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Lifestyle and Dietary Correlates of Plasma Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1), Leptin, and C-Peptide: The Multiethnic Cohort

Katherine DeLellis Henderson, Sabina Rinaldi, Rudolf Kaaks, Laurence Kolonel, Brian Henderson, and Loïc Le Marchand

Abstract: *Circulating insulin-like growth factor binding protein 1 (IGFBP-1), leptin, and insulin are 3 proteins modified by obesity and have been associated with cancer at several sites in past studies. We conducted a cross-sectional study to describe the correlation of these proteins with gender, race/ethnicity, anthropometric indexes, and dietary and lifestyle factors. We measured fasting plasma levels of IGFBP-1, leptin, and C-peptide, used here as a stable measure of insulin secretion, in a random sample of 450 male and 352 postmenopausal female Hawaii and Los Angeles Multiethnic Cohort Study (MEC) participants (age range 47–82 yr at blood draw). Through a series of multiple linear regressions, we found that the most parsimonious model for plasma IGFBP-1 included inverse associations with age, body mass index (BMI), and regular soda intake. A term for interaction between age and BMI was positively associated with plasma IGFBP-1. Adjusted mean plasma leptins were highest among Whites and African Americans and lowest among Hawaiians and Japanese Leptin was also inversely associated with age and positively associated with the interaction between age and race/ethnicity, female gender, and BMI. A model with only race/ethnicity and BMI (positive association) was best for plasma C-peptide. Adjusted means for C-peptide were highest for Japanese and Whites and lowest for African Americans. The overall percent of variance in protein levels explained by these models was low for IGFBP-1($R^2 = 0.17$) and C-peptide ($R^2 = 0.11$) and higher for leptin ($R^2 = 0.57$). We saw no clear correlation between racial/ethnic trends in protein levels with those of colorectal, breast, or prostate cancer incidence rates in the MEC. Research to clarify factors associated with determination of these proteins and their relationship with cancer etiology is warranted.*

Introduction

The insulin-like growth factor (IGF) and insulin pathways have been implicated in cancer etiology at certain organ sites and provide a possible mechanistic link between obesity and cancer. The mechanism linking each of these pathways to cancer has not been fully elucidated, but it is hypothesized that proteins involved in regulation of these pathways, such as IGF binding protein-1 (IGFBP-1), leptin, and insulin, may act in concert to play a crucial role (for review, see Refs. 1–3). Insulin has been shown to suppress IGFBP-1 expression, thereby potentially increasing the bioactivity of IGF-I (4). Leptin, another important regulator of energy balance, has also been shown to interact with the IGF system (5,6). Specifically, leptin has been shown to be inversely associated with IGFBP-1 in a body mass index (BMI) dependent fashion (6). Proteins in these pathways are important regulators of human metabolic processes and may be involved in metabolically derived human carcinogenesis.

Although the hypothesis for the importance of these proteins in cancer etiology is strong, and the factors that determine circulating levels of these proteins are generally understood, it is unclear whether the relationship between these factors and circulating levels differs by obesity status and how these factors operate in the pathway between obesity and cancer. In the literature, IGFBP-1 appears to be inversely associated with age and anthropometric measures including BMI, weight (7,8), and alcohol intake (9) and directly correlated with total energy intake, carbohydrate intake (7), and leisure-time physical activity (8). Leptin appears to be positively correlated with anthropometric measures such as BMI and body fat content (10). Females have been shown to have higher leptin levels than males (11). Leptin levels have demonstrated

K. DeLellis and B. Henderson are affiliated with the University of Southern California, Keck School of Medicine, Los Angeles, CA 90033; S. Rinaldi is affiliated with the International Agency for Research on Cancer, Lyon, France; R. Kaaks is affiliated with the Division of Cancer Epidemiology, German Cancer Research Center (Deutsches Krebsforschungszentrum; DKFZ), Im Neuenheimer Feld 280 Heidelberg, Germany; and L. Kolonel and Loïc Le Marchange are affiliated with the Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI.

variation with reproductive factors such as at pubertal onset (12), across the normal menstrual cycle (13,14), during pregnancy (15), and with years since menopause (16). Leptin levels are sensitive to energy balance, increasing with energy intake (17) and decreasing with increased physical activity (18). C-peptide is a stable, surrogate measure for insulin in the circulation (19). Thus, the determinants of C-peptide should be largely the same as for insulin itself. However, there is evidence that higher C-peptide is associated with increasing BMI, lower physical activity, and a western diet (20–23), factors in line with those thought to be driving insulin levels, primarily energy balance and diet.

We performed a cross-sectional investigation of the correlates of plasma IGFBP-1, leptin, and C-peptide in a multiethnic population-based sample of middle-aged to older men and postmenopausal women. We set out to determine what factors were correlated with these 3 proteins in these data and whether such factors differed by obesity, gender, or racial/ethnic status. By better characterizing the factors that determine circulating IGFBP-1, leptin, and C-peptide levels, we hope to advance our understanding of the interaction of these pathways in cancer etiology and perhaps to identify some modifiable risk factors for prevention of obesity-related chronic diseases such as cancer.

Materials and Methods

Study Subjects

Participants included in these analyses were selected from a large population-based cohort study, The Hawaii and Los Angeles Multiethnic Cohort (MEC) study. The primary aim of the MEC is to evaluate the dietary and other environmental contributions to the racial/ethnic variability in cancer risk. The MEC consists of 215,251 men and women, mainly Japanese Americans, Whites, and Native Hawaiians in Hawaii and African Americans and Latinos in Los Angeles. Subjects were recruited between 1993 and 1996 primarily through driver's license files. All participants were between the ages of 45 and 75 yr at the time of enrollment. Baseline data were collected on cohort participants via a mailed questionnaire that contained sections on medical and family cancer history, diet, physical activity, and female reproductive history. For this study, a single blood sample was collected on a subcohort of about 5,000 randomly selected participants stratified by sex and race/ethnicity. These participants have been used as controls in nested case-control studies. Participants were instructed to fast before the blood draw, which was typically completed in the morning at the person's home, after informed consent was obtained. Handling of samples was achieved with attention to minimization of time between draw and processing; 90% of samples were processed within 4 h of the blood draw, and 98% were processed within 24 h of draw. Sodium heparin was used as an anticoagulant in blood collection tubes. The participation rate for providing a blood sample was approximately 66% and did not vary greatly across different racial/ethnic groups.

For this study, 100 subjects from each of 10 gender-racial/ethnic groups with equal representation of each 5-yr age group at blood draw were randomly selected from the MEC blood subcohort. Women who reported that they were taking estrogen replacement therapy at the time of blood draw; women of unknown menopausal status; subjects with prevalent breast, prostate, or colorectal cancer, or with missing or invalid baseline questionnaire data for calculation of BMI were excluded: 802 men and postmenopausal women were left for the analysis (Table 1).

IGFBP-1, Leptin, and C-Peptide Measurements in Plasma

Samples were analyzed blindly as to race/ethnicity, sex of the participant, and case-control status. To reduce the effect of laboratory variability, each analytical batch included equal numbers of subjects from each sex and racial/ethnic group. Plasma IGFBP-1 was measured by an immunoradiometric assay (IRMA) by Diagnostic System Laboratories (DSL, Webster, TX). The sensitivity of the assay is 0.20 ng/ml. Human plasma leptin was measured by a direct double-antibody radioimmunoassay (RIA) by LINCO Research Inc. (St. Charles, MI). The theoretic sensitivity of the assay (as stated by the manufacturer) is 0.5 ng/ml. Plasma C-peptide was also measured by a direct double-antibody RIA but with reagents from DSL (Webster, TX). The detection limit of the assay is 0.05 ng/ml. Reproducibility of the assays were documented by analyzing blind duplicate samples (10%) with the study samples. The average overall intrabatch coefficients of variation for IGFBP-1, leptin, and C-peptide were 4.8%, 6.3%, and 6.6%, respectively. The average overall interbatch coefficients of variation were 14.6%, 11.9%, and 13.7% for IGFBP-1, leptin, and C-peptide, respectively.

Statistical Analysis

Analysis of variance and analysis of covariance were used to test for differences in crude and adjusted mean protein levels by sex and racial/ethnic group and covariate level. In Table 1, the crude levels shown were not transformed. However, in Tables 2 and 4 and in the regression models, protein values were transformed to produce the best approximate normal distribution among all subjects. For plasma leptin and C-peptide, the natural log transformation produced the best fit to normal. Raising plasma IGFBP-1 levels by the exponent 0.2 produced the best fit to normal. All values have been transformed back to normal physiological levels in the tables for the purpose of presentation.

Diet intake data were adjusted for total calorie intake either by the calculation of a nutrient density or by calculation of a percent of calories from the diet component such as carbohydrates. A nutrient density was calculated by multiplying the daily diet component intake in grams by the inverse of total daily energy intake in calories $\times$ 1,000 (24). The percent

Table 1. Distribution of Characteristics and Crude Plasma IGFBP-1, Leptin, and C-Peptide Levels From 802 Multiethnic Cohort Participants by Sex and Racial/Ethnic Group[a]

Characteristic	Native Hawaiian	African American	Japanese American	Latino	White	P^b
			Women			
No. subjects (% of women)	58 (16.5)	73 (20.7)	69 (19.6)	81 (23.0)	71 (20.2)	
Age, yr [LS Mean (95%CL)]	61.4 (59.7,63.2)	62.0 (60.5,63.6)	64.2 (62.6,65.9)	61.4 (59.9,62.9)	62.3 (60.7,63.9)	0.098
BMI, kg/m^2[LS Mean (95% CL)]	27.1 (25.9,28.4)	28.4 (27.2,29.5)	23.8 (22.6,24.9)	28.3 (27.2,29.4)	26.1 (24.9,27.2)	<0.0001
IGFBP-1(ng/ml)[LS Mean (95% CL)]	24.5 (19.6,30.3)	26.6 (21.9,32.1)	37.5 (31.1,44.9)	30.6 (25.6,36.5)	32.7 (27.1,39.3)	0.025
Leptin(ng/ml)[LS Mean (95% CL)]	20.6 (17.2,24.6)	27.9 (23.8,32.7)	15.5 (13.1,18.2)	25.9 (22.2,30.1)	21.4 (18.2,25.2)	<0.0001
C-peptide(ng/ml)[LS Mean (95% CL)]	4.20 (3.67,4.81)	3.49 (3.09,3.94)	3.83 (3.39,4.34)	4.20 (3.74,4.70)	4.12 (3.64,4.65)	0.17
			Men			
No. subjects (% of women)	100 (22.2)	73 (16.2)	93 (20.7)	90 (20.0)	94 (20.9)	
Age, years[LS Mean (95% CL)]	59.2 (57.5,60.9)	58.2 (56.1,60.2)	59.5 (57.7,61.3)	59.0 (57.2,60.9)	59.4 (57.6,61.2)	0.88
BMI,kg/m^2[LS Mean (95% CL)]	28.4 (27.6,29.3)	27.5 (26.5,28.5)	24.7 (23.8,25.6)	27.1 (26.2,28.0)	26.9 (26.1,27.8)	<0.0001
IGFBP-1(ng/ml)[LS Mean (95% CL)]	22.9 (18.5,28.1)	20.3 (15.7,25.9)	28.6 (23.1,35.0)	23.7 (18.9,29.4)	23.0 (18.5,28.4)	0.32
Leptin(ng/ml)[LS Mean (95% CL)]	8.80 (7.70,10.07)	8.61 (7.36,10.07)	6.35 (5.52,7.29)	8.14 (7.07,9.37)	9.84 (8.57,11.30)	0.0004
C-Peptide(ng/ml)[LS Mean (95% CL)]	4.16 (3.73,4.64)	3.50 (3.08,3.98)	3.92 (3.50,4.40)	4.07 (3.63,4.57)	4.23 (3.78,4.74)	0.23

[a] Abbreviations are as follows: IGFBP, insulin-like growth factor binding protein; LS, least squares; CL, confidence limit; BMI, body mass index.
[b] P value testing homogeneity of LS Means across race/ethnicity derived from analysis of variance models.

of calories from a diet component was calculated by dividing calories from the specific diet component, multiplied by 100, by the sum of calories ingested from the major macronutrients per day. The macronutrients included fat, protein, carbohydrates, and alcohol. Means presented are least-squares means (LS means). In the univariate analysis (see Table 2), continuous variables were cut at quartiles except in cases in which a large percent of subjects had a zero value; in which case, the continuous variable was cut as shown in Table 2.

Multiple linear regressions using Proc GLM in SAS version 9.0 (SAS Institute, Cary, NC) were performed to determine which covariates were associated with plasma IGFBP-1, leptin, and C-peptide levels. We used past reports (6–9,11,21,25–27) and known biology to guide the selection of variables to be considered in the models. The following factors were tested for possible association with plasma IGFBP-1, leptin, and C-peptide: gender, race/ethnicity, age at baseline questionnaire, BMI, physical activity, smoking, total energy intake (kcal/day), percents of daily calories from saturated fat, fat from meat and protein, percent of daily calories from carbohydrates, added sugar intake (teaspoons added sugar per day), energy density of the diet (total calories and/or total grams food and caloric beverages per day), and the following dietary intake variables as densities: total dairy, skim milk, lowfat milk, whole milk, vitamin D, calcium from food sources, dietary fiber, total fruit and vegetable, regular soda, and diet soda. In the multiple regressions, continuous variables were tested unless otherwise specified. Four measures of physical activity were considered: hours in vigorous work per day (physical activity 1), hours in vigorous activity per day (physical activity 2), hours in moderate activity per day (physical activity 3), and metabolic equivalents (METs) of physical activity per day. These variables reflect usual physical activity over the previous year. Smoking status was tested as a 3-level variable with the possible values of never, past, or current.

In the multiple regressions, the R-squared selection method was used in conjunction with Mallow's Cp to identify important associated variables. All variables were allowed to compete in the multiple regressions. Plots of the Cp statistic as well as examination of incremental changes in the R-squared assisted in the final selection of variables for inclusion in the models. Although the sampling proportions were not equal for each race/ethnic group, we adjusted the models for this variable when indicated and therefore expected the results to be generalizable to the entire cohort.

The cancer incidence rates in Table 4 were computed for cancer surveillance through December 31, 2002. These rates were truncated to ages 50–74 per 100,000 and age adjusted to the United States 1970 standard population. Details of the method of calculation of these rates have been published previously.

Results

Table 1 shows the number of men and women in each racial/ethnic group as well as the LS means and 95% confidence limits for age, BMI, and crude plasma protein levels for the 352 healthy women and 450 healthy men included in this analysis by racial/ethnic group. Body size characteristics such as height, weight, and BMI differed significantly across racial/ethnic groups in both women and men and were in line with what has been reported previously for this group of controls and for the entire cohort (28,29). In Table 1, the crude LS mean level of plasma IGFBP-1 is significantly different across races among females, whereas the crude LS mean level of plasma leptin differs significantly in both genders.

Table 2. Least Squares (LS) Mean Plasma IGFBP-1, Leptin, and C-Peptide Levels by Gender, Race/Ethnicity, Age, BMI, Lifestyle and Dietary Variables[a]

Variable		IGFBP-1	Leptin	C-Peptide
Gender				
Male		23.73	8.26	3.99
Female		30.30	22.02	3.73
P^b		0.0003	<0.0001	0.791
Race/ethnicity				
Native Hawaiian		23.49	13.68	4.17
African American		23.29	15.50	3.50
Japanese American		32.14	9.66	3.89
Latino		26.80	14.43	4.13
White		26.85	14.71	4.18
P^b		0.019	<0.0001	0.019
Age(yr)				
≤ 54	Q1	17.78	13.86	4.07
55–60	Q2	23.98	14.48	4.24
61–67	Q3	31.97	12.34	3.7
68+	Q4	35.45	13.55	3.90
P^c		<0.0001	0.308	0.140
BMI (kg/m2)				
≤ 23.56	Q1	41.65	7.53	3.06
23.57–26.13	Q2	28.81	12.39	3.86
26.131–29.393	Q3	20.98	15.19	4.31
29.3931+	Q4	18.70	23.59	4.92
P^c		<0.0001	<0.0001	<0.0001
Physical activity 1 (h in vigorous work per day on average during last year)				
0	L1	28.49	14.05	4.00
0.10–0.30	L2	26.78	13.31	4.07
0.31+	L3	20.78	12.34	3.85
P^c		0.0007	0.059	0.535
Physical activity 2 (h in vigorous activity per day on average during last year)				
0	L1	28.92	14.08	4.09
0.10–0.36	L2	24.42	14.42	4.04
0.37+	L3	23.95	11.21	3.73
P^c		0.020	0.016	0.088
Physical activity 3 (h in moderate activity per day on average during last year)				
≤ 0.299	Q1	25.79	14.72	4.15
0.3–0.699	Q2	27.99	13.64	3.88
0.7–0.999	Q3	27.78	13.29	3.92
1.0+	Q4	24.62	12.49	3.97
P^c		0.544	0.022	0.540
Smoking				
Never	L1	27.54	12.82	3.83
Past	L2	26.11	14.55	4.05
Current	L3	24.50	12.43	4.21
P^c		0.239	0.708	0.073
Alcohol intake(% daily calories from alcohol)				
0	L1	28.22	14.19	4.05
0.07–2.27	L2	25.91	12.91	3.82
2.28+	L3	23.68	12.67	4.00
P^c		0.035	<0.0001	0.639
Energy intake (kcal/day)				
≤ 1420	Q1	29.22	14.78	3.95
1421–2042	Q2	29.63	13.21	3.81
2043–2672	Q3	23.29	13.38	4.12
2673+	Q4	24.16	12.62	4.03
P^c		0.008	0.041	0.432
Saturated fat intake(% daily calories from saturated fat)				
≤ 7.10	Q1	28.21	11.51	3.79
7.11–8.92	Q2	25.32	14.00	4.00
8.93–10.886	Q3	26.18	13.67	4.13
10.8861+	Q4	25.40	15.02	3.95
P^c		0.361	0.0006	0.373

(*Continued on next page*.)

Table 2. Least Squares (LS) Mean Plasma IGFBP-1, Leptin, and C-Peptide Levels by Gender, Race/Ethnicity, Age, BMI, Lifestyle and Dietary Variables[a] *(Continued)*

Variable		IGFBP-1	Leptin	C-Peptide
Fat from meat intake (% daily calories from fat from meat)				
≤ 2.721	Q1	32.28	11.33	3.55
2.722–4.670	Q2	24.05	14.14	4.09
4.671–6.869	Q3	24.92	13.95	4.34
6.870+	Q4	24.45	14.89	3.92
P[c]		0.009	0.0004	0.032
Total protein intake (% daily calories from protein)				
≤ 13.01	Q1	26.39	12.40	3.90
13.011–14.71	Q2	25.03	13.27	4.10
14.72–16.27	Q3	27.91	13.08	3.79
16.28+	Q4	25.76	15.31	4.07
P[c]		0.904	0.006	0.789
Total dairy intake density (g/1,000 kcal/day)				
≤ 38.5	Q1	26.74	12.61	4.00
38.51–80.9	Q2	24.53	13.15	3.85
81.0–146.61	Q3	24.89	14.70	4.10
146.62+	Q4	29.05	13.52	3.92
P[c]		0.388	0.163	0.984
Dietary fiber intake density (g/1,000 kcal/day)				
≤ 8.31	Q1	21.30	13.94	4.42
8.32–10.66	Q2	24.28	14.09	3.86
10.67–13.92	Q3	29.21	13.58	3.76
13.93+	Q4	31.14	12.45	3.86
P[c]		<0.0001	0.101	0.015
Total carbohydrate intake (% daily calories from carbohydrates)				
≤ 46.58	Q1	24.70	14.45	3.91
46.59–51.86	Q2	26.77	13.62	3.89
51.861–58.062	Q3	27.24	13.89	4.02
58.063+	Q4	26.27	12.14	4.04
P[c]		0.508	0.026	0.455
Added sugar intake (teaspoons added sugar per day)				
≤ 5.70	Q1	28.29	14.25	4.00
5.71–9.67	Q2	27.67	13.15	3.83
9.68–16.127	Q3	24.63	13.56	4.08
16.128+	Q4	24.62	12.99	3.96
P[c]		0.083	0.275	0.842
Total fruit and vegetable intake density (g/1,000 kcal/day)				
≤ 187.82	Q1	20.48	13.62	4.39
187.83–272.59	Q2	25.89	14.35	3.83
272.60–382.96	Q3	30.36	13.38	3.86
382.97+	Q4	29.21	12.71	3.81
P[c]		<0.0001	0.240	0.017
Regular soda intake density (g/1,000 kcal/day)				
0	Q1	31.90	13.36	3.75
1.00–8.79	Q2	26.02	12.76	4.29
8.80–60.40	Q3	23.93	13.36	4.07
60.41+	Q4	20.71	14.11	4.13
P[c]		<0.0001	0.448	0.040
Diet soda intake density (g/1,000 kcal/day)				
0	L1	25.89	12.48	3.87
1.00–56.37	L2	27.77	12.98	4.00
56.38+	L3	25.76	16.42	4.15
P[c]		0.924	<0.0001	0.138
METs (METs of activity per day)				
≤ 1.4382	Q1	24.78	15.34	4.13
1.4383–1.6212	Q2	25.02	13.83	4.06
1.6213–1.7976	Q3	29.68	12.91	3.98
1.7977+	Q4	21.90	10.62	3.80
P[c]		0.476	0.0009	0.135

Table 2. Least Squares (LS) Mean Plasma IGFBP-1, Leptin, and C-Peptide Levels by Gender, Race/Ethnicity, Age, BMI, Lifestyle and Dietary Variables[a]

Variable		IGFBP-1	Leptin	C-Peptide
Energy density (total calories per total grams food and caloric beverages/day)				
≤ 0.974	Q1	24.13	12.58	3.91
0.975–1.125	Q2	30.17	13.35	4.01
1.126–1.304	Q3	27.22	13.58	3.83
1.305+	Q4	23.88	14.49	4.12
P^c		0.658	0.050	0.522

[a] Means presented are LS means of transformed hormone levels exponentiated back to physiological levels. IGFBP-1 and C-peptide levels are crude levels. Leptin levels are adjusted for gender only. Abbreviations are as follows: IGFBP, insulin-like growth factor binding protein; Q, quartile; L,; BMI, body mass index; METs, metabolic units.
[b] P values for homogeneity, categorical variable.
[c] P values for trend, categorical continuous variable.

Crude plasma C-peptide levels did not differ significantly by race.

In a univariate analysis of these data (see Table 2), females had higher plasma IGFBP-1 versus males ($P = 0.0003$). Plasma IGFBP-1 differed significantly by race/ethnicity in the univariate analysis. Japanese Americans had the highest plasma IGFBP-1, with Latinos and Whites intermediate, and Native Hawaiians and African Americans at the bottom of the groups tested. Age, fiber intake, and total fruit and vegetable intake have positive relationships with plasma IGFBP-1, whereas BMI, hours per week of vigorous work (physical activity 1) and hours per week of vigorous activity (physical activity 2), energy intake and regular soda have inverse relationships with plasma IGFBP-1 in these data. The relationship between plasma IGFBP-1 and percent daily calories from fat from meat appears to be driven by the high plasma IGFBP-1 LS mean in the lowest quartile of fat from meat rather than by a dose response effect.

Several variables were statistically significantly associated with plasma leptin levels in a univariate analysis.

Table 3. Multiple Regression of Plasma IGFBP-1, Leptin, and C-Peptide on Variables Selected Using Mallow's Cp Selection and R^2 Selection[a]

Variable	Regression Coefficient	P^b	Adjusted
IGFBP-1(ng/ml)			
Age	−0.0056	0.5180	
BMI(kg/m^2)	−0.0577	0.0026	
Age × BMI(kg/m^2)	0.0006	0.0449	
Regular soda intake(g/1,000 kcal/day)	−0.0003	0.0007	
			0.171
Leptin (ng/ml)			
Age	−0.0030	0.5776	
Race/ethnicity African American	−0.9182	0.0487	
Race/ethnicity Latino	0.4007	0.3784	
Race/ethnicity Japanese	−1.0963	0.0200	
Race/ethnicity Hawaiian	−0.6702	0.1653	
Age × Race/ethnicity African American	0.0142	0.0642	
Age × Race/ethnicity Latino	−0.0086	0.2520	
Age × Race/ethnicity Japanese	0.0147	0.0549	
Age × Race/ethnicity Hawaiian	0.0079	0.3176	
Gender, female	0.9737	<0.0001	
BMI(kg/m^2)	0.0856	<0.0001	
			0.572
C-Peptide (ng/ml)			
Race/ethnicity African American	−0.2290	0.0001	
Race/ethnicity Latino	−0.0518	0.3614	
Race/ethnicity Japanese	0.0088	0.8801	
Race/ethnicity Hawaiian	−0.0525	0.3649	
BMI(kg/m^2)	0.0364	<0.0001	
			0.105

[a] Abbreviations are as follows: IGFBP, insulin-like growth factor binding protein; BMI, body mass index. Whites are reference group.
[b] P testing the null hypothesis that the parameter is not significantly different from zero.
[c] Multivariate R^2adjusting for all variables in model.

Table 4. LS Mean Plasma Protein Levels From 802 Hawaii and Los Angeles Multiethnic Cohort (MEC) Controls and Age-Adjusted Incidence Rates in the MEC Through 2002 by Gender and Race/Ethnicity[a]

IGFBP-1[a]		Leptin[a]		C-Peptide[b]		Colorectal Cancer[b]		Breast Cancer[b]	
				Women					
J	32.9 (27.1,39.7)	AA	24.3 (21.4,27.6)	J	4.21 (3.74,4.73)	AA	134.5	H	539.0
W	32.1 (26.7,38.3)	W	22.8 (20.0,25.9)	W	4.20 (3.71,4.75)	H	112.3	J	417.8
L	32.0 (26.9,37.9)	L	22.6 (20.0,25.5)	H	4.15 (3.65,4.73)	J	107.9	W	372.3
AA	27.5 (22.7,33.0)	H	20.1 (17.4,23.1)	L	4.01 (3.59,4.48)	W	80.7	AA	322.6
H	25.0 (20.2,30.7)	J	19.5 (17.0,22.5)	AA	3.33 (2.96,3.74)	L	80.2	L	233.7
				Men					
H	25.4 (20.9,30.5)	W	9.9 (8.8,11.1)	J	4.32 (3.87,4.82)	J	200.5	AA	1152.8
L	24.0 (19.6,29.1)	AA	8.4 (7.3,9.5)	W	4.23 (3.80,4.71)	H	182.9	L	479.9
J	23.8 (19.4,29.0)	L	8.0 (7.1,9.0)	L	4.05 (3.63,4.51)	AA	162.4	W	453.8
W	22.3 (18.3,27.1)	H	7.8 (6.9,8.7)	H	3.90 (3.51,4.33)	W	119.9	H	411.8
AA	22.3 (17.7,27.8)	J	7.6 (6.8,8.6)	AA	3.42 (3.03,3.86)	L	114.8	J	343.2

[a] Abbreviations are as follows: LS, least squares; IGFBP, insulin-like growth factor binding protein; AA, African Americans; H, Native Hawaiians; J, Japanese Americans; W, Whites; L, Latinos. Protein concentrations are adjusted for covariates found to be significant in multivariate model (see Table 3). LS Mean levels in ng/ml.

[b] Cancer incidence rates are truncated to ages 50–74 per 100,000 and age adjusted to the U.S. 1970 standard population.

Because of the strong gender effect, females had much higher plasma leptin compared to males ($P < 0.0001$); plasma leptin values in Table 2 have been adjusted for gender. After adjustment for gender, African Americans had the highest plasma leptin, followed by Whites, Latinos, Native Hawaiians, and Japanese Americans. BMI, saturated fat, fat from meat, total protein, and diet soda intake had the strongest positive relationships with plasma leptin. Hours per week of vigorous activity (physical activity 2), METs, alcohol intake, and energy intake had inverse relationships with plasma leptin in this analysis.

For plasma C-peptide, race/ethnicity, BMI, percent daily calories from fat from meat, dietary fiber intake density, total fruit and vegetable intake density, and regular soda intake density were associated at the $P < 0.05$ level. Whites had the highest plasma C-peptide levels, and African Americans the lowest. BMI and plasma C-peptide were directly correlated, whereas fat from meat was indirectly correlated with plasma C-peptide.

In addition, the intake densities for skim milk, lowfat milk, whole milk, vitamin D from food sources, and calcium from food sources were tested for possible association with IGFBP-1, leptin, and C-peptide. No clear relationships were seen between these variables and IGFBP-1, leptin, or C-peptide (data not shown).

The results of multiple regressions of plasma IGFBP-1, leptin, and C-peptide on all factors considered resulted in the models shown in Table 3. Whites were used as the reference group since they have been the most often studied population in past reports. Age, BMI, the interaction between age and BMI, and regular soda intake were independently associated with plasma IGFBP-1. Age, race/ethnicity, the interaction between age × race/ethnicity, gender, and BMI were independently associated with plasma leptin. The P value for the group of Age × Race interaction terms was 0.0009, and 2 of the individual Age × Race/Ethnicity interaction terms were of borderline significance (Age × African American Race/Ethnicity and Age × Japanese Race/Ethnicity). Race/ethnicity and BMI were statistically significantly associated with plasma C-peptide. The addition of total energy intake to each of the 3 final models produced no significant change to the R^2 values. The slopes for the relationships of age with IGFBP-1 and leptin did not change significantly when BMI was included in the models.

Table 4 shows the LS mean plasma protein levels, adjusted for covariates found to be significant in the multivariate models, by gender and race/ethnicity. We found no clear correlation in either gender between LS mean plasma IGFBP-1, leptin, or C-peptide levels and colorectal, breast, or prostate cancer incidence rates within the MEC by race/ethnicity. There appears to be no difference in plasma IGFBP-1 across races in either gender. In plasma leptin, the difference across races is statistically significant among males only ($P = 0.002$). In plasma C-peptide, the difference across racial/ethnic groups was clearly significant in females ($P = 0.031$) but not in males ($P = 0.053$).

Discussion

In this cross-sectional analysis, a host of potential correlates was examined for possible association with plasma IGFBP-1, leptin, and C-peptide levels in a multiethnic population-based sample. Multivariate analyses produced a simple model for each of these 3 proteins. The most parsimonious model for plasma IGFBP-1 included age, BMI, the interaction between age and BMI, and regular soda intake. That for plasma leptin included age, race/ethnicity, the interaction between age and race/ethnicity, gender, and BMI. The best model for plasma C-peptide included only race/ethnicity and BMI. The percent of variance explained by these models was low for plasma IGFBP-1 and C-peptide, suggesting that other determining factors have yet to be identified. The

percent of variance explained by the model for plasma leptin was much higher. Finally, a crude analysis of LS mean plasma protein levels by gender and race/ethnicity, adjusted for covariates found to be significant in the multivariate models, produced no clear correlation with colorectal, breast, or prostate cancer incidence rates within the MEC.

In the context of the literature, our results for plasma IGFBP-1 are generally in line with those of past studies and offer some new insight. IGFBP-1 has been shown to be inversely associated with both age and BMI in previous reports (7,8); however, no study has reported finding an interaction between age and BMI. IGFBP-1 has also been reported to be inversely associated with alcohol intake (9). In our analysis, plasma IGFBP-1 was inversely associated with alcohol intake in the univariate analysis ($P = 0.035$) only. In other investigations of diet and IGFBP-1, a positive correlation was reported between IGFBP-1 and total energy and carbohydrate intake (7). We found evidence for a linear relationship between plasma IGFBP-1 and total energy in the univariate relationship, which was not statistically significant in the multivariate model. We found no strong relationship between plasma IGFBP-1 and carbohydrate intake in these data. We did however find a negative association between plasma IGFBP-1 and regular soda intake, which was independent of adjustment for the other factors in the multivariate model. Another component of energy balance that has been reported to be associated with IGFBP-1 is physical activity (8). Lukanova et al. (8) reported that IGFBP-1, after adjustment for age and batch, was significantly higher in female subjects reporting higher levels of bicycling but not jogging or swimming. Our data suggested an inverse association between vigorous work and vigorous activity and plasma IGFBP-1; however, these associations were not statistically significant in the multivariate model. In the literature, IGFBP-1 has not been examined for differences across races in any study we have found. In our data, it is important to note that while a difference across ethnicities/races did appear in the univariate analysis, this effect was not independent of other factors in the multivariate analysis. Thus, it appears that circulating IGFBP-1 may be driven primarily by age, BMI, and some measure of energy intake. In addition, the total variance explained by the multivariate model was relatively low, indicating that although we examined a wide range of possible correlates, we have yet to identify the factor or factors that explain the majority of variation in this protein.

Plasma leptin, like plasma IGFBP-1, was correlated with age and BMI. However, while both proteins showed an inverse association with age, and plasma IGFBP-1 was inversely associated with BMI, plasma leptin was positively associated with BMI. Using percent body fatness as a measure of adiposity, Donohue et al. (11) reported that adiposity was the strongest correlate of leptin levels. In addition, these investigators showed that male gender, higher alcohol intake, higher physical activity, and smoking were all inversely correlated with plasma leptin after adjustment for age, gender, and ethnicity. In our data, gender was statistically significantly associated with plasma leptin levels, but alcohol, physical activity, and smoking were not independently associated with plasma leptin levels in the multivariate model. However, physical activity and alcohol intake were significantly inversely associated with leptin in the univariate analysis. Donohue et al. (11) reported no significant difference in leptin levels across races in their study, which included African Americans, Cuban Americans, and non-Hispanic Whites. In our data, we saw an independent, statistically significant difference in plasma leptin levels across races. Whites and African Americans had the highest and Hawaiians and Japanese the lowest mean plasma leptin levels after adjustment for covariates. The interaction between age and race/ethnicity was unexpected and warrants further investigation.

A stable, biological surrogate for insulin (19), plasma C-peptide has been measured in this cross-sectional study to better understand the relationship between diet and cancer. Giovannucci et al. (21) reported that C-peptide was associated with increasing BMI, lower physical activity, and a Western diet. This study did not use a positive measure of C-peptide in the analysis but rather used a C-peptide score derived from likely determinants. In our analysis of directly measured plasma C-peptide, we found that only race/ethnicity and BMI were independently correlated with plasma C-peptide in a multivariate regression. Adjusted means for plasma C-peptide were highest for Japanese and Whites and lowest for African Americans. However, only a small percentage of variance is explained by these factors, and thus, much of the normal variation in C-peptide in the circulation has yet to be explained.

Effects of diet and age on circulating levels of these proteins may be primarily through alterations in adiposity. These data suggest that dietary variables, with the exception of soda consumption, may not relate to circulating levels of these peptides independently of BMI. Age, however, does appear to relate to levels of IGFBP-1 and leptin after adjustment for BMI. The relationship of age with these 2 proteins is not attenuated significantly when BMI is included in the models. Therefore, it is less likely that the associations of age with IGFBP-1 and leptin are due to residual confounding by adiposity in light of the fact that BMI and body fat content are correlated at an R^2 of about 0.9 (30). However, residual confounding cannot be ruled out entirely because BMI in not an exact measure of adiposity.

Finally, although the hypothesis that the overlapping impact of nutrition and lifestyle on insulin and insulin-like growth factor levels may be important in the etiology of obesity-related cancer (for reviews, see Refs. 1 and 31), we saw no clear correlation with any of these 3 proteins and colorectal, breast, or prostate cancer incidence rates by race and gender within the MEC.

One main limitation of the current study is its reliance on a single time point measurement of plasma IGFBP-1, leptin, and C-peptide. While studies have shown measurable interindividual variation in these 3 proteins (6,7,9,22,25,32–34), Kaaks et al. (22) have shown that intraindividual

variation across 1 yr in IGFBP-1 and C-peptide is moderate, suggesting that a single measurement may be adequate to predict long-term circulating levels. No such data appear to be available for leptin measurement.

Another limitation of this study is its cross-sectional design. Due to the fact that blood was drawn from these subjects at essentially the same time that questionnaire data was collected, we cannot be sure that these factors are determinants of protein levels. Rather, we can only assess these factors as potential correlates. The identification of determinants should be addressed in future, prospectively designed studies. Understanding the factors that determine the levels of these proteins in the circulation may offer insight into the mechanism linking diet and obesity to cancer and perhaps into possible cancer prevention strategies; however, one critical caveat is whether circulating levels are highly correlated with tissue levels, a question that has yet to be definitively answered.

In conclusion, plasma IGFBP-1, leptin, and C-peptide are correlated with a number of factors in these data. However, the final models resulting from these multivariate regressions suggest that important modifiable determinants of these proteins have yet to be identified, especially for IGFBP-1 and C-peptide. Future studies looking at additional factors, such as glycemic index and the role for genetics in determining these protein levels are planned.

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References

1. Kaaks R and Lukanova A: Energy balance and cancer: the role of insulin and insulin-like growth factor-I. *Proceedings of the nutrition society* **60**, 91-106, 2001.
2. Marshall S: Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. *Science's Stke [Electronic Resource]: Signal Transduction Knowledge Environment* 2006.
3. Stephenson GD and Rose DP: Breast cancer and obesity: an update. *Nutr Cancer* **45,** 1–16, 2003.
4. Unterman TG, Oehler DT, Murphy LJ, and Lacson RG: Multihormonal regulation of insulin-like growth factor-binding protein-1 in rat H4IIE hepatoma cells: the dominant role of insulin. *Endocrinology* **128,** 2693–2701, 1991.
5. Seck T, Englaro P, Blum WF, et al.: Leptin concentrations in serum from a randomly recruited sample of 50- to 80-year-old men and women: positive association with plasma insulin-like growth factors (IGFs) and IGF-binding protein-3 in lean, but not in obese, individuals. *Eur J Endocrinol* **138,** 70–75, 1998.
6. Soderberg S, Ahren B, Eliasson M, Dinesen B, Brismar K, et al.: Circulating IGF binding protein-1 is inversely associated with leptin in non-obese men and obese postmenopausal women. *Eur J Endocrinol* **144,** 283–290, 2001.
7. Wolk K, Larsson SC, Vessby B, Wolk A, and Brismar K: Metabolic, anthropometric, and nutritional factors as predictors of circulating insulin-like growth factor binding protein-1 levels in middle-aged and elderly men. *J Clin Endocrinol Metab* **89,** 1879–1884, 2004.
8. Lukanova A, Toniolo P, Akhmedkhanov A, et al.: A cross-sectional study of IGF-I determinants in women. *Eur J Cancer Prev* **10,** 443–452, 2001.
9. Vrieling A, Voskuil DW, Bueno de Mesquita HB, et al.: Dietary determinants of circulating insulin-like growth factor (IGF)-I and IGF binding proteins 1, -2, and -3 in women in the Netherlands. *Cancer Causes Control* **15,** 787–796, 2004.
10. Considine RV, Sinha MK, Heiman ML, et al.: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334,** 292–295, 1996.
11. Donahue RP, Zimmet P, Bean JA, et al.: Cigarette smoking, alcohol use, and physical activity in relation to serum leptin levels in a multiethnic population: the Miami Community Health Study. *Ann Epidemiol* **9,** 108–113, 1999.
12. Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, et al.: Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. *J Clin Endocrinol Metab* **82,** 2849–2855, 1997.
13. Quinton ND, Laird SM, Okon MA, et al.: Serum leptin levels during the menstrual cycle of healthy fertile women. *Brit J Biomed Sci* **56,** 16–19, 1999.
14. Salkeld BD, MacAulay JC, Ball RW, and Cannon JG: Modulation of body temperature, interleukin-6 and leptin by oral contraceptive use. *Neuroimmunomodulation* **9,** 319–325, 2001.
15. Stock SM, Sande EM, and Bremme KA: Leptin levels vary significantly during the menstrual cycle, pregnancy, and in vitro fertilization treatment: possible relation to estradiol. *Fertil Sterility* **72,** 657–662, 1999.
16. Brooks-Asplund EM, Tupper CE, Daun JM, Kenney WL, and Cannon JG: Determinants of plasma leptin concentration in postmenopausal women. *Mech Ageing Dev* **125,** 117–119, 2004.
17. Boden G, Chen X, Mozzoli M, and Ryan I: Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab* **81,** 3419–3423, 1996.
18. Fung TT, Hu FB, Yu J, et al.: Leisure-time physical activity, television watching, and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Epidemiol* **152,** 1171–1178, 2000.
19. Hovorka R and Jones RH: How to measure insulin secretion. *Diabetes Metab Res Rev* **10,** 91–117, 1994.
20. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, et al.: Prediagnostic levels of C-peptide, IGF-I, IGFBP-1, -2 and -3 and risk of endometrial cancer. *Int J Cancer* **108,** 262–268, 2004.
21. Giovannucci E, Rimm EB, Liu Y, and Willett WC: Height, predictors of C-peptide and cancer risk in men. *Int J Epidemiol* **33,** 217–225, 2004.
22. Kaaks R, Toniolo P, Akhmedkhanov A, et al.: Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. *J Natl Cancer Inst* **92,** 1592–1600, 2000.
23. Verheus M, Peeters PH, Rinaldi S, et al.: Serum C-peptide and breast cancer risk: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int J Cancer* **119,** 659–667, 2006.
24. Willett W. *Nutritional Epidemiology. Monographs in Epidemiology and Biostatistics*. Oxford, England: Oxford University Press, 1998.

25. Allen NE, Appleby PN, Davey GK, Key TJ, Rinaldi S, et al.: The effect of diet on serum insulin-like growth-factor-I and its main binding proteins. *IARC Sci Pub* **156,** 295–296, 2002.
26. Allen NE, Appleby PN, Kaaks R, Rinaldi S, Davey GK, et al.: Lifestyle determinants of serum insulin-like growth-factor-I (IGF-I), C-peptide and hormone binding protein levels in British women. *Cancer Causes Control* **14,** 65–74, 2003.
27. Giovannucci E: Nutrition, insulin, insulin-like growth factors and cancer. *Horm Metab Res* **35,** 694–704, 2003.
28. DeLellis K, Rinaldi S, Kaaks RJ, Kolonel LN, Henderson B, et al.: Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the multiethnic cohort.[Erratum appears in *Cancer Epidemiol Biomarkers Prev* 2004 **13(11 Pt 1),** 1825]. *Cancer Epidemiol Biomarkers Prev* **13,** 1444–1451, 2004.
29. Kolonel LN, Henderson BE, Hankin JH, et al.: A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol* **151,** 346–357, 2000.
30. Garrow JS and Webster J: Quetelet's index (W/H2) as a measure of fatness. *Int J Obes* **9,** 147–153, 1985.
31. Calle EE and Kaaks R: Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* **4,** 579–591, 2004.
32. Lukanova A, Toniolo P, Akhmedkhanov A, et al.: A prospective study of insulin-like growth factor-I, IGF-binding proteins-1, -2, and -3 and lung cancer risk in women. *Int J Cancer* **92,** 888–892, 2001.
33. Juul A, Bang P, Hertel NT, et al.: Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab* **78,** 744–752, 1994.
34. Juul A, Main K, Blum WF, Lindholm J, Ranke MB, et al.: The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. *Clin Endocrinol* **41,** 85–93, 1994