI-OS. Additional details for these studies are available elsewhere (NHS ref. 15, HPFS ref. 16, PHS ref. 17, WHI-OS ref. 18). The National Death Index is frequently searched for nonrespondents; this method has been shown to have a sensitivity of 98% (19).

Blood Collection. Blood samples were collected from 32,826 of the NHS participants in 1989 to 1990, from 18,018 participants of the HPFS in 1993 to 1995, from 14,916 of the PHS at baseline (1982-1984), and on all 93,676 WHI participants at the first screening visit (1994-1998). All blood samples were continuously stored in well-monitored liquid nitrogen freezers from blood collection to their retrieval for analysis. Details on blood draw, transportation, and storage in these cohorts are provided elsewhere [NHS (20), HPFS (21), PHS (22), and WHI (23)].

Pancreatic Cancer Cases and Matched Controls. For this analysis, we included cases of pancreatic cancer that were diagnosed at least 2 years after the date of blood draw and through 2004. Incident cases were initially self-reported by cohort participants on annual or biennial questionnaires and then confirmed with medical records or pathology reports. In addition, given the high fatality rate of pancreatic cancer, cases were often identified through follow-up of reported deaths (notified by postal authorities or next-of-kin) or when searching National Death Index for nonrespondents. Medical records were also requested for deceased cases. Pancreatic cancer cases with a prior history of malignancy (other than non-melanoma skin cancer) were excluded from these analyses.

Eligible controls were cohort participants who were still alive and free of cancer at the date of the case's diagnosis and who had provided a blood sample. From among these participants, we randomly selected three controls for every case matching on cohort (which concurrently matched on sex), year of birth, smoking status (current, past, or never), fasting status (>8 h), and month of blood draw.

Given that C-peptide and insulin levels among diabetics are unlikely to reflect long-term exposure levels (due to changes from hyperinsulinemia to hypoinsulinemia during progress of disease), we excluded cases and controls who reported being diabetic before or at the time of blood draw (no exclusions were made for prior cardiovascular disease).

Laboratory Assays. Plasma C-peptide and insulin were assayed using ELISA with reagents from Diagnostic Systems Laboratory in the laboratory of Dr. Michael Pollak. Samples from matched sets were handled together, shipped together, and assayed in the same analytic run along with randomly inserted masked quality control samples. All laboratory personnel were blinded to the case/control/quality control status of the samples. The mean intra-assay coefficients of variation from the internal quality control samples (n = 24) were 7.3% for C-peptide and 5.5% for insulin.

Statistical Analysis. C-peptide and insulin were log-transformed to improve normality. To compare mean concentrations of C-peptide and insulin between matched cases and controls, we used the paired t test. To compare continuous characteristics between matched cases and controls, we used the nonparametric Wilcoxon signed rank test and for categorical variables we used the χ^2 test.

Table 1. Description of the four cohorts included in this analysis with details on blood collection

	HPFS	NHS I	PHS	WHI
Years of enrollment	1986	1976	1982	1994-1998
Age at entry (y)	40-75	30-55	40-84	50-79
Study design	Observational	Observational	Trial	Observational
Years of blood acquisition	1993-1995	1989-1990	1982-1984	1994-1998
No. bloods obtained	18,018	32,826	14,916	93,676
No. pancreatic cancer cases (with bloods)	29	46	54	68
No. pancreatic cancer cases (fasting)	21	32	0	68

To estimate matched odds ratios (OR) of pancreatic cancer, we used conditional logistic regression models and included indicator variables for quartiles of C-peptide and insulin levels with cutpoints based on the distributions of the controls. For C-peptide, cutpoints were determined to be cohort-specific a priori due to substantial differences in population characteristics (e.g., age, sex, study design; Table 1) and in storage time, collection and instructions of blood samples. For insulin, given small numbers (as only fasting insulin was measured), cutpoints were based on the three cohorts combined (as only a couple of cases in PHS had blood collected during fasting, insulin was not measured in this cohort). In multivariate analyses, we adjusted for body mass index (BMI), multivitamin use, physical activity, time between last meal and blood draw, and high smoking dose among current smokers (25+ cigarettes per day in HPFS and NHS, 20+ cigarettes/d in PHS; note that this is in addition to matching by smoking status for tighter control but due to small numbers of current smokers, further stratification of smoking dose was not feasible as the statistical models were too unstable). All covariates included in the multivariate models were measured before blood collection (1988 for NHS and 1992 for HPFS) or at the time of blood collection (PHS and WHI). To test for trend, we entered the quartile-specific median value as a continuous value into the logistic regression model. Stratified analyses were done for factors that could modify the relation between C-peptide and pancreatic cancer (i.e., BMI, sex, age, smoking, fasting status, and physical activity). To estimate the stratum-specific ORs and to maximize power, we ran the stratified unconditional logistic regression models adjusting for matching factors in addition to the other covariates in the conditional logistic model. For the stratified analysis by fasting status, we created new cutpoints based on the distribution of C-peptide in the controls of fasting and nonfasting separately, as the C-peptide levels in these two subgroups vary. To test for statistical interaction, we entered into the model the main effect terms and a term of the cross-product, the coefficient for which was evaluated by the Wald test. We examined the possibly nonlinear relation between C-peptide and the OR for pancreatic cancer nonparametrically with restricted cubic splines (24). Tests for nonlinearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. Analyses were conducted using SAS release 9.1 (SAS Institute). Two-sided P values are given for all tests.

Results

After excluding cases diagnosed within 2 years of blood collection or with baseline diabetes, 197 pancreatic cancer cases and 571 matched controls with C-peptide levels were available for this analysis. Insulin levels were measured in 120 cases and 343 matched controls with fasting blood samples. Table 2 provides characteristics for the four individual cohorts included in the analyses. BMI was substantially higher in cases than controls in the PHS cohort, but not in the other three cohorts. For each cohort individually, cases tended to exercise less and were slightly more likely to be multivitamin users than controls. Age, smoking, and fasting status were matching factors. C-peptide levels were slightly higher in cases than controls in the HPFS, NHS, and PHS cohorts, but these differences were not statistically significant. In the combined data, cases had significantly higher C-peptide levels than controls when measured in nonfasting bloods, but C-peptide levels were similar in cases and controls in fasting blood (bottom of Table 2).

In a conditional analysis, individuals in the highest quartile of plasma C-peptide experienced a significant increase in the risk of pancreatic cancer when compared with participants in the lowest quartile (RR, 1.63; 95% CI, 1.03-2.57; P_{trend} = 0.002; Table 3). This association was slightly attenuated after controlling for potential confounders (RR, 1.52, mostly due to controlling for BMI); but the multivariate test for trend remained statistically significant (P = 0.005). Although the associations between C-peptide and pancreatic cancer were not homogeneous across cohorts, statistical power to examine the individual cohorts was low (comparing highest versus lowest quartiles, NHS cases, 46; RR, 6.64; 95% CI, 1.65-26.7; HPFS cases, 29; RR, 1.07; 95% CI, 0.26-4.31; PHS cases, 54; RR, 2.36; 95% CI, 0.76-7.34; WHI cases, 68; RR, 0.70; 95% CI, 0.27-1.85). The strength of the association in the highest quartile of C-peptide was slightly greater after excluding cases and matched controls diagnosed within 4 years of blood collection (MV OR, 1.90; 95% CI, 0.97-3.73; Table 3).

Table 4 provides associations between C-peptide and pancreatic cancer risk in stratified analyses. No significant differences were observed when stratifying by age, sex, physical activity, and BMI. C-peptide seemed to be more strongly related to pancreatic cancer among never smokers (OR, 3.13; 95% CI, 1.30-7.54), than ever smokers (OR, 1.15; 95% CI, 0.58-2.28), but the interaction by smoking status was not statistically significant ($P_{interaction}$ = 0.33). In addition, plasma C-peptide was strongly related to pancreatic cancer in the nonfasting blood samples (OR, 4.24; 95% CI, 1.30-13.8, top versus bottom quartile, 60 cases), but not in the fasting blood samples (136 cases). The difference in the association with plasma C-peptide between nonfasting and fasting status was statistically significant ($P_{interaction}$ = 0.03; OR, 1.21; 95% CI, 0.66-2.24 for fasting bloods, for highest versus lowest C-peptide quartile). We observed a similarly null association for fasting C-peptide and pancreatic cancer after removing WHI data (OR, 1.17; 95% CI, 0.46-3.00, for highest versus lowest C-peptide quartile comparison), indicating that the null association for fasting C-peptide was not driven by a null finding in the WHI cohort study (as this cohort has the largest sample size and all had fasting bloods).

We further examined the shape of the relation between C-peptide and pancreatic cancer by fasting status using splines (Figs. 1 and 2). The association between C-peptide and pancreatic cancer was linear (P = 0.02) among the nonfasting bloods, but no association was observed in fasting bloods.

Fasting plasma insulin levels were not related to pancreatic cancer risk in this study in the main analysis or after excluding cases and matched controls from the first four years of follow-up (Table 5). The Spearman correlation between C-peptide and insulin levels was 0.49. In a secondary analysis, we examined whether individuals with both high insulin and high C-peptide

Table 2. Baseline characteristics of pancreatic cancer cases and matched controls nested in the HPFS (1992), NHS I (1988), PHS (1982), and WHI (1993-1998)

	Cases	Controls	*P**
HPFS	29	82	
C-peptide (ng/mL) geometric mean (IQ range)†	1.85 (0.92-2.81)	1.66 (1.19-2.01)	0.84
Insulin (μIU/mL) geometric mean (interquartile range)‡	11.5 (5.66-27.6)	12.0 (7.12-18.7)	0.40
Age (y)	63.7 (8.5)	63.1 (8.2)	
Height (in.)	70.1 (2.6)	69.7 (2.5)	0.32
BMI (kg/m^2)	25.6 (3.8)	25.6 (3.5)	0.85
MET-h/wk§	37.3 (47.4)	48.7 (56.9)	0.11
Smoking (%)			
Never	20.7	22.0	
Past	65.5	61.0	
Current	13.8	17.1	
Multivitamin use	51.7	50.6	0.92
Fasting samples (%)	72.4	73.2	
NHS	46	136	
C-peptide (ng/mL) geometric mean (IQ range)†	1.86 (1.20-2.85)	1.55 (1.07-1.98)	0.07
Insulin (μIU/mL) geometric mean (IQ range)‡	8.52 (3.29-19.3)	5.68 (1.00-12.4)	0.12
Age (y)	59.4 (6.4)	59.4 (6.4)	
Height (in.)	64.5 (2.3)	64.6 (2.4)	0.92
BMI (kg/m^2)	26.1 (5.8)	25.4 (4.5)	0.33
MET-h/wk§	12.7 (14.6)	17.9 (18.6)	0.008
Smoking (%)‖			
Never	37.0	37.5	
Past	34.8	40.4	
Current	28.2	20.6	
Multivitamin use (%)	52.2	44.4	0.36
Fasting samples (%)	69.6	70.2	
PHS	54	160	
C-peptide (ng/mL) geometric mean (IQ range)†	2.14 (1.31-3.58)	1.92 (1.22-2.83)	0.37
Age (y)	57.1 (8.0)	55.2 (6.8)	
Height (in.)	70.3 (2.4)	70.5 (2.7)	0.51
BMI (kg/m^2)	26.1 (3.0)	24.8 (2.6)	<0.0001
Smoking (%)			
Never	40.7	41.5	
Past	35.2	35.9	
Current	24.2	22.6	
Exercise (%)			
≥5 times/wk	13.0	16.3	0.83
2-4 times/wk	25.9	26.3	
1 time/wk	22.2	21.9	
<1 time/wk	38.9	35.6	
Multivitamin use (%)	29.6	19.0	0.10
Fasting samples (%)	29.6	28.1	
WHI	68	193	
C-peptide (ng/mL) geometric mean (IQ range)†	1.36 (0.95-1.92)	1.33 (0.96-1.94)	0.79
Insulin (μIU/mL) geometric mean (IQ range)‡	9.57 (5.10-17.5)	9.11 (4.66-18.8)	0.77
Age (y)	66.8 (7.0)	67.1 (7.0)	
Height (in.)	64.3 (2.8)	64.3 (3.2)	0.99
BMI (kg/m^2)	27.5 (5.3)	27.8 (7.1)	0.79
Smoking (%)			
Never	41.2	41.0	
Past	50.0	50.3	
Current	8.8	8.7	
Exercise (%)			
≥5 times/wk	9.0	13.5	0.72
2-4 times/wk	43.3	43.5	
1 time/wk	14.9	15.5	
<1 time/wk	32.8	27.5	
Multivitamin use (%)	39.7	35.9	0.58
Fasting samples (%)	100	100	
Cohorts combined	197	571	
C-peptide (ng/mL) geometric mean (IQ)†			
Nonfasting	2.71 (2.02-4.44)	2.17 (1.44-3.26)	0.03
Fasting	1.40 (1.01-2.03)	1.39 (0.99-1.86)	0.80

*P values for plasma C-peptide and insulin are estimated from paired t test, Wilcoxon signed rank for all other continuous variables, and χ^2 for categorical variables.

†Interquartile range, 25% to 75%.

‡Insulin was only measured in fasting bloods (NHS 31 cases, 91 controls; HPFS 21 cases, 60 controls; WHI 68 cases, 192 controls; PHS, nonfasting samples not sent for analysis).

§Metabolic equivalent-hour (MET-h)/wk, sum of the average time/wk spent in each activity × metabolic equivalent value of each activity.

‖Percent does not add up to 100 due to missing data.

Table 3. RR (95% CI) for pancreatic cancer according to quartiles of plasma C-peptide in HPFS, NHS, PHS, and WHI cohorts

Cases/controls	Quartiles of serum C-peptide				P_{trend}
	1 (low)	2	3	4 (high)	
	46/144	40/141	36/143	75/143	
RR (95% CI)					
Matching factors*	1.0	0.86 (0.53-1.41)	0.79 (0.48-1.30)	1.63 (1.03-2.57)	0.002
Multivariable †	1.0	0.82 (0.49-1.38)	0.78 (0.45-1.35)	1.52 (0.87-2.64)	0.005
Excluding cases diagnosed <4 y after blood draw					
Cases/controls	29/103	30/91	27/107	51/98	
RR (95% CI)					
Matching factors*	1.0	1.13 (0.63-2.04)	0.92 (0.51-1.66)	1.79 (1.02-3.13)	0.01
Multivariable †	1.0	1.10 (0.59-2.05)	0.99 (0.52-1.86)	1.90 (0.97-3.73)	0.01

NOTE: Cohort specific cutpoints (quartiles 1-4): HPFS <1.20, 1.20-1.52, 1.53-2.01, >2.01; NHS <1.08, 1.08-1.44, 1.45-1.98, >1.98; PHS <1.23, 1.23-1.82, 1.83-2.83, >2.83; WHI <0.98, 0.98-1.32, 1.33-1.94, >1.94.

*Matching factors include age, month of blood draw, smoking status (never, past, current), cohort, and fasting status.

†Additionally controlling for BMI (quartiles), physical activity (quartiles), current multivitamin use, high smoking dose (25+ cigarettes/d for HPFS and NHS; 20+ cigarettes/d for PHS), and time between last meal and blood draw.

(top quartile for both) were at a higher risk compared with those with low insulin and low C-peptide (bottom three quartiles for both) but we observed no significant association for this comparison (OR, 1.30; 95% CI, 0.62-2.66).

Discussion

In this nested case-control study of four large prospective cohort studies, we observed a statistically significant positive trend between plasma C-peptide levels and the subsequent risk of pancreatic cancer. The association was more apparent among never smokers, although the test for interaction was not statistically significant. Of particular interest, the association between C-peptide and pancreatic cancer was limited to those participants who provided nonfasting blood specimens. Fasting levels of plasma C-peptide as well as fasting plasma insulin levels were not associated with pancreatic cancer.

To date, elevated postload and fasting glucose levels have been consistently associated with an increased risk of pancreatic cancer in prospective studies with RRs ranging between 1.7 and 4.0 (12, 25-27). Furthermore, a high glycemic load (based on dietary intake) was associated with elevated pancreatic cancer risk in the NHS (28), suggesting that diet can similarly influence pancreatic carcinogenesis.

Table 4. RRs (unconditional regression) for pancreatic cancer according to quartiles of plasma C-peptide in HPFS, NHS, PHS, and WHI cohorts, by age, sex, smoking status, BMI, and physical activity level

	Cases/controls	RR* for quartiles of serum C-peptide				P_{trend}
		1	2	3	4	
Age (y)						
≤62	96/312	1.0	0.65 (0.31-1.33)	0.72 (0.32-1.59)	1.64 (0.75-3.59) †	0.01
>62	101/259	1.0	1.33 (0.62-2.82)	1.04 (0.49-2.21)	1.92 (0.89-4.15)	0.03
Sex						
Women	114/329	1.0	1.11 (0.58-2.15)	0.96 (0.48-1.95)	1.65 (0.80-3.40)	0.05
Men	83/242	1.0	0.46 (0.20-1.06)	0.46 (0.19-1.07)	1.39 (0.61-3.17)	0.03
Smoking status						
Never ‡	73/215	1.0	0.65 (0.27-1.54)	0.86 (0.34-2.21)	3.13 (1.30-7.54)	<0.001
Ever	124/356	1.0	1.07 (0.56-2.03)	0.77 (0.40-1.48)	1.15 (0.58-2.28)	0.46
BMI						
<25	82/274	1.0	0.79 (0.40-1.56)	0.70 (0.31-1.58)	2.14 (0.91-5.03)	0.08
≥25	114/296	1.0	0.79 (0.35-1.81)	0.74 (0.33-1.62)	1.28 (0.60-2.73)	0.03
Physical activity						
Low	116/297	1.0	0.88 (0.44-1.76)	0.49 (0.23-1.06)	1.32 (0.64-2.72)	0.04
High	81/274	1.0	0.73 (0.33-1.59)	1.13 (0.53-2.43)	1.82 (0.78-4.25)	0.04
Fasting§						
Yes	136/393	1.0	0.79 (0.46-1.37)	0.81 (0.45-1.44)	1.21 (0.66-2.24)	0.19
No	60/177	1.0	1.21 (0.35-4.25)	0.86 (0.22-3.29)	4.24 (1.30-13.8)	<0.001

*Controlling for age, month of blood draw, smoking status (never, past, current), cohort, fasting status, BMI (quartiles), physical activity (quartiles), current multivitamin use, and high smoking dose (25+ cigarettes/d for HPFS and NHS; 20+ cigarettes/d for PHS).

†95% CI for top quartile.

‡Controlling for age, month of blood draw, cohort, fasting status, BMI (quartiles), physical activity (quartiles), current multivitamin use, and time between last meal and blood draw.

§Test for interaction $P = 0.02$.

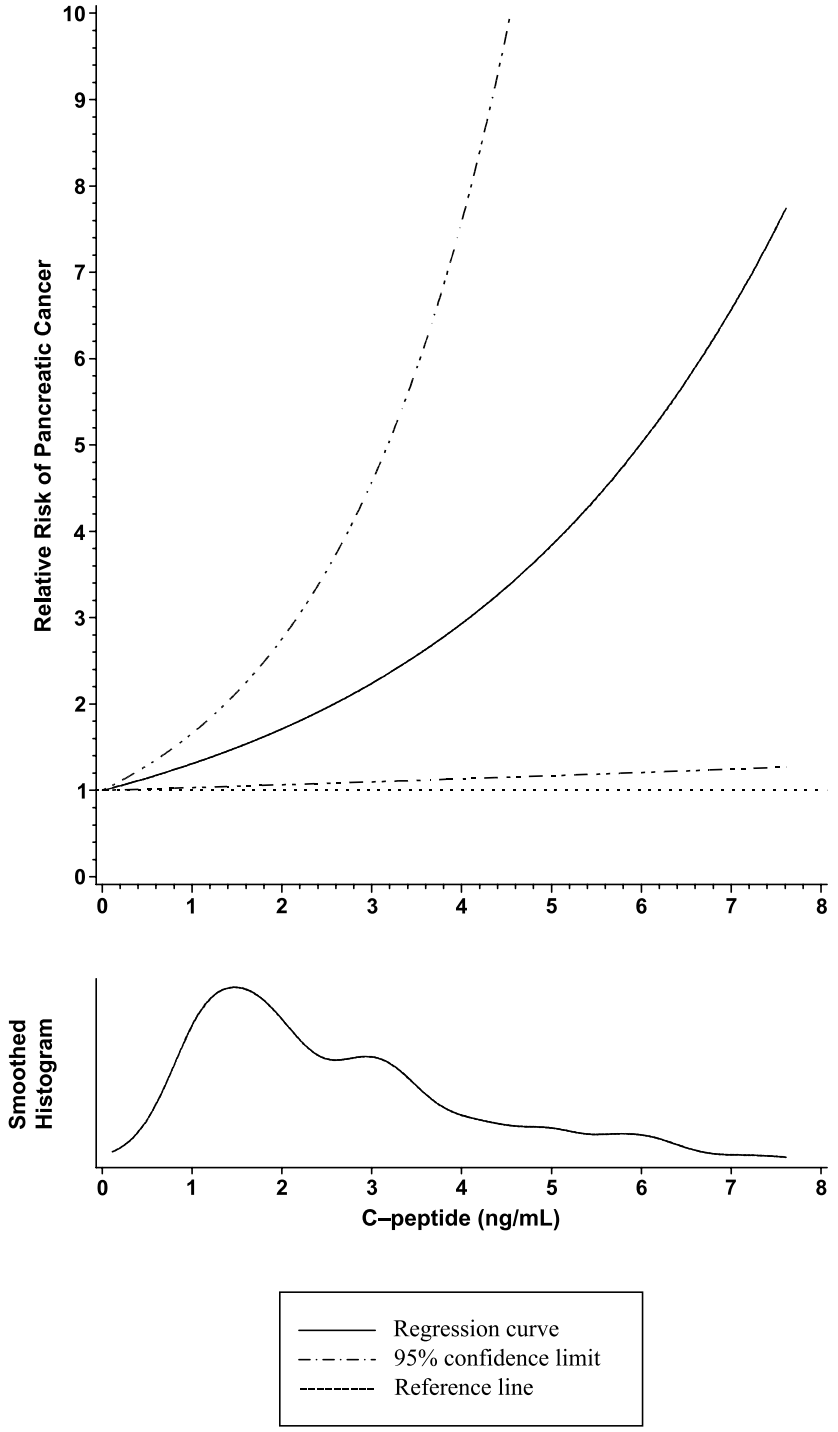

Figure 1. Plasma C-peptide and pancreatic cancer risk among nonfasting individuals.

Although some data support a direct role for insulin in pancreatic carcinogenesis, mainly through its growth-promoting properties (29, 30), it is not known whether insulin per se is the underlying factor that explains the higher rates of pancreatic cancer risk among diabetics and obese individuals. Other studies suggest that peripheral insulin resistance and islet cell proliferation, but not insulin itself, play an important role in exocrine pancreatic cancer (11). In a recent prospective study of male smokers, fasting insulin levels were more strongly associated with pancreatic cancer risk than glucose levels or estimated insulin resistance, although all three were significantly associated with risk. Nonetheless, in that study, the relation between fasting insulin and pancreatic cancer was significant only among cases diagnosed 10 or more years after the baseline blood draw (12).

In the current study, the positive association between plasma C-peptide was limited to bloods obtained in a

nonfasting state. The influence of fasting status on the relation between C-peptide and pancreatic cancer may explain why, in the main analysis that included both fasting and nonfasting specimens, the overall association did not appear linear and was somewhat modest in strength. In a recent NHS analysis of colorectal cancer, the observed association with C-peptide was similarly stronger among nonfasting blood specimens and was markedly attenuated in fasting participants (21). Other studies on colon cancer risk have observed similar findings for insulin as well as for C-peptide (31), suggesting that postprandial insulin may be a better measure for the association with cancer risk than fasting insulin levels. In a previous study, fewer meals per day and a lower caloric intake both conferred a lower risk of pancreatic cancer (OR, 0.5; 95% CI, 0.3-0.96, for 1 versus ≥3 meals per day; ref. 32), supporting the hypothesis that postprandial insulin exposure may be important.

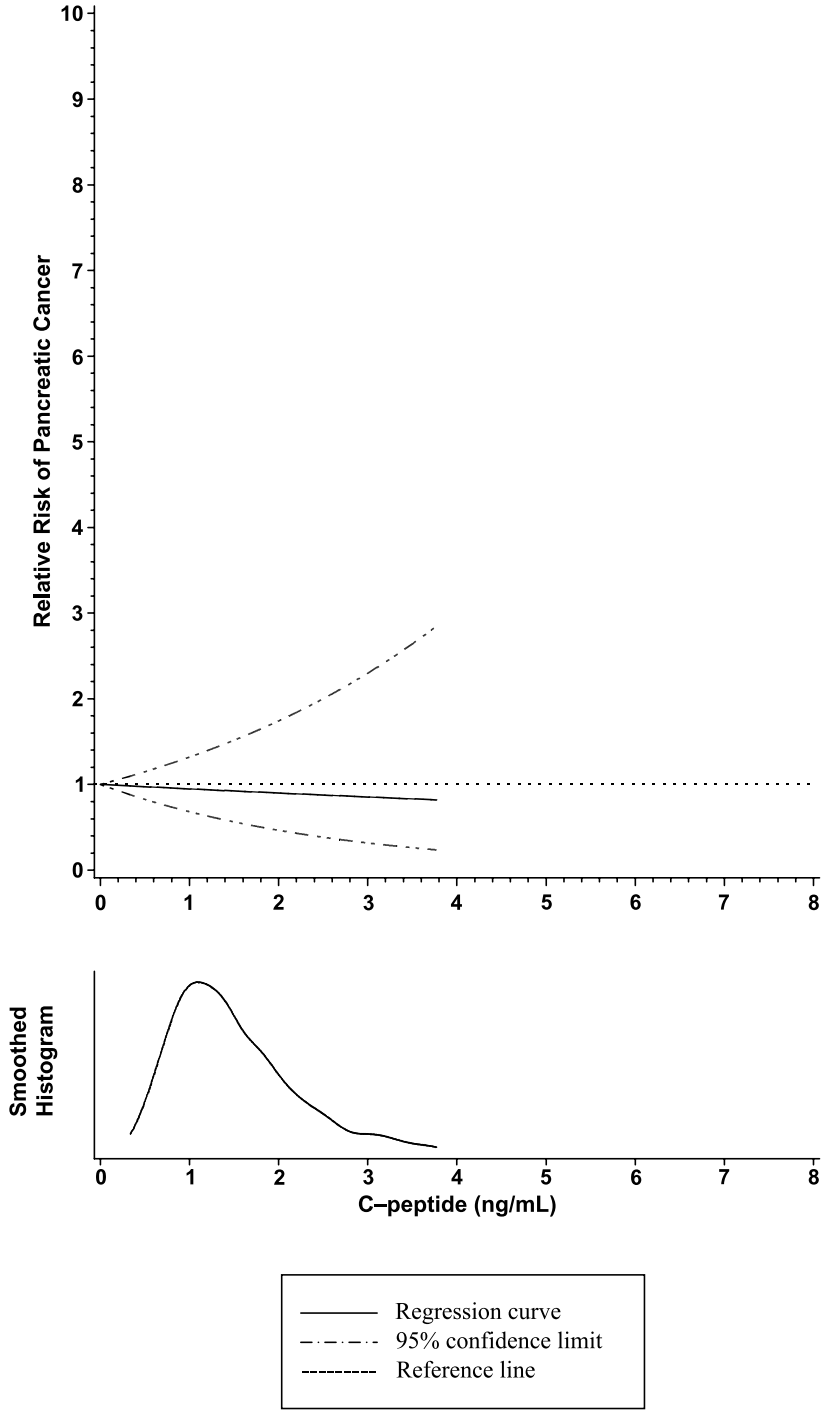

Figure 2. Plasma C-peptide and pancreatic cancer risk among fasting individuals.

Table 5. RRs (95% confidence intervals) for pancreatic cancer according to quartiles of plasma insulin in HPFS, NHS and WHI cohorts

	Quartiles of insulin				P_{trend}
	1	2	3	4	
Insulin (μIU/mL), median	1.0	6.5	12.3	31.8	
Cases/controls	27/85	32/86	29/87	32/85	
RR (95% CI)					
Match*	1.0	1.19 (0.65-2.21)	1.06 (0.57-1.99)	1.24 (0.68-2.28)	0.57
Multivariable †	1.0	1.12 (0.59-2.13)	0.99 (0.51-1.91)	1.08 (0.57-2.04)	0.90
Excluding cases diagnosed <4 y after blood draw					
Cases/controls	19/53	21/56	17/55	14/41	
RR (95% CI)					
Match*	1.0	1.06 (0.50-2.26)	0.89 (0.40-1.95)	1.02 (0.45-2.31)	0.98
Multivariable †	1.0	0.92 (0.41-2.07)	0.76 (0.32-1.80)	0.86 (0.36-2.09)	0.77

*Matching factors include age, month of blood draw, smoking status, cohort. The PHS cohort is not included as fasting samples were not available on the majority of participants.
†Additionally controlling for BMI (quartiles), physical activity (quartiles), current multivitamin use, and high smoking dose (25+ cigarettes/d).

We did not observe an association between fasting plasma insulin levels and pancreatic cancer in this study (insulin was not measured in nonfasting bloods). This finding is consistent with the lack of association between fasting plasma C-peptide and pancreatic cancer in our study population. However, it is not consistent with the previous observation from the prospective study of Finnish male smokers where fasting insulin was positively associated with pancreatic cancer (12). However, as stated above, that study failed to detect a significant influence of fasting insulin when limited to cancers detected within 5 to 10 years after blood collection, which is similar to our range of follow-up. Moreover, these two populations are not directly comparable; one consists of Finnish male smokers, whereas our study represents healthy men and women with a low prevalence of current smokers at baseline. Of interest is that no association was observed for BMI and pancreatic cancer in the Finnish population (33), whereas we have previously reported strong positive associations for BMI in two of the four cohorts included in this analysis (the NHS and HPFS; ref. 4). One may speculate whether mechanisms of pancreatic carcinogenesis differ in these substantially different populations.

The strengths of our study include its prospective design, high follow-up rates in the individual cohorts, relatively large number of incident pancreatic cancer cases, matching on potential confounders to increase efficiency, and additionally controlling for potential confounders. The limitations of this study include potential residual confounding as a result of combining data from four cohorts; specifically some of the variables, such as physical activity, were not measured in the same manner across the four different cohorts. Another limitation is that we had only one plasma measurement per individual, which could potentially generate misclassification over time. However, previous studies suggest that plasma C-peptide levels are relatively stable over time (within-person correlation coefficient was 0.57 between two measures of fasting bloods taken 4 years apart; ref. 34). In addition, we cannot exclude the possibility that measurement error was introduced in the laboratory assays, although our internal quality control data, which showed a relatively low coefficient of variability, suggest that the measurements were highly reliable. Moreover, any misclassification in plasma C-peptide that resulted from either change in levels over time or random measurement error would have biased our results toward the null. Finally, because 75% of cases in our study were diagnosed within 8 years of blood collection, we did not have the power to examine the association between plasma C-peptide and pancreatic cancer after a 10-year lag period. However, to minimize possible reverse causation, we excluded cases diagnosed in the first 2 years of follow-up from all our analyses; moreover, when we repeated our analysis after excluding cases diagnosed within the first 4-years, we observed similar associations.

Because this analysis pooled blood specimens from four distinct cohort populations, we used cohort-specific quantiles for C-peptide rather than pooling the raw data across cohorts, given the differences in the study participants and specimen collection. Although our analysis strongly supports the hypothesis that ''high'' levels of nonfasting C-peptide influence the risk of pancreatic cancer, the determination of a specific ''high-risk'' level of plasma C-peptide for future preventive guidelines would likely require a study of plasma C-peptide within a single, large prospective cohort with a sufficiently homogenous method of sample collection.

The 4-fold elevation in pancreatic cancer risk associated with elevated nonfasting plasma C-peptide levels in our study suggest that insulin levels in the postprandial state may be the most relevant exposure for pancreatic carcinogenesis. Additional studies are needed to examine the role of postprandial insulin and confirm our findings with nonfasting plasma C-peptide. More generally, however, our findings provide additional evidence that insulin is involved in pancreatic carcinogenesis and confirm a potential mechanism for the relationship between diabetes, obesity, and pancreatic cancer.

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