Association of C-peptide and leptin with prostate cancer incidence in the Health Professionals Follow-up Study

Gabriel Y. Lai · Edward L. Giovannucci · Michael N. Pollak · Sarah B. Peskoe · Meir J. Stampfer · Walter C. Willett · Elizabeth A. Platz

Abstract

Purpose Hyperinsulinemia is hypothesized to influence prostate cancer risk. Thus, we evaluated the association of circulating C-peptide, which is a marker of insulin secretion, and leptin, which is secreted in response to insulin and influences insulin sensitivity, with prostate cancer risk.

Methods We identified prostate cancer cases ($n = 1{,}314$) diagnosed a mean of 5.4 years after blood draw and matched controls ($n = 1{,}314$) in the Health Professionals Follow-up Study. Plasma C-peptide and leptin concentrations were measured by ELISA. Odds ratios (ORs) and 95 % confidence intervals (CI) were estimated taking into account the matching factors age and history of a PSA test before blood draw and further adjusting for body mass index, diabetes, and other factors.

Results Neither C-peptide (quartile [Q]4 vs. Q1: OR 1.05, 95 % CI 0.82–1.34, p-trend $= 0.95$) nor leptin (Q4 vs. Q1: OR 0.85, 95 % CI 0.65–1.12, p-trend $= 0.14$) was associated with prostate cancer risk. Further, neither was associated with risk of advanced or lethal disease ($n = 156$ cases; C-peptide: Q4 vs. Q1, OR 1.18, 95 % CI 0.69–2.03, p-trend $= 0.78$; leptin: Q4 vs. Q1, OR 0.74, 95 % CI 0.41–1.36, p-trend $= 0.34$).

Conclusions In this large prospective study, circulating C-peptide and leptin concentrations were not clearly associated with risk of prostate cancer overall or aggressive disease. Well into the PSA era, our findings do not appear to be supportive of the hypothesis that hyperinsulinemia influences risk of total or aggressive prostate cancer.

Keywords Prostate cancer · C-peptide · Leptin · Nested case–control study · Risk

Introduction

Hyperinsulinemia, often a consequence of obesity and diabetes, is hypothesized to influence prostate cancer risk. Insulin's ability to stimulate cellular proliferation and inhibit

G. Y. Lai · S. B. Peskoe · E. A. Platz (✉)
Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Rm E6132, 615 N. Wolfe Street, Baltimore, MD 21205, USA
e-mail: eplatz@jhsph.edu

G. Y. Lai
Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA

E. L. Giovannucci · M. J. Stampfer · W. C. Willett
Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA, USA

E. L. Giovannucci · M. J. Stampfer · W. C. Willett
Channing Division of Network Medicine, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA

M. N. Pollak
Department of Oncology, McGill University and Lady David Institute for Medical Research, Jewish General Hospital, Montreal, QC, Canada

E. A. Platz
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

E. A. Platz
The James Buchanan Brady Urological Institute and the Department of Urology, Johns Hopkins School of Medicine, Baltimore, MD, USA

apoptosis is well described [1]. Results are not consistent across studies that evaluated the association of hyperinsulinemia or insulin resistance with prostate cancer (e.g., [2–6]). Because many cohort studies have not collected fasting blood samples, some investigators have measured C-peptide, an indicator of insulin secretion with a longer half-life than insulin [7]. Of the prospective studies, one reported no association with total prostate cancer [8], two reported an inverse association with localized/low-grade disease and a nonsignificant positive association with aggressive disease [9, 10], and another reported no association with low-grade disease, but a positive association with high-grade disease (in the placebo arm of a chemoprevention trial) [11]. A separate prospective study found that men with higher baseline C-peptide concentration who were subsequently diagnosed with prostate cancer were more likely to die of their disease [12]. To evaluate the independent role of C-peptide with prostate cancer, these studies took obesity into account. Although not entirely consistent, these studies, when considered together, generally support the hypothesis that hyperinsulinemia, like obesity [13, 14], may be differently associated with aggressive (positive direction) versus nonaggressive (inverse direction) prostate cancer.

In this context, some groups have studied leptin. Leptin is a hormone secreted by adipocytes that regulates energy intake and expenditure and also influences insulin sensitivity [15]. Leptin was not associated with prostate cancer in small case–control studies [16–18] and three nested case–control studies [19–21] and was not associated with stage at diagnosis [22], although it was positively associated with prostate cancer in a small nested case–control study conducted in a population without routine PSA screening [23] and with low-grade disease in a case–control study [24].

Taking together the studies on C-peptide and leptin, a consistent pattern is not yet apparent for the link of these correlates of insulin secretion and action with prostate cancer risk. It is also possible that the smaller sample size of previous studies limited the ability to evaluate what may be a modest association of C-peptide and leptin with prostate cancer. Thus, to understand better the etiologic role of these metabolic markers, we evaluated the association of C-peptide and leptin with prostate cancer risk overall and by stage and grade in the Health Professionals Follow-up Study (HPFS), a large prospective study with a large number of prostate cancer cases, both nonaggressive and aggressive.

Methods

Study population and design

The HPFS is an ongoing, prospective cohort study of diet and lifestyle in the etiology of chronic diseases. At baseline in 1986, 51,529 US male health professionals aged 40–75 years completed a mailed questionnaire on demographics, anthropometrics, lifestyle, and medical history, and a semiquantitative food frequency questionnaire. Subsequently, every 2 years, we mailed participants a questionnaire to update exposures and disease information and every 4 years updated their diet. Participants' deaths were identified by searches of the National Death Index, reports from the US Postal Service, or response by next of kin to the mailed follow-up questionnaires. Between 1993 and 1995, 18,018 of the men provided blood samples collected in tubes containing sodium EDTA and shipped by overnight courier in a chilled container. After centrifugation, the samples were aliquoted into plasma, buffy coat, and erythrocytes and frozen in liquid nitrogen.

Men were eligible for inclusion in the nested case–control study if they provided a blood sample, did not have a cancer diagnosis (aside from nonmelanoma skin cancer) at time of blood draw, and provided a valid baseline food frequency questionnaire.

Prostate cancer cases and controls

We asked the men to report on the questionnaires whether they had a prostate cancer diagnosis in the past 2 years. We requested pertinent medical records and pathology reports from physicians and hospitals after receiving permission from the men, or next of kin, if the first indication of the diagnosis was on the death certificate. We reviewed these records to confirm the diagnosis and to abstract stage and Gleason sum. Over 90 % of the cases have been confirmed by medical and pathology records.

We excluded T1a disease because small volume tumors incidentally detected during benign prostatic hyperplasia surgery are susceptible to detection bias. Between date of blood draw and 31 January 2004, we identified 1,331 non-T1a prostate cancer cases. For analyses by stage and grade, we categorized cases as localized (T1b–T2c and N0M0); advanced stage at diagnosis ($\geq$T3b or N1 or M1), progression to distant metastases or death from prostate cancer during follow-up ("advanced or lethal"); lower grade (Gleason sum <7); and higher grade (Gleason sum ≥ 7); we did not include T3a N0M0 cases in either the localized or advanced or lethal categories to increase the specificity of organ-confined disease ($\leq$T2b) and advanced disease ($\geq$T3b).

For each case, we selected one control who was alive, not diagnosed with cancer by the case's diagnosis date, and had had a PSA test after the date of blood draw. We matched controls to cases on age, PSA test before blood draw, and year, time of day, and season of blood draw. We excluded men with insufficient stored plasma and their matched pair leaving 1,314 prostate cancer cases and 1,314 controls. The Institutional Review Boards at the Harvard

School of Public Health and the Johns Hopkins Bloomberg School of Public Health approved this work.

Laboratory assays

Plasma concentrations of C-peptide and leptin were measured in duplicate by ELISA (Diagnostic Systems Laboratories/Beckman Coulter, Webster, TX) in the laboratory of Dr. Pollak; the pair mean was used in the analysis. Case–control pairs were analyzed together but in random within-pair order. The cases and matched controls were assayed in four batches based on dates of diagnosis. Laboratory personnel were unaware of the case–control status of each sample. Mean intrapair coefficients of variation for the replicates were 2.3 % for C-peptide and 1.5 % for leptin.

Statistical analysis

We compared means and proportions of known and suspected prostate cancer risk factors and correlates of obesity and diabetes between matched cases and controls using the paired t test and McNemar's test, respectively. Concentrations were right skewed; thus, we used the Wilcoxon sign-rank test to compare them between cases and controls. We used conditional logistic regression to estimate odds ratios (OR) and 95 % confidence intervals (95 % CI) of total prostate cancer. We entered into the models indicator variables for C-peptide and leptin quartiles with cutpoints based on the distributions among the controls for each batch. To test for trend, we entered into the model a single ordinal variable with values of 1–4 corresponding to the quartile into which a man's concentration fell.

First, we estimated matched ORs. Next, we adjusted for body mass index (BMI; calculated from self-reported height and weight) and history of diabetes around the time of blood draw to determine whether the C-peptide and leptin were associated with prostate cancer risk beyond their correlations with obesity (Spearman's correlations with BMI adjusted for age in the controls, C-peptide: $r = 0.34$; leptin: $r = 0.61$; both $p < 0.0001$) and diabetes (geometric mean adjusted for age in controls, C-peptide: diabetes = 1.99 ng/mL, no diabetes = 2.05 ng/mL, $p = 0.70$; leptin: diabetes = 15.27 ng/mL, no diabetes = 9.67 ng/mL, $p < 0.0001$). Then, we additionally adjusted for factors known or suspected to be associated with C-peptide or leptin [25, 26] and other factors that were either known or suspected risk factors or were previously found to be associated with prostate cancer in this cohort [27, 28]: height, first degree family history of prostate cancer, vigorous physical activity, smoking in the past 10 years, history of vasectomy, total energy intake, alcohol intake, energy-adjusted intake of calcium, alpha-linolenic acid, lycopene, and fructose, cumulative updated intake (1986–1994) of red meat and fish, and use of a vitamin E or selenium supplement. We also adjusted for other circulating factors perturbed in obesity and diabetes (IGF-1, IGFBP-3 [29], testosterone, estradiol, sex hormone binding globulin—SHBG [30], and total cholesterol [31]) using indicator variables in the multivariable model.

To determine whether the associations differed by age at diagnosis, we stratified at the median and ran conditional logistic regression models. In additional subanalyses, we broke the matching and ran logistic regression models adjusting for the matching factors age, history of a PSA test prior to blood draw, and other covariates. We repeated the primary analyses with localized, advanced or lethal, low-grade, and high-grade disease as the outcomes. To determine whether the associations differed by adiposity, we stratified by BMI (<25, ≥ 25 kg/m^2). Because type 2 diabetes is characterized by hyperinsulinemia early in its natural history and insufficient insulin subsequently, and because insulin influences levels of C-peptide and leptin, in a subanalysis, we restricted to men without a history of diabetes. Because the men were not asked to fast and C-peptide and leptin levels differed by time since last meal (age-adjusted geometric mean C-peptide and leptin [ng/mL] in controls, fasting: 1.69, 9.98; not fasting: 2.94, 9.34, respectively, [all $p < 0.0001$]), we stratified by fasting status. We defined fasting as not having eaten for ≥ 8 h (cases 64.5 %, controls 66.8 %). To test for differences in the stratum-specific associations, we entered terms for C-peptide or leptin and the covariate (binary) along with a term for their product into the multivariable models. The coefficient for the product term was evaluated by the Wald test. Analyses were conducted using SAS release 9.1 (SAS Institute, Cary, NC). Two-sided p values are reported.

Results

Most cases had localized disease (86.4 % of the 1,231 cases with stage information). A total of 39.3 % had Gleason sum ≥ 7 disease (of the 1,213 cases with grade information). Median age at diagnosis was 69.5 years [interquartile range (IQR): 64.6–74.8 years]. Median time between blood draw and diagnosis was 5.4 years (IQR 3.1–7.7 years). Case and control characteristics were similar except for family history of prostate cancer (Table 1). Cases and controls had similar median C-peptide ($p = 0.92$) levels (Table 1). Median leptin concentration was lower in cases than controls ($p = 0.03$). The Spearman correlation (adjusted for age) between C-peptide and leptin was $r = 0.35$ ($p < 0.0001$) in the controls.

Prostate cancer overall

C-peptide was not associated with prostate cancer in the matched analysis; after adjusting for BMI and diabetes; or

Table 1 Characteristics of prostate cancer cases and matched controls, including plasma C-peptide and leptin concentrations, HPFS

	Cases	Controls	*p*
No.	1,314	1,314	
Mean age (year)	64.2	64.2	Matched
White (%)	94.2	92.9	0.17
Mean height in 1986 (in)	70.2	70.1	0.77
Mean body mass index (kg/m^2)	25.8	25.9	0.50
Family history of prostate cancer by 1996 (%)	14.3	10.5	0.003
Ever had a screening PSA test (%)	72.6	72.8	0.79
History of diabetes (%)	5.1	4.6	0.52
History of vasectomy (%)	27.6	28.8	0.47
Mean vigorous physical activity (MET-h/week)	12.8	12.4	0.65
Smoked cigarettes in past 10 years (%)	15.1	16.7	0.24
Mean intake			
Total energy (kcal/day)	2,031	2,030	0.97
Alcohol (g/day)	12.3	12.0	0.63
Lycopene in 1990 (μg/day)	6,762	6,769	0.97
Red meat (servings/week)[a]	7.6	7.6	0.97
Fish (servings/week)[a]	2.2	2.3	0.32
Calcium (mg/day)	950	945	0.79
Alpha-linolenic acid (g/day)	1.05	1.05	0.87
Energy-adjusted fructose in 1990 (g/day)	48.7	48.7	0.95
Use of vitamin E supplement (%)	37.1	36.8	0.84
Use of selenium supplement (%)	7.6	8.0	0.72
Median (interquartile range) plasma concentration			
C-peptide (ng/mL)	1.91 (1.33–3.06)	1.90 (1.28–3.10)	0.92
Leptin (ng/mL)	9.30 (5.58–16.52)	9.78 (5.87–17.93)	0.03

Demographic, medical, and lifestyle characteristics were assessed in 1994 unless otherwise noted. Plasma concentrations were assessed in 1993–1995. Cases and controls matched on age, PSA test before blood draw, and year, time of day, and season of blood draw

[a] Cumulative average intake from 1986 to 1994

after multivariable adjustment (Table 2). For leptin, the ORs of prostate cancer were nonstatistically significantly <1.00 in quartiles 3 and 4 versus 1. The results for C-peptide and leptin were not notably altered after their mutual adjustment, or after adjustment for IGF-1 and IGFBP-3; for testosterone, estradiol, and SHBG; or for cholesterol in the multivariable model (data not shown). To reduce the possibility that undiagnosed prostate cancer may have influenced concentrations of C-peptide and leptin, we excluded cases diagnosed within 2 years after blood draw and their matched controls; the inferences were unchanged (data not shown).

Prostate cancer by stage and grade

Neither C-peptide nor leptin was associated with prostate cancer that was localized, advanced or lethal, low-grade, or high-grade in any of the models (Table 3). The results for C-peptide and leptin were unchanged after their mutual adjustment in the multivariable model (data not shown). Further adjustment for IGF-1 and IGFBP-3; testosterone, estradiol, and SHBG; or cholesterol generally did not alter the results for either C-peptide or leptin (data not shown). The exception was a possible inverse association between leptin and high-grade disease (Q3 vs. Q1: OR 0.53, 95 % CI 0.32–0.86, Q4 vs. Q1: OR 0.59, 95 % CI 0.33–1.04) that was observed after adjusting for testosterone, estradiol, and SHBG.

Subanalyses

The associations of C-peptide and leptin with prostate cancer overall or by stage and grade did not differ between strata of age at diagnosis (data not shown) with one exception. Among older men, leptin was inversely associated with advanced or lethal disease (Q4 vs. Q1: OR 0.45, 95 % CI 0.20–1.02, p-trend = 0.04), whereas among younger men, the association was null or possibly in the positive direction (Q4 vs. Q1: OR 1.62, 95 % CI 0.62–4.25, p-trend = 0.24; p-interaction = 0.05). The association between C-peptide and prostate cancer overall or by stage and grade did not differ between strata of BMI. However, BMI appeared to modify the association of leptin with low- (p-interaction = 0.009) and high-grade (p-interaction = 0.15) disease (Table 4). In particular, leptin was statistically significantly inversely associated with high-grade disease among men with BMI <25 kg/m^2, but was not associated among men with higher BMI (Table 4). Among men without a history of diabetes, the associations for C-peptide and leptin with prostate cancer and by stage and grade did not notably differ from overall (data not shown). The C-peptide association did not differ by fasting status (all p-interaction >0.18).

Table 2 Association of plasma C-peptide and leptin concentrations with prostate cancer overall, HPFS

	OR (95 % CI) by quartile of the distribution				OR per quartile increase (95 % CI)	*p*-trend
	1 (Ref)	2	3	4		
C-peptide[a]						
No. cases/controls	304/327	378/334	321/327	311/326		
OR[b] (95 % CI)	1.00	1.20 (0.98–1.49)	1.05 (0.84–1.31)	1.02 (0.82–1.28)	0.99 (0.92–1.07)	0.81
OR[c] (95 % CI)	1.00	1.22 (0.98–1.50)	1.07 (0.85–1.35)	1.05 (0.83–1.33)	1.00 (0.93–1.08)	0.99
OR[d] 95 % CI)	1.00	1.21 (0.98–1.50)	1.09 (0.87–1.38)	1.05 (0.82–1.34)	1.00 (0.93–1.08)	0.95
Leptin[a]						
No. cases/controls	357/329	345/328	309/333	303/324		
OR[b] (95 % CI)	1.00	0.97 (0.78–1.20)	0.85 (0.68–1.06)	0.86 (0.69–1.06)	0.94 (0.88–1.01)	0.10
OR[c] (95 % CI)	1.00	0.96 (0.78–1.20)	0.84 (0.67–1.06)	0.84 (0.64–1.10)	0.93 (0.86–1.02)	0.12
OR[d] (95 % CI)	1.00	0.97 (0.77–1.21)	0.83 (0.66–1.06)	0.85 (0.65–1.12)	0.94 (0.86–1.02)	0.14

[a] Case–control pairs assayed in 4 batches. Quartile cutpoints were as follows. Batch 1—C-peptide 1.16, 1.72, and 2.84 (ng/mL), and leptin 8.72, 15.34, and 24.05 (ng/mL). Batch 2—C-peptide 1.29, 1.80, and 2.73 (ng/mL), and leptin 8.63, 14.45, and 25.24 (ng/mL). Batch 3—C-peptide 1.10, 1.60, and 2.44 (ng/mL), and leptin 8.39, 13.95, and 21.82 (ng/mL). Batch 4—C-peptide 1.41, 2.18, and 3.77 (ng/mL), and leptin 4.42, 6.83, and 11.41 (ng/mL)

[b] Matched analysis. Cases and controls matched on age, PSA test before blood draw, and year, time of day, and season of blood draw

[c] Same analysis as (b), additionally adjusted for BMI (continuous) and history of diabetes

[d] Same analysis as (c), additionally adjusted for height (continuous), family history of prostate cancer, vasectomy, vigorous physical activity (continuous), smoking in the past 10 years, intakes (continuous) of total energy, alcohol, lycopene, red meat, fish, calcium, alpha-linolenic acid, fructose, use of a vitamin E, or selenium supplement

Table 3 Association of plasma C-peptide and leptin concentrations with prostate cancer by stage and grade, HPFS

	Comparing Q4 versus Q1 and adjusted for					
	Matching factors[a]		Matching factors, BMI, and diabetes[b]		Multivariable[c]	
	OR (95 % CI)	*p*-trend	OR (95 % CI)	*p*-trend	OR (95 % CI)	*p*-trend
C-peptide						
Localized (T1b–T2c and N0M0) (1,064 cases)	0.97 (0.77–1.22)	0.48	1.01 (0.79–1.30)	0.77	1.03 (0.80–1.33)	0.88
Advanced (≥T3b or N1 or M1) or lethal (156 cases)	1.22 (0.74–2.03)	0.64	1.18 (0.70–2.00)	0.76	1.18 (0.69–2.03)	0.78
Low-grade (Gleason sum <7) (736 cases)	0.91 (0.70–1.19)	0.20	0.96 (0.73–1.27)	0.39	0.98 (0.74–1.30)	0.50
High-grade (Gleason sum ≥7) (477 cases)	1.14 (0.84–1.54)	0.42	1.18 (0.86–1.62)	0.30	1.20 (0.87–1.66)	0.28
Leptin						
Localized (T1b–T2c and N0M0) (1,064 cases)	0.85 (0.67–1.06)	0.09	0.88 (0.66–1.17)	0.24	0.90 (0.67–1.20)	0.31
Advanced (≥T3b or N1 or M1) or lethal (156 cases)	0.94 (0.58–1.50)	0.78	0.77 (0.43–1.38)	0.37	0.74 (0.41–1.36)	0.34
Low-grade (Gleason sum <7) (736 cases)	0.85 (0.66–1.10)	0.13	0.92 (0.67–1.26)	0.41	0.91 (0.66–1.26)	0.39
High-grade (Gleason sum ≥7) (477 cases)	0.85 (0.63–1.14)	0.19	0.81 (0.56–1.18)	0.18	0.84 (0.57–1.23)	0.28

Matching broken and all 1,314 controls included. ORs and 95 % CIs estimated from logistic regression models

[a] Analysis adjusted for age and PSA test before blood draw

[b] Same as (b), additionally adjusted for BMI (continuous) and history of diabetes

[c] Same as (c), additionally adjusted for height (continuous), family history of prostate cancer, vasectomy, vigorous physical activity (continuous), smoking in the past 10 years, intakes (continuous) of total energy, alcohol, lycopene, red meat, fish, calcium, alpha-linolenic acid, fructose, use of a vitamin E, or selenium supplement

Discussion

In this prospective study, neither C-peptide nor leptin was associated with prostate cancer risk, including aggressive disease. In some subgroups, leptin was inversely associated with risk, including high-grade disease in lean men and advanced or lethal disease in older men.

Our null results for C-peptide and prostate cancer are consistent with some prospective studies reporting no association for C-peptide [8] or insulin [3, 32]; these

Table 4 Association of plasma C-peptide and leptin concentrations with prostate cancer by BMI, HPFS

	Lower BMI (<25 kg/m^2) (controls = 572)			Higher BMI (≥25 kg/m^2) (controls = 742)			*p*-interaction
	Cases	OR (95 % CI) Q4 vs. Q1	*p*-trend	Cases	OR (95 % CI) Q4 vs. Q1	*p*-trend	
C-peptide							
Total prostate cancer	575	0.96 (0.68–1.37)	0.93	739	1.08 (0.77–1.53)	0.83	0.78
Localized (T1b–T2c and N0M0)	474	0.91 (0.62–1.31)	0.85	590	1.08 (0.75–1.56)	0.73	0.85
Low-grade (Gleason sum <7)	320	0.84 (0.55–1.30)	0.91	416	0.96 (0.64–1.43)	0.24	0.19
High-grade (Gleason sum ≥7)	218	1.07 (0.67–1.70)	0.78	259	1.43 (0.86–2.37)	0.32	0.16
Leptin							
Total prostate cancer	575	0.89 (0.51–1.55)	0.25	739	0.82 (0.55–1.20)	0.20	0.38
Localized (T1b–T2c and N0M0)	474	1.02 (0.58–1.81)	0.59	590	0.85 (0.56–1.28)	0.31	0.23
Low-grade (Gleason sum <7)	320	1.39 (0.76–2.54)	0.71	416	0.74 (0.48–1.16)	0.093	0.009
High-grade (Gleason sum ≥7)	218	0.36 (0.13–0.98)	0.024	259	1.13 (0.63–2.00)	0.59	0.15

Adjusted for the matching factors and BMI (continuous), height (continuous), history of diabetes, family history of prostate cancer, vasectomy, vigorous physical activity (continuous), smoking in the past 10 years, intakes (continuous) of total energy, alcohol, lycopene, red meat, fish, calcium, alpha-linolenic acid, fructose, use of a vitamin E, or selenium supplement

Results are not shown for advanced or lethal disease because of small sample size (67 cases in the lower BMI group, 89 cases in the higher BMI group). None of the stratum-specific *p*-trends for advanced or lethal disease was statistically significant except for a possible inverse association for leptin in the lower BMI group (*p*-trend = 0.07); the *p*-interaction was 0.27

studies did not evaluate associations by stage and grade. Our results are not consistent with prospective studies observing possible inverse associations with nonaggressive and positive associations with aggressive disease [9–11]. Other studies reported a positive association between C-peptide and incident prostate cancer [2] or between C-peptide [12] or insulin [4] and prostate cancer death among men with the diagnosis.

Our results for leptin are mostly consistent with the studies reporting no association with prostate cancer risk [16–21], but not with a nested case–control study in Sweden, where PSA screening is not routine, that reported a positive association [23]. Because circulating leptin level is positively correlated with fat mass [33], we might have expected that the leptin–prostate cancer association would have the same pattern by aggressiveness as has been observed for obesity and prostate cancer: an inverse association with nonaggressive and a positive association with aggressive disease [13]. We did not observe this pattern, although we possibly observed the pattern of effect modification by age at diagnosis for the leptin–prostate cancer association (i.e., an inverse association for younger men and a positive association for older men) that is similar to what was observed for the BMI–prostate cancer association in the HPFS [27].

We hypothesized that a chronically elevated insulin level, including as a sequela of obesity and diabetes (early in its natural history), might mediate their associations with prostate cancer. We expected that the link between insulin, and thus its correlates, and prostate cancer might be complex given that (1) obesity appears to be inversely associated with localized and low-grade prostate cancer and positively associated with advanced and high-grade prostate cancer [13] and yet (2) diabetes, which is often a consequence of obesity, appears to be inversely associated with prostate cancer irrespective of stage and grade [34]. Along with its role in energy regulation, insulin is a mitogen [1] that would be expected to preferentially influence the growth of cells that have already lost growth control (e.g., neoplastic cells). Yet, the observed null association between C-peptide, a marker for insulin, suggests that insulin itself may not influence prostate cancer risk, although we cannot rule out that we did not capture insulin exposure during the etiologically relevant time. Increased levels of insulin generally exist alongside other metabolic abnormalities that, themselves, may influence prostate cancer development. The possible lower risk of prostate cancer in men with higher circulating leptin levels in some subgroups (e.g., lean mean, older men) may have two explanations, one causal and one bias, but both related to the lower testosterone concentration in men with elevated leptin levels [35]: (1) Lower testosterone level may have reduced the likelihood of prostate cancer development in men with elevated leptin levels. (2) Because PSA expression is under androgenic regulation and screening for elevated PSA is very common in the HPFS, men with elevated leptin levels may have had a lower circulating PSA level than they would have otherwise and thus reduced likelihood of undergoing diagnostic work-up for prostate cancer.

At this time, it is unclear what aspect of chronically elevated insulin, for example, usual nonfasting level, usual fasting level, or the area under the curve defined by insulin level across time, may influence prostate cancer risk. We used a single measurement of the insulin correlates to reflect usual levels, and although we matched on time of day of blood draw, some men were fasting and some were not. To reduce heterogeneity in C-peptide and leptin levels due to differences in time since last having eaten, we stratified by the fasting status. The association for C-peptide was in the positive direction for all endpoints in those who were fasting (null in nonfasting), and the association for leptin was in the inverse direction for all endpoints in those who were not fasting (null in fasting), but none of these associations was statistically significant.

Our study had a number of strengths, including its prospective nature, large size, separate evaluation by disease aggressiveness, and ability to take into account the correlations of C-peptide and leptin with other circulating factors that are perturbed in obesity and diabetes. Our study also had some limitations. Because our study was conducted in the PSA era, we had fewer advanced stage or lethal cases. We limited one potential source of detection bias by requiring the matched controls to have had a PSA test after the date of blood draw, but we cannot rule out that our results may be affected by different sensitivities of the PSA test in men who are obese, diabetic, or who are otherwise hyperinsulinemic versus men who are lean and not diabetic. We did not take into account other circulating factors that are altered in men who are obese or who are diabetic, including glucose and inflammatory mediators. Because we did not measure fasting insulin, we cannot determine whether the levels of C-peptide and leptin in the top quartile equate to abnormally high insulin levels. Finally, leptin levels in our study (IQR for controls: 6–18 ng/mL) were higher than in others [25, 36]. Differences in assay method between other studies (radioimmunoassay) and ours (ELISA) may have contributed to differences in findings. In addition, we performed multiple tests, and our findings could be due to chance.

While the HPFS participants are predominantly white, have high educational attainment, and, on average, are somewhat more health conscious than the general population of US men, the range of many exposures—including BMI, smoking levels, physical activity, and diet—among the HPFS participants overlap with the general population. Thus, we do not expect the nature of the observed associations in this study to differ systematically from what would be observed in other men. We conclude that our findings are not compatible with the hypothesis that hyperinsulinemia influences prostate cancer risk, although we could not rule out an inverse association for leptin and more aggressive disease in some subgroups. Because this work was conducted in the PSA era, the number of lethal cases was very small; thus, future studies of lethal disease and hyperinsulinemia are needed.

Acknowledgments We would like to thank the participants and staff of the Health Professionals Follow-up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. In addition, this study was approved by the Connecticut Department of Public Health (DPH) Human Investigations Committee. Certain data used in this publication were obtained from the DPH. The authors assume full responsibility for analyses and interpretation of these data. We also thank Yuzhen Tao in the Dr. Pollak's laboratory for running the assays. This work was funded by Public Health Service research Grants R01 CA55075, R01 CA141298, and R01 CA133891 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Gabriel Lai was supported by a National Research Service Award T32 CA009314 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kaaks R, Lukanova A (2001) Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc 60: 91–106
2. Hsing AW, Gao YT, Chua S, Deng J, Stanczyk FZ (2003) Insulin resistance and prostate cancer risk. J Natl Cancer Inst 95:67–71
3. Hubbard JS, Rohrmann S, Landis PK et al (2004) Association of prostate cancer risk with insulin, glucose, and anthropometry in the Baltimore Longitudinal Study of Aging. Urology 63:253–258
4. Hammarsten J, Hogstedt B (2005) Hyperinsulinaemia: a prospective risk factor for lethal clinical prostate cancer. Eur J Cancer 41:2887–2895
5. Hammarsten J, Damber JE, Peeker R, Mellstrom D, Hogstedt B (2010) A higher prediagnostic insulin level is a prospective risk factor for incident prostate cancer. Cancer epidemiol 34:574–579
6. Dankner R, Shanik MH, Keinan-Boker L, Cohen C, Chetrit A (2012) Effect of elevated basal insulin on cancer incidence and mortality in cancer incident patients: the Israel GOH 29-year follow-up study. Diabetes Care 35:1538–1543
7. Bonser AM, Garcia-Webb P (1984) C-peptide measurement: methods and clinical utility. Crit Rev Clin Lab Sci 19:297–352
8. Borugian MJ, Spinelli JJ, Sun Z et al (2007) Prediagnostic C-peptide and risk of prostate cancer. Cancer Epidemiol Biomark Prev 16:2164–2165
9. Stocks T, Lukanova A, Rinaldi S et al (2007) Insulin resistance is inversely related to prostate cancer: a prospective study in Northern Sweden. Int J Cancer 120:2678–2686
10. Lai GY, Helzlsouer KJ, Clipp SL, Rifai N, Platz EA (2010) Association between C-peptide concentration and prostate cancer incidence in the CLUE II cohort study. Cancer Prev Res (Phila). 3:1334–1341
11. Neuhouser ML, Till C, Kristal A et al (2010) Finasteride modifies the relation between serum C-peptide and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Cancer Prev Res (Phila) 3:279–289

12. Ma J, Li H, Giovannucci E et al (2008) Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. Lancet Oncol 9:1039–1047
13. Freedland SJ, Giovannucci E, Platz EA (2006) Are findings from studies of obesity and prostate cancer really in conflict? Cancer Causes Control 17:5–9
14. MacInnis RJ, English DR (2006) Body size and composition and prostate cancer risk: systematic review and meta-regression analysis. Cancer Causes Control 17:989–1003
15. Ceddia RB, Koistinen HA, Zierath JR, Sweeney G (2002) Analysis of paradoxical observations on the association between leptin and insulin resistance. Faseb J. 16:1163–1176
16. Lagiou P, Signorello LB, Trichopoulos D, Tzonou A, Trichopoulou A, Mantozoros CS (1998) Leptin in relation to prostate cancer and benign prostatic hyperplasia. Int J Cancer 76:25–28
17. Hsing AW, Chua S, Gao YT et al (2001) Prostate cancer risk and serum levels of insulin and leptin: a population-based study. J Natl Cancer Inst 93:783–789
18. Stattin P, Kaaks R, Johansson R et al (2003) Plasma leptin is not associated with prostate cancer risk. Cancer Epidemiol Biomark Prev 12:474–475
19. Baillargeon J, Platz EA, Rose DP et al (2006) Obesity, adipokines, and prostate cancer in a prospective population-based study. Cancer Epidemiol Biomark Prev 15:1331–1335
20. Li H, Stampfer MJ, Mucci L et al (2010) A 25-year prospective study of plasma adiponectin and leptin concentrations and prostate cancer risk and survival. Clin Chem 56:34–43
21. Touvier M, Fezeu L, Ahluwalia N et al (2013) Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. Am J Epidemiol 177:3–13
22. Burton A, Martin RM, Holly J et al (2013) Associations of adiponectin and leptin with stage and grade of PSA-detected prostate cancer: the Protect study. Cancer Causes Control 24:323–334
23. Stattin P, Soderberg S, Hallmans G et al (2001) Leptin is associated with increased prostate cancer risk: a nested case-referent study. J Clin Endocrinol Metab 86:1341–1345
24. Fowke JH, Motley S, Dai Q, Concepcion R, Barocas DA (2013) Association between biomarkers of obesity and risk of high-grade prostatic intraepithelial neoplasia and prostate cancer: evidence of effect modification by prostate size. Cancer Lett 328:345–352
25. DeLellis HK, Rinaldi S, Kaaks R, Kolonel L, Henderson B, Le Marchand L (2007) Lifestyle and dietary correlates of plasma insulin-like growth factor binding protein-1 (IGFBP-1), leptin, and C-peptide: the multiethnic cohort. Nutr Cancer 58:136–145
26. Bandaru P, Shankar A (2011) Association between plasma leptin levels and diabetes mellitus. Metab Syndr Relat Disord 9:19–23
27. Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC (2007) Risk factors for prostate cancer incidence and progression in the Health Professionals Follow-up Study. Int J Cancer 121: 1571–1578
28. Patel AR, Klein EA (2009) Risk factors for prostate cancer. Nat Clin Pract 6:87–95
29. Platz EA, Pollak MN, Leitzmann MF, Stampfer MJ, Willett WC, Giovannucci E (2005) Plasma insulin-like growth factor-1 and binding protein-3 and subsequent risk of prostate cancer in the PSA era. Cancer Causes Control 16:255–262
30. Platz EA, Leitzmann MF, Rifai N et al (2005) Sex steroid hormones and the androgen receptor gene CAG repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. Cancer Epidemiol Biomark Prev 14:1262–1269
31. Platz EA, Clinton SK, Giovannucci E (2008) Association between plasma cholesterol and prostate cancer in the PSA era. Int J Cancer 123:1693–1698
32. Tande AJ, Platz EA, Folsom AR (2006) The metabolic syndrome is associated with reduced risk of prostate cancer. Am J Epidemiol 164:1094–1102
33. Considine RV (2005) Human leptin: an adipocyte hormone with weight-regulatory and endocrine functions. Semin Vasc Med 5: 15–24
34. Kasper JS, Giovannucci E (2006) A meta-analysis of diabetes mellitus and the risk of prostate cancer. Cancer Epidemiol Biomark Prev 15:2056–2062
35. Kaaks R, Lukanova A, Rinaldi S et al (2003) Interrelationships between plasma testosterone, SHBG, IGF-I, insulin and leptin in prostate cancer cases and controls. Eur J Cancer Prev 12:309–315
36. Ruhl CE, Everhart JE (2001) Leptin concentrations in the United States: relations with demographic and anthropometric measures. Am J Clin Nutr 74:295–301