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Pancreatic Cancer Risk Associated with Prediagnostic Plasma Levels of Leptin and Leptin Receptor Genetic Polymorphisms

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Abstract

Leptin is an adipokine involved in regulating energy balance, which has been identified as a potential biologic link in the development of obesity-associated cancers, such as pancreatic cancer. In this prospective, nested case–control study of 470 cases and 1,094 controls from five U.S. cohorts, we used conditional logistic regression to evaluate pancreatic cancer risk by prediagnostic plasma leptin, adjusting for race/ethnicity, diabetes, body mass index, physical activity, plasma C-peptide, adiponectin, and 25-hydroxyvitamin D. Because of known differences in leptin levels by gender, analyses were conducted separately for men and women. We also evaluated associations between 32 tagging SNPs in the leptin receptor (*LEPR*) gene and pancreatic cancer risk. Leptin levels were higher in female versus male control participants (median, 20.8 vs. 6.7 ng/mL; $P < 0.0001$). Among men, plasma leptin was positively associated with pancreatic cancer risk and those in the top quintile had a multivariable-adjusted OR of 3.02 [95% confidence interval (CI), 1.27–7.16; $P_{trend} = 0.02$] compared with men in the bottom quintile. Among women, circulating leptin was not associated with pancreatic cancer risk ($P_{trend} = 0.21$). Results were similar across cohorts ($P_{heterogeneity} = 0.88$ for two male cohorts and 0.35 for three female cohorts). In genetic analyses, rs10493380 in *LEPR* was associated with increased pancreatic cancer risk among women, with an OR per minor allele of 1.54 (95% CI, 1.18–2.02; multiple hypothesis-corrected $P = 0.03$). No SNPs were significantly associated with risk in men. In conclusion, higher prediagnostic levels of plasma leptin were associated with an elevated risk of pancreatic cancer among men, but not among women. *Cancer Res; 76(24); 7160–7. ©2016 AACR.*

Introduction

Pancreatic cancer is the third leading cause of cancer-related death in the United States (1). Obesity is associated with increased pancreatic cancer risk (2, 3), but the underlying mechanisms are poorly understood. Leptin was initially discovered in studies of obese mice, which were noted to have increased caloric intake. Subsequent studies demonstrated that the underlying cause of the obese phenotype in these mice was a truncating mutation in the gene encoding for leptin (4). Leptin is a hormone synthesized almost exclusively in adipocytes, and plasma levels of leptin in humans are proportional to total body adipose tissue (5). When circulating levels of leptin are increased, downstream signaling is activated through the ObR [HUGO Gene Nomenclature Committee (HGNC) Symbol LEPR] transmembrane receptor on target cells (6). The leptin receptor is present on cells within the hypothalamus, which is the mechanism by which leptin is thought to

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regulate caloric intake (7). In addition, leptin receptor expression is distributed widely throughout the body, including on cells within the pancreas (8). Leptin receptors are also present on tumor cells and activation of these receptors increases cancer cell proliferation and reduces rates of cancer cell apoptosis (9).

Given its essential role in regulating energy balance, leptin may be an important biologic link in the development of obesity-associated malignancies, including pancreatic cancer. To further investigate the role of leptin in pancreatic carcinogenesis, we examined the association between prediagnostic plasma leptin and subsequent pancreatic cancer risk in 5 prospective cohorts with up to 26 years of follow-up since blood collection. We additionally evaluated the association between polymorphisms in *LEPR* with risk of pancreatic cancer.

Patients and Methods

Study participants

We pooled blood samples and data from 5 U.S. prospective cohorts. The Health Professionals Follow-up Study (HPFS) enrolled 51,529 male health professionals aged 40–75 in 1986. The Nurses' Health Study (NHS) enrolled 121,700 female nurses aged 30–55 in 1976. The Physicians' Health Study I (PHS) was a randomized clinical trial of aspirin and β-carotene that enrolled 22,071 healthy male physicians aged 40–84 in 1982. The aspirin component of the trial ended in 1988, whereas the β-carotene component ended in 1995, and PHS I participants continue follow-up as an observational cohort. The Women's Health Initiative (WHI)-Observational Study enrolled 93,676 postmenopausal women aged 50–79 between 1994 and 1998. The Women's Health Study (WHS) was a randomized clinical trial of low-dose aspirin and vitamin E that enrolled 39,876 healthy female health professionals aged ≥45 in 1992. The trial was completed in 2004 and WHS participants continue to be followed as an observational cohort.

Individual characteristics and lifestyle factors were obtained from baseline questionnaires at enrollment in PHS, WHI, and WHS and from the questionnaires preceding blood draw in HPFS and NHS. Details of these cohorts have been described previously (10–14). The current study was approved by the Human Research Committee at the Brigham and Women's Hospital (Boston, MA) and participants provided informed consent.

Blood collection and plasma assays

Blood samples were collected from 18,225 men in HPFS from 1993–1995, 32,826 women in NHS from 1989–1990, 14,916 men in PHS from 1982–1984, 93,676 women in WHI from 1994–1998, and 28,345 women in WHS from 1992–1995. Details on blood draw, transportation, and storage have been described previously (12, 14–16).

Plasma leptin was assayed in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA), using reagents from R&D Systems. Measurement of plasma adiponectin and C-peptide was described previously (17). All samples for leptin and adiponectin were handled identically in a single batch and C-peptide was handled in 2 batches. Laboratory personnel were blinded to case or control status. The mean intra-assay coefficients of variance for quality control samples were ≤10% for each biomarker.

Pancreatic cancer cases and matched controls

We included cases of pancreatic adenocarcinoma diagnosed through 2008 with prediagnostic blood and no prior history of cancer, except non-melanoma skin cancer. Incident cases were identified by self-report or during follow-up of a participant's death. Deaths were ascertained from next-of-kin or the U.S. postal service and by searching the National Death Index. Medical records of the cases were requested and reviewed by study physicians blinded to exposure data.

Eligible controls were cohort participants who provided blood and were alive and free of cancer at the date of the case's diagnosis. We randomly selected 2–3 controls for each case, matching on year of birth (±5 years), prospective cohort (which concurrently matched on sex), smoking status (never, past, current), fasting status (fasting, non-fasting), and month/year of blood draw.

For the present analysis, 488 pancreatic cancer cases and 1,132 matched controls with plasma were available. Because of concern regarding the possible influence of subclinical malignancy, we excluded pancreatic cancer cases diagnosed within 1 year of blood draw ($n = 19$) and their matched controls ($n = 38$), resulting in a total of 470 cases and 1,094 controls (Supplementary Table S1). Of these 470 cases, 465 (99%) were confirmed by review of medical records, tumor registry data, or death certificates.

SNP selection and genotyping

A total of 39 SNPs in the *LEPR* gene ± 20 kb was selected with the tagger algorithm in Haploview, using $r^2 = 0.8$ and minor allele frequency (MAF) ≥5% among Whites from the HapMap Project database. Five SNPs associated with: serum amyloid A (rs1275319; ref. 18), soluble Ob-R (rs2767485; ref. 19), and CRP: [rs4420065 (ref. 20), rs6700896 (ref. 21), and rs1892534 (ref. 22)] were forced in. From 412 cases (Supplementary Table S1), DNA was extracted from buffy coat using QIAGEN QIAmp and whole-genome amplified using GE Healthcare GenomiPhi. Genotyping was performed at Partners HealthCare Center for Personalized Genetic Medicine using a custom-designed Illumina Golden Gate genotyping assay. Seven tagging SNPs were not supported by the platform. One SNP (rs913199) deviated from Hardy–Weinberg equilibrium at $P = 0.008$. Replicate samples tested for quality control ($n = 44$ groups) had a mean genotype concordance of 98.2%.

Statistical analysis

Median leptin levels among cases and controls were compared using the Wilcoxon rank-sum test. Because men and women have different distributions of leptin levels (23), we performed separate analyses by gender using pooled gender-specific quintiles from controls. To compute ORs and 95% confidence intervals (CI), we used conditional logistic regression. In multivariate models, we adjusted for potential confounding factors, including race (White, Black, other), multivitamin use (yes, no), diabetes (yes, no), body mass index (BMI; kg/m^2), physical activity (MET-h/wk), plasma C-peptide (continuous), plasma adiponectin (quartiles, as previous analysis demonstrated non-linear association of adiponectin and pancreatic cancer risk; ref. 17), and plasma 25-hydroxyvitamin D [25(OH)D; continuous]. P_{trend} values were calculated by the Wald test of a score variable that contained median values of quintiles. We also conducted a meta-analysis of cohort-level data among men and women. We calculated ORs for each cohort and then pooled the ORs to compute a summary OR by gender using the random effects model (24). Heterogeneity across studies was tested using the *Q*-statistic (24). To evaluate whether the association between leptin and pancreatic cancer risk was log-linear, we compared the model fit including linear and cubic spline terms to

the model fit with only the linear term using the likelihood ratio test (25). We conducted subgroup analyses using unconditional logistic regression adjusted for the matching factors and covariates. Tests for interaction were performed by the Wald test of cross-product terms. We conducted sensitivity analyses excluding diabetics or cases diagnosed within 2 or 4 years from blood draw.

We examined the association between *LEPR* SNPs and pancreatic cancer risk by modeling each genotype as number of copies of the minor allele (additive model) using conditional logistic regression. We used R software (version R.3.2.2.) to calculate the corrected *P* value by taking into account the total number of comparisons, as well as correlations between 32 SNPs (26). We used HaploReg v4.1 to explore the noncoding functional characteristics of identified and highly correlated SNPs ($r^2 > 0.6$ in 1000G CEU data). Statistical analyses were performed with SAS 9.1 (SAS Institute), and all *P* values are 2-sided.

Results

The median time between blood collection and cancer diagnosis was 7.1 years among cases. Among controls, median plasma leptin was 20.8 ng/mL for women and 6.7 ng/mL for men. Leptin levels were comparable across studies for men (HPFS and PHS) and for women (NHS, WHI, and WHS; Supplementary Table S2). Individuals with higher leptin levels had higher BMI, plasma C-peptide, and prevalence of diabetes (Table 1). After adjusting for age, fasting status, and cohort, Spearman correlation coefficients for plasma leptin and BMI were 0.50 ($P < 0.0001$) among men and 0.73 ($P < 0.0001$) among women (Fig. 1; Supplementary Table S3), similar to those reported in other populations (27, 28).

We observed a positive association between plasma leptin and pancreatic cancer risk among men, but not among women ($P_{heterogeneity} = 0.02$; Table 2). In the base model conditioned on matching factors, compared with the bottom quintile, men in the top quintile had an OR of 2.77 (1.37–5.61; $P_{trend} = 0.01$; Table 2). In comparison, women in the top quintile had an OR of 1.27 (0.84–1.91; $P_{trend} = 0.64$; Table 2). Further adjustment for race, multivitamin use, plasma 25(OH)D, history of diabetes, BMI, physical activity, plasma C-peptide, and plasma adiponectin yielded similar results (Table 2). Similar associations were observed in sensitivity analyses when we excluded cases with

Table 1. Age- and study-standardized baseline characteristics according to plasma leptin levels among controls

	Quintiles of plasma leptin				
Characteristic[a]	**1**	**2**	**3**	**4**	**5**
Men					
Plasma leptin level, ng/mL	≤3.0	3.1–4.8	4.9–7.8	7.9–11.7	≥11.8
No. of controls	69	71	71	72	70
Age at blood draw, y	60.7 (10.1)	60.1 (8.9)	60.2 (10.0)	60.8 (8.9)	61.0 (9.1)
Race, %					
White	94.0	93.2	85.5	93.7	91.3
Black	0	1.0	0	0	0
Other	6.0	5.9	14.5	6.3	8.7
BMI, kg/m^2	23.4 (2.6)	24.7 (2.1)	24.8 (2.3)	26.0 (2.5)	27.6 (3.6)
Physical activity, MET-h/wk	34.6 (34.9)	29.1 (31.6)	30.6 (48.6)	27.2 (34.6)	18.1 (22.6)
Cigarette smoking, %					
Never	37.0	36.9	37.3	40.9	33.6
Past	52.9	50.4	40.7	41.1	47.8
Current	10.2	12.7	22.0	18.1	18.6
Missing	0	0	0	0	0
History of diabetes mellitus, %	1.0	2.5	2.2	1.6	7.3
Regular multivitamin use, %	37.8	37.1	36.0	30.2	30.2
Plasma 25(OH)D, nmol/L	76.9 (28.9)	74.5 (32.0)	72.3 (20.7)	70.2 (20.9)	66.3 (19.5)
Plasma C-peptide levels, ng/mL	1.8 (1.1)	2.2 (1.4)	2.5 (1.6)	2.8 (3.4)	3.1 (2.9)
Plasma adiponectin levels, μg/mL	6.0 (3.2)	5.9 (2.8)	6.0 (3.3)	5.8 (3.2)	5.7 (4.1)
Women					
Plasma leptin level, ng/mL	≤9.5	9.6–17.7	17.8–24.8	24.9–37.7	≥37.8
No. of controls	148	146	150	148	149
Age at blood draw, y	63.3 (8.3)	63.2 (7.9)	64.3 (8.3)	63.1 (7.3)	63.1 (7.8)
Race, %					
White	89.2	95.8	94.9	92.1	93.9
Black	2.4	0.6	0.9	4.6	4.1
Other	8.4	3.6	4.2	3.4	2.1
BMI, kg/m^2	21.8 (2.6)	24.2 (3.9)	25.7 (3.7)	27.4 (3.2)	31.3 (5.2)
Physical activity, MET-h/wk	21.7 (20.4)	17.4 (18.2)	15.3 (16.8)	13.0 (13.8)	12.0 (11.8)
Cigarette smoking, %					
Never	41.1	43.6	52.9	37.7	50.7
Past	42.8	44.3	38.5	48.7	42.4
Current	14.7	10.7	8.1	13.0	6.0
Missing	1.4	1.5	0.5	0.7	1.0
History of diabetes mellitus, %	1.7	0	3.9	3.9	7.7
Regular multivitamin use, %	47.3	42.6	45.2	43.6	36.3
Plasma 25(OH)D, nmol/L	70.0 (25.3)	65.7 (21.1)	62.7 (20.3)	57.4 (32.4)	54.6 (18.3)
Plasma C-peptide levels, ng/mL	1.3 (0.6)	1.6 (0.7)	2.0 (1.2)	2.2 (1.0)	2.5 (1.2)
Plasma adiponectin levels, μg/mL	11.1 (6.0)	9.6 (4.8)	8.7 (4.7)	7.7 (4.7)	8.0 (4.6)

[a]Mean (SD) for all continuous variables.

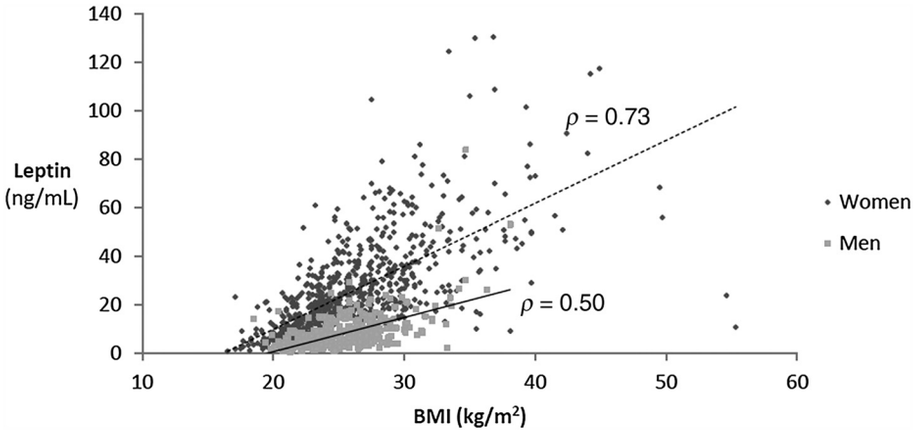

Figure 1.
Correlation between BMI and plasma leptin in men and women. Scatterplot of BMI versus plasma leptin for female (dark gray squares) and male (light gray squares) control subjects. Trendline (line of best fit) is shown as full (men) or dashed line (women). Spearman correlation coefficients are adjusted for age, cohort, and fasting status.
ρ, Spearman correlation coefficient.

diabetes or cases diagnosed within 2 or 4 years of blood collection and their matched controls (Supplementary Table S4).

Spline curves were consistent with log-linear associations ($P_{nonlinear} = 0.81$ for men; $P_{nonlinear} = 0.14$ for women). Therefore, in subsequent meta-analyses and subgroup analyses, we modeled leptin as a continuous variable. The multivariate ORs for an increment of 5 ng/mL in plasma leptin were 1.25 (1.02–1.54) for men and 0.98 (0.93–1.03) for women (Supplementary Table S5). ORs were similar within the 2 male and the 3 female cohorts (Fig. 2; $P_{heterogeneity} = 0.88$ for HPFS and PHS, and $P_{heterogeneity} = 0.35$ for NHS, WHI, and WHS). In stratified analyses, no statistically significant effect modification was observed (Supplementary Table S5).

Several SNPs at the *LEPR* gene were associated with pancreatic cancer risk among women to $P < 0.05$ in an additive genetic model (Table 3, Supplementary Fig. S1). After adjusting for multiple comparisons, rs10493380 located intronic to *LEPR* remained statistically significantly associated with increased risk of pancreatic cancer (OR per minor allele = 1.54; 95% CI = 1.18–2.02, multiple hypothesis-corrected $P = 0.03$; Table 3). This association was consistent across the 3 female cohorts ($P_{heterogeneity} = 0.28$). The association for rs10493380 was not statistically significant among men (OR = 1.19, 95% CI = 0.79–1.78, multiple hypothesis-corrected $P = 1.00$; Supplementary Table S6). Analysis of rs10493380 and highly correlated SNPs using HaploReg identified multiple transcription factor binding sites altered by these SNPs (Supplementary Table S7). Furthermore, in a blood eQTL database (29), rs10493380 (index SNP) and rs3790429 (in high LD with rs10493380, $r^2 = 0.90$ in 1000G EUR) were found to have *cis* eQTL effects on *LEPR* gene expression. No statistically significant association was identified between SNPs at the *LEPR* gene and plasma leptin levels among controls (Supplementary Table S8).

Table 2. ORs and 95% CIs for pancreatic cancer according to quintiles of plasma leptin

	Quintiles of plasma leptin					
	1	2	3	4	5	P_{trend}[a]
Men						
Leptin levels, ng/mL						
Range	≤3.0	3.1–4.8	4.9–7.8	7.9–11.7	≥11.8	
Median	2.2	4.0	6.7	9.2	15.3	
No. of cases	17	26	32	27	42	
No. of controls	69	71	71	72	70	
Base model[b]	1.0	1.57 (0.78–3.16)	1.90 (0.95–3.80)	1.61 (0.80–3.24)	2.77 (1.37–5.61)	0.01
Adjusted model I[b]	1.0	1.48 (0.72–3.05)	1.80 (0.89–3.64)	1.59 (0.78–3.25)	2.55 (1.23–5.27)	0.02
Adjusted model II[c]	1.0	1.48 (0.72–3.05)	1.80 (0.88–3.67)	1.60 (0.77–3.33)	2.54 (1.13–5.72)	0.03
Adjusted model III[d]	1.0	1.68 (0.79–3.54)	2.03 (0.96–4.29)	1.84 (0.86–3.95)	3.02 (1.27–7.16)	0.02
Women						
Leptin levels, ng/mL						
Range	≤9.5	9.6–17.7	17.8–24.8	24.9–37.7	≥37.8	
Median	6.2	14.0	20.8	30.6	51.5	
No. of cases	60	80	45	60	81	
No. of controls	148	146	150	148	149	
Base model[e]	1.0	1.36 (0.91–2.03)	0.76 (0.49–1.19)	0.98 (0.64–1.50)	1.27 (0.84–1.91)	0.64
Adjusted model I[b]	1.0	1.36 (0.91–2.03)	0.74 (0.48–1.16)	0.95 (0.61–1.47)	1.19 (0.78–1.82)	0.90
Adjusted model II[c]	1.0	1.27 (0.84–1.92)	0.68 (0.43–1.09)	0.82 (0.50–1.33)	0.94 (0.55–1.62)	0.37
Adjusted model III[d]	1.0	1.26 (0.82–1.93)	0.63 (0.38–1.03)	0.72 (0.43–1.21)	0.84 (0.46–1.51)	0.21

[a]P_{trend} values were calculated by the Wald test of a score variable that contained median values of quintiles.
[b]Further adjusted for race (White, Black, other), history of diabetes mellitus (yes, no), current multivitamin use (yes, no), and plasma 25(OH)D (continuous).
[c]Further adjusted for BMI (continuous) and physical activity (continuous).
[d]Further adjusted for plasma C-peptide (continuous) and plasma adiponectin (quartiles).
[e]ORs and 95% CI were estimated by conditional logistic regression conditioned on the matching factors including year of birth, prospective cohort (HPFS, NHS, PHS, WHI, WHS), smoking status (never, past, current), fasting status (fasting, non-fasting), and month/year of blood draw.

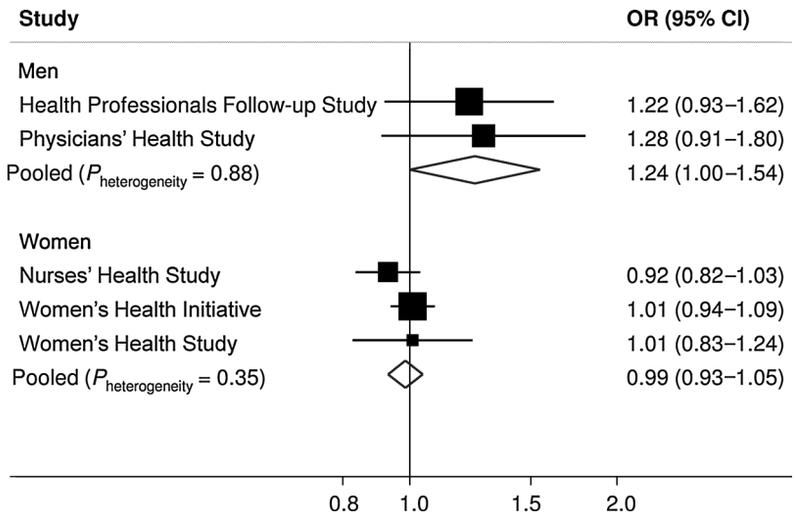

Figure 2.
Cohort-specific and meta-analysis of pooled ORs for pancreatic cancer according to plasma leptin levels (per 5 ng/mL increase in plasma leptin). Cohort-specific multivariate ORs conditioned on matching factors including year of birth, prospective cohort (HPFS, NHS, PHS, WHI, WHS), smoking status (never, past, current), fasting status (fasting, non-fasting), and month/year of blood draw and adjusted for covariates including race (White, Black, other), history of diabetes mellitus (yes, no), current multivitamin use (yes, no), plasma 25(OH)D (continuous), BMI (continuous), physical activity (continuous), plasma C-peptide (continuous), and plasma adiponectin (quartiles). The pooled OR is calculated by the DerSimonian and Laird random effects model. The solid squares and horizontal lines correspond to the cohort-specific multivariate ORs and 95% CIs, respectively. The area of the solid square reflects the cohort-specific weight (inverse of the variance). The open diamond represents the pooled multivariate OR and 95% CI. The solid vertical line indicates an OR of 1.0.

Discussion

As seen in prior studies (23), plasma leptin levels were higher in our female than in male control subjects, with a median level approximately 3 times higher in women versus men. Interestingly, higher prediagnostic plasma leptin was associated with an increased risk of pancreatic cancer in men, while no increase in risk was observed in women. This positive association among men was independent of other known risk factors for pancreatic cancer, including characteristics and plasma markers associated with obesity and insulin resistance. Furthermore, the association was highly consistent across 2 cohorts with male participants

Table 3. Association between SNPs in the leptin receptor (*LEPR*) gene and risk of pancreatic cancer among women

		Controls		Cases		Additive Model		
SNP	**Minor allele**	***n***	**MAF (%)**	***n***	**MAF (%)**	**OR[a] (95% CI)**	**Raw *P***	**Corrected *P***
rs10493380	C	646	0.16	272	0.21	1.54 (1.18–2.02)	0.001	0.032
rs3790424	G	655	0.31	280	0.24	0.69 (0.55–0.89)	0.003	0.069
rs6673324	G	650	0.48	277	0.55	1.38 (1.11–1.71)	0.003	0.072
rs2154381	G	653	0.34	281	0.27	0.72 (0.57–0.90)	0.004	0.088
rs6662904	A	648	0.42	278	0.48	1.32 (1.07–1.62)	0.009	0.170
rs9436746	A	653	0.45	280	0.39	0.75 (0.61–0.93)	0.010	0.180
rs9436747	A	653	0.42	278	0.35	0.75 (0.60–0.93)	0.010	0.184
rs11801408	A	657	0.18	283	0.23	1.36 (1.06–1.75)	0.014	0.243
rs41459646	C	654	0.16	279	0.20	1.36 (1.05–1.77)	0.021	0.329
rs2767485	G	650	0.18	280	0.22	1.27 (0.99–1.63)	0.059	0.647
rs9436301	G	650	0.23	280	0.27	1.25 (0.99–1.58)	0.063	0.672
rs7524834	G	659	0.42	282	0.46	1.21 (0.98–1.49)	0.074	0.724
rs12025906	G	657	0.21	280	0.18	0.79 (0.61–1.03)	0.084	0.770
rs9436748	A	654	0.39	278	0.43	1.18 (0.95–1.46)	0.134	0.901
rs10128072	C	650	0.15	277	0.18	1.19 (0.90–1.58)	0.210	0.975
rs7602	A	655	0.20	279	0.23	1.17 (0.91–1.50)	0.222	0.981
rs1887285	G	657	0.09	277	0.11	1.23 (0.88–1.72)	0.233	0.984
rs3828033	A	647	0.37	276	0.35	0.89 (0.72–1.10)	0.293	0.996
rs913199	A	652	0.45	279	0.49	1.09 (0.89–1.33)	0.420	1.000
rs1892534	A	653	0.39	278	0.41	1.08 (0.87–1.34)	0.474	1.000
rs3790431	G	651	0.20	279	0.19	0.91 (0.71–1.18)	0.482	1.000
rs3806318	G	654	0.26	279	0.28	1.08 (0.86–1.36)	0.519	1.000
rs2148683	G	655	0.47	282	0.49	1.06 (0.87–1.30)	0.537	1.000
rs2148682	G	655	0.35	280	0.37	1.05 (0.85–1.30)	0.649	1.000
rs12753193	G	659	0.39	279	0.40	1.05 (0.85–1.30)	0.663	1.000
rs4420065	A	651	0.39	279	0.40	1.04 (0.84–1.29)	0.691	1.000
rs4655537	A	654	0.36	275	0.37	1.04 (0.84–1.29)	0.736	1.000
rs6700896	A	654	0.39	280	0.40	1.03 (0.83–1.27)	0.776	1.000
rs11585329	A	655	0.16	279	0.14	0.97 (0.72–1.29)	0.817	1.000
rs9436737	G	650	0.14	274	0.14	1.02 (0.76–1.36)	0.900	1.000
rs3790436	C	653	0.45	277	0.46	1.01 (0.82–1.25)	0.920	1.000
rs17127601	G	649	0.13	278	0.13	1.01 (0.75–1.37)	0.926	1.000

[a]ORs and 95% CI were estimated using conditional logistic regression, conditioning on matching factors including year of birth, prospective cohort (HPFS, NHS, PHS, WHI, WHS), which also conditions on gender, smoking status (never, past, current), fasting status (fasting, non fasting), and month/year of blood draw, and adjusted for race (White, Black, other), history of diabetes mellitus (yes, no), current multivitamin use (yes, no), plasma 25(OH)D (continuous), BMI (continuous), physical activity (continuous), plasma C-peptide (continuous) and plasma adiponectin (quartiles).

(positive association in HPFS and PHS) and 3 cohorts with female participants (no association in NHS, WHI, and WHS). In contrast, single-nucleotide variants at the leptin receptor (*LEPR*) gene were associated with pancreatic cancer risk only in women, but not in men. This association was consistent across the 3 female cohorts. Notably, the most statistically significant SNP (rs10493380) and highly correlated variants at *LEPR* may alter leptin receptor gene expression on the basis of bioinformatic analyses. In aggregate, these data support the importance of adipokines and adipokine signaling in pancreatic cancer risk in men and women, even though baseline circulating leptin levels differ greatly by gender.

A previous nested case–control study evaluated the association of prediagnostic plasma leptin with risk of pancreatic cancer (30). In this pooled analysis of 3 cohorts, plasma leptin was not associated with risk of pancreatic cancer during the first 10 years of follow-up, using gender-specific quintiles of plasma leptin. However, a statistically significant positive association was observed among men (OR, 2.94; 95% CI, 1.34–6.46; P_{trend} = 0.001; comparing extreme quintiles) with longer follow-up time ($\geq$10 years), whereas the association among women for this duration could not be evaluated because of small sample size. Similarly, in our cohorts, a positive association of plasma leptin with pancreatic cancer risk was observed in men. However, in stratified analyses by time between blood collection and cancer diagnosis, statistically significant effect modification was not seen. This difference may be partly due to our *a priori* exclusion of cases with blood collected within 12 months of cancer diagnosis, the time period during which cancer-associated weight loss most commonly occurs (31). The stratified analyses in men were also limited by smaller sample sizes within strata. Three previous cross-sectional studies observed that plasma leptin levels were lower in pancreatic cancer patients than in controls (32–34). However, in these retrospective studies, hypoleptinemia may have been due to the weight loss that is commonly experienced by patients with pancreatic cancer (5). Therefore, it is difficult to determine whether the observed low leptin levels contributed to pancreatic carcinogenesis or were a consequence of the cancer.

Several lines of evidence support a biologic link between leptin and pancreatic carcinogenesis. Leptin plays a central role in the regulation of insulin sensitivity (35), and studies have demonstrated associations between hyperglycemia, insulin resistance, and future risk of pancreatic cancer (17, 36–38). Therefore, one mechanism by which leptin may influence pancreatic cancer risk is through its modulation of insulin sensitivity (39). Leptin is synthesized by adipose tissue, and its concentration correlates with total body fat. Leptin may therefore act as a better marker for the relevant states of adiposity than BMI, which does not discriminate between fat and muscle mass (40). Target tissue effects of circulating leptin are not solely mediated centrally on cells within the hypothalamus, as leptin receptors are widely distributed in the body (7). Leptin receptors have been identified on the surface of tumor cells, including pluripotent cells thought to function as tumor-initiating cells (41). Therefore, leptin signaling may have an important direct role in promoting tumor initiation and growth, independent of its role in insulin sensitivity (42). As a consequence of this potential direct effect, inhibitors of the leptin receptor are being explored as novel therapeutics to inhibit tumor growth in patients (43).

We observed a positive association between plasma leptin and pancreatic cancer risk only in men. Interestingly, leptin levels are considerably lower in men than in women, even for the same age and BMI (44). Although the underlying reasons for this difference in circulating leptin are unclear, sex differences in reproductive hormones and body fat distribution have been proposed as possible etiologies. Particularly, women tend to have higher total and subcutaneous fat, whereas men have a greater percentage of visceral fat, which may influence circulating levels of leptin (40). Given the substantially lower leptin levels and differing metabolic environment in men, the actions of leptin may be sex-specific, with implications for disease development. Alternatively, physiologic differences related to circulating leptin may be detectable only when compared in the lower ranges of circulating leptin, which are seen predominantly in men. Notably, a number of prospective cohort studies have examined the association of prediagnostic plasma leptin and the subsequent diabetes risk. Similar to the current study of pancreatic cancer, most of these studies demonstrated positive associations of leptin and diabetes in men, but not in women (45). Of note, crosstalk between estrogen and leptin signaling has been shown previously (46). Other than modulating synthesis of leptin (47) and leptin receptor (48), estrogen receptor-α enhances leptin-induced activation of downstream signaling pathways, including the JAK/STAT pathway (46). Alternatively, the different association between leptin levels and pancreatic cancer risk between genders in our study could be due to chance, and these findings should be confirmed in future studies.

The current study has several strengths. The prospective design and exclusion of cases diagnosed within 1 year of blood collection reduced the potential impact of reverse causation on our results. Furthermore, similar associations were observed when we excluded cases diagnosed within 2 or 4 years of blood collection. Leptin was measured in a single laboratory as a single batch, with low coefficients of variance for quality control samples. In our analyses, we adjusted for BMI, physical activity, and other biomarkers related to insulin resistance, including C-peptide and adiponectin, to rigorously control for confounding. We evaluated not only circulating leptin levels, but also genetic variants in the leptin receptor, which may affect signal transduction after ligand binding. Additional strengths included a large sample size, long follow-up period, and inclusion of men and women.

Plasma leptin was measured at only one point in time, so leptin levels may not fully reflect long-term plasma concentrations. However, leptin levels are relatively stable over time in healthy subjects; repeated plasma leptin measurements 1-year apart demonstrated a high intraclass correlation coefficient of 0.74 (21). We cannot rule out that residual confounding by adiposity not captured by BMI may be present; however, adjustment for plasma adiponectin, 25(OH)D, and C-peptide as markers of adiposity and insulin resistance did not materially alter our results. We identified an association of rs10493380 with pancreatic cancer risk in women. This SNP was not identified as genome-wide significant in 2 recent pancreatic cancer genome-wide association studies (49, 50). However, these studies were required to meet a stringent multiple-hypothesis testing threshold for statistical significance due to testing of more than 500,000 SNPs, included approximately 60% men, and included a majority of patients from tertiary center case–control studies. Finally, our study population consisted primarily of White participants and further studies are required to evaluate circulating leptin in participants of different race/ethnicity.

In conclusion, we identified a positive association between prediagnostic circulating leptin levels and pancreatic cancer risk in men, independent of BMI and other risk factors of pancreatic

cancer. Although this association was not observed in women, single-nucleotide variants in the leptin receptor were associated with pancreatic cancer risk in women, and bioinformatic analyses suggested differences in leptin receptor expression with these variants. Our data provide additional evidence for a biologic link between obesity, insulin resistance, and pancreatic cancer risk, specifically focusing attention on adipokines and adipokine signaling in pancreatic cancer development.

Disclosure of Potential Conflicts of Interest

N. Rifai is the editor-in-chief and has contributed to clinical chemistry in the journal. M.L. Anderson is the consultant/advisory board member of Tesari. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The study sponsors had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the article; or the decision to submit the article for publication.

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