VOLUME 27 · NUMBER 2 · JANUARY 10 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Insulin, the Insulin-Like Growth Factor Axis, and Mortality in Patients With Nonmetastatic Colorectal Cancer

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Submitted May 5, 2008; accepted September 5, 2008; published online ahead of print at www.jco.org on December 8, 2008.

Supported by Grants No. CA118553, CA87969, CA108341, CA127003-01, and CA09001 from the National Cancer Institute, National Institutes of Health, Bethesda, MD.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2702-176/$20.00

DOI: 10.1200/JCO.2008.17.9945

ABSTRACT

Purpose
Obesity, sedentary lifestyle, and Western dietary pattern have been linked to increased risk of cancer recurrence and mortality among patients with surgically resected colorectal cancer. Excess energy balance leads to increased circulating insulin and depressed levels of circulating insulin-like growth factor binding protein (IGFBP) -1, which promote cancer cell growth in preclinical models.

Patients and Methods
Among 373 patients diagnosed with nonmetastatic colorectal cancer between 1991 and 2004, we performed a prospective observational study nested within two large US cohorts to evaluate the association between mortality and prediagnosis circulating C-peptide (a marker of insulin secretion), IGFBP-1, insulin-like growth factor-I (IGF-I), and IGFBP-3.

Results
Compared with patients in the bottom quartile, patients in the top quartile of plasma C-peptide had an age-adjusted hazard ratio (HR) for death of 1.87 (95% CI, 1.04 to 3.36; $P = .03$ for trend), whereas those in the top quartile of circulating IGFBP-1 had a significant reduction in mortality (HR = 0.48; 95% CI, 0.28 to 0.84; $P = .02$ for trend). Little change in these estimates was noted after adjusting for other covariates known or suspected to influence survival. No associations were noted between mortality and IGF-I or IGFBP-3, which are two components of the IGF axis not closely correlated with lifestyle factors.

Conclusion
Among patients with surgically resected colorectal cancer, higher levels of prediagnosis plasma C-peptide and lower levels of prediagnosis plasma IGFBP-1 were associated with increased mortality. Circulating insulin and IGFBP-1 are potential mediators of the association between lifestyle factors and mortality after colorectal cancer resection.

J Clin Oncol 27:176-185. © 2008 by American Society of Clinical Oncology

INTRODUCTION

Numerous epidemiologic studies have demonstrated an association between lifestyle factors, such as adiposity,[1-7] physical activity,[2,3,8-12] and diet,[13-19] and the risk of incident colorectal cancer. More recently, studies have demonstrated that these factors are associated with the risk of cancer recurrence and mortality after primary surgical resection of colorectal cancer.[20-25] High body mass index (BMI) and total body adiposity, sedentary lifestyle, and consumption of a Western pattern diet lead to elevated levels of circulating insulin and low levels of circulating insulin-like growth factor binding protein (IGFBP) -1.[26-29] In contrast, these factors have little effect on plasma levels of other components of the insulin-like growth factor (IGF) axis, such as IGF-I and IGFBP-3.[30]

In experimental models, insulin promotes the growth and survival of colorectal cancer cells,[31,32] whereas IGFBP-1 inhibits cancer cell growth and migration, both directly and through local modulation of other components of the IGF axis.[33-35] Prospective observational studies have demonstrated that higher baseline C-peptide (a more stable marker of insulin exposure) and lower IGFBP-1 are associated with a significant increase in colorectal cancer risk, supporting their possible role as mediators of the association between lifestyle factors and colorectal cancer.[36-40] In patients with early-stage breast cancer, elevated circulating levels of insulin and C-peptide and the presence of metabolic syndrome have been linked to an increased risk for tumor recurrence and mortality.[41-43] However, the effect of circulating C-peptide and IGFBP-1 on survival after surgical resection of colorectal cancer is unknown. Therefore, we prospectively assessed the

influence of prediagnosis plasma levels of C-peptide, IGFBP-1, IGF-I, and IGFBP-3 on mortality in patients with nonmetastatic colorectal cancer enrolled onto two large prospective cohort studies.

PATIENTS AND METHODS

Study Population

The Nurses' Health Study (NHS) began in 1976, when 121,700 female nurses between 30 and 55 years of age completed a baseline questionnaire about their lifestyles and medical histories. Subsequently, these women have completed a self-administered, mailed questionnaire biennially to update information on their lifestyle, medical history, and diet. A total of 32,826 women between 43 and 69 years of age returned a mailed blood collection kit by overnight courier in 1989 and 1990. The Health Professionals Follow-Up Study (HPFS) was initiated in 1986 when 51,529 US men age 40 to 75 years responded to a mailed questionnaire. Subsequently, these men have completed a self-administered, mailed questionnaire biennially to update information on their lifestyle, medical history, and diet. Blood was collected from 18,225 men and returned in a mailed blood collection kit by overnight courier in 1993 through 1995. In both cohorts, blood samples were centrifuged on arrival and separated into plasma, WBCs, and RBCs. Approximately 95% of samples were received within 24 hours of blood collection. The current study was approved by the Human Research Committee at the Brigham and Women's Hospital (Boston, MA), and all participants provided consent.

Identification of Study Patients

When a participant (or next of kin for decedents) reported a diagnosis of colorectal cancer on a follow-up questionnaire, we obtained hospital records and pathology reports. Study physicians blinded to exposure data reviewed medical records. For nonrespondents, we searched the National Death Index to discover deaths and ascertain diagnoses of colorectal cancer. In the NHS and HPFS, we included all participants diagnosed with colorectal cancer between the date of blood collection and May 31, 2004 or January 31, 2002, respectively. Because of the possibility of subclinical cancer leading to alterations in plasma biomarker levels, we excluded from our analyses those participants diagnosed within 12 months of blood collection.

Measurement of Mortality

Women were observed until death or June 2005. Men were observed until death or January 2005. Ascertainment of deaths included reporting by family or postal authorities. Names of persistent nonresponders were searched in the National Death Index. More than 98% of deaths have been identified by these methods.[44] Physician reviewers assigned the cause of death.

Laboratory Analyses

Plasma levels of C-peptide, IGFBP-1, IGF-I, and IGFBP-3 were assayed by enzyme-linked immunosorbent assay with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory of one of the authors (M.N.P.). To assess laboratory precision, we included masked, randomly inserted aliquots from a pool of quality control plasma. The mean intra-assay coefficients of variation for C-peptide, IGFBP-1, IGF-I, and IGFBP-3 were less than 13%, 13%, 15%, and 12%, respectively.

Covariates

Tumor stage, grade of differentiation, and location (colon or rectum) were extracted from the medical record. Starting in 1993, women were asked about colorectal cancer treatment in a supplemental questionnaire in the NHS. Further covariates were obtained from the questionnaire returned before measurement of the plasma markers, except for postdiagnosis BMI and physical activity, which were obtained from the questionnaire returned after cancer diagnosis. As part of a validated assessment of physical activity,[45,46] participants were asked to average the time spent per week in a total of eight different activities over the previous year, and a weekly physical activity score was derived by multiplying the time spent in each activity per week by its typical energy expenditure requirements expressed in metabolic equivalents.

Statistical Analyses

The primary exposures were prediagnosis levels of plasma markers. Patients with diabetes mellitus were excluded from all analyses of C-peptide. Baseline characteristics were determined for participants in each quartile, and differences across quartiles were evaluated with analysis of variance for continuous variables and χ^2 tests for categoric variables. We calculated Spearman correlation coefficients to investigate relationships between plasma markers and covariates relevant to energy balance.

Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% CIs for overall and colorectal cancer–specific mortality, according to quartile of plasma marker. Follow-up time was calculated from the date of colorectal cancer diagnosis to the date of death or June 2005 in the NHS and to the date of death or January 2005 in the HPFS. In the analyses of colorectal cancer–specific mortality, patients who died as a result of causes other than colorectal cancer were censored at the time of death. Two-tailed P values for linear trend tests across categories were calculated by modeling the log of each plasma marker as a continuous variable. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of plasma marker categories with time.

To provide increased power for subgroup analyses, plasma markers were categorized into tertiles. Tests of interaction were assessed by entering into the model the cross-product of the plasma marker tertile and the dichotomized covariate. We also investigated relationships between plasma markers by dividing each marker at the median and making four-category variables for each pair of markers. All statistical analyses were performed using the SAS 9.1 statistical package (SAS Institute, Cary, NC), and all P values are two sided.

RESULTS

Baseline Characteristics

Among the 373 eligible participants with colorectal cancer, there were 108 deaths, of which 69 were colorectal cancer–specific deaths. Plasma collection was performed at a median of 6.6 years (standard deviation, 3.6 years) before colorectal cancer diagnosis. The median follow-up time from the date of diagnosis was 5.9 years (standard deviation, 3.2 years) for participants who were alive. The majority of deaths from other causes were a result of cardiovascular disease (23%), neurologic disease (21%), or other cancers (15%). Baseline patient characteristics by quartiles of plasma C-peptide and IGFBP-1 are listed in Tables 1 and 2, respectively. Higher plasma levels of C-peptide and lower levels of plasma IGFBP-1 were associated with higher BMI. Patients with higher levels of IGFBP-1 were also more likely to use postmenopausal hormones and multivitamins and more likely to have stage I and III disease.

We further assessed the relationships of plasma markers and selected covariates by calculating Spearman correlation coefficients (Appendix Table A1, online only). C-peptide was positively correlated with prediagnosis BMI ($r = 0.35$) and postdiagnosis BMI ($r = 0.26$) and inversely correlated with plasma IGFBP-1 ($r = -0.53$). Plasma IGFBP-1 was inversely correlated with prediagnosis BMI ($r = -0.40$) and postdiagnosis BMI ($r = -0.32$; all $P < .001$). IGF-I and IGFBP-3 were positively correlated ($r = 0.63$, $P < .001$) but were not significantly correlated with either BMI or physical activity.

Plasma Markers and Mortality

We assessed the influence of prediagnosis plasma C-peptide and IGFBP-1 on patient survival (Table 3). Compared with patients in the

Table 1. Baseline Characteristics of Women and Men With Nonmetastatic Colorectal Cancer According to Quartile of Prediagnosis Plasma C-Peptide

Characteristic	Quartiles of Plasma C-Peptide				*P**
	1 (n = 88)	2 (n = 86)	3 (n = 89)	4 (n = 87)	
Median plasma C-peptide, ng/mL	1.04	1.67	2.42	4.45	—
Age, years					.68
Median	69.5	68.5	69.0	69.0	
SD	7.4	8.6	7.3	7.3	
Sex, % of patients					—
Female	64	63	62	62	
Male	36	37	38	38	
Body mass index, kg/m^2					
Prediagnosis					< .001
Median	23.8	24.8	25.3	27.0	
SD	3.2	3.3	4.4	5.1	
Postdiagnosis					< .001
Median	23.7	25.1	25.9	27.0	
SD	4.1	4.2	4.8	4.7	
Activity level, MET-h/wk					
Prediagnosis					.19
Median	17.1	16.4	15.8	9.1	
SD	23.5	39.6	30.5	22.3	
Postdiagnosis					.58
Median	10.7	12.1	9.2	9.0	
SD	29.4	19.5	25.8	19.4	
Smoking status, % of patients					.27
Never	47	35	43	40	
Past	45	52	38	45	
Current	8	13	19	15	
Alcohol consumption, g/d					.13
Median	3.5	2.0	3.5	1.8	
SD	15.1	8.7	13.1	10.7	
Aspirin use, ≥ twice per week, % of patients	32	31	27	26	.79
Postmenopausal hormone use, % of patients†					.15
Premenopausal	11	22	11	4	
Never used	30	30	42	43	
Current user	27	35	25	24	
Past user	32	13	22	29	
Regular multivitamin use, % of patients	38	48	39	35	.33
Total vitamin D intake, U/d					.57
Median	287.1	287.9	307.1	239.0	
SD	269.0	238.3	249.0	256.9	
Tumor location, % of patients					.06
Colon	70	69	82	82	
Rectum	30	31	18	18	
Tumor differentiation, % of patients					.51
Well	11	14	15	8	
Moderate	54	64	55	61	
Poor	16	6	12	9	
Unknown	19	16	18	22	
Stage of disease, % of patients					.45
I	39	28	35	28	
II	24	33	27	30	
III	24	23	28	21	
Unknown	13	16	10	21	
Chemotherapy received, % of patients†					.65
Yes	13	14	19	10	
No	11	9	11	15	
Unknown	76	77	70	75	
No. of years between plasma collection and cancer diagnosis					.43
Median	7.4	6.2	6.8	6.7	
SD	3.7	3.9	3.5	3.4	

Abbreviations: SD, standard deviation; MET, metabolic equivalent.
*Covariates were analyzed using analysis of variance for continuous variables and χ^2 tests for categorical variables.
†Data are available for Nurses' Health Study participants only.

Table 2. Baseline Characteristics of Women and Men With Nonmetastatic Colorectal Cancer According to Quartile of Prediagnosis Plasma IGFBP-1

Characteristic	Quartiles of Plasma IGFBP-1				*P**
	1 (n = 94)	2 (n = 92)	3 (n = 92)	4 (n = 90)	
Median plasma IGFBP-1, ng/mL	5.0	13.8	27.5	53.7	—
Age, years					.02
Median	69.0	67.0	70.0	71.0	
SD	7.4	7.8	6.9	7.8	
Sex, % of patients					—
Female	63	63	64	64	
Male	37	37	36	36	
Body mass index, kg/m^2					
Prediagnosis					< .001
Median	28.1	26.0	24.6	23.7	
SD	5.5	3.4	3.7	3.4	
Postdiagnosis					< .001
Median	28.2	25.9	24.6	23.4	
SD	5.1	3.9	4.5	4.3	
Activity level, MET-h/wk					
Prediagnosis					.75
Median	14.0	14.1	11.5	20.7	
SD	24.2	25.6	39.5	25.3	
Postdiagnosis					.61
Median	10.6	8.9	10.1	10.2	
SD	26.1	16.8	22.2	26.9	
Smoking status, % of patients					.18
Never	38	43	39	46	
Past	44	50	42	44	
Current	18	7	19	10	
Alcohol consumption, g/d					.43
Median	2.0	2.3	2.0	3.4	
SD	11.5	13.4	10.6	12.8	
Aspirin use, ≥ twice per week, % of patients	23	34	32	32	.42
Postmenopausal hormone use, % of patients†					.01
Premenopausal	8	21	9	7	
Never used	48	36	42	21	
Current user	17	21	29	48	
Past user	27	22	20	24	
Regular multivitamin use, % of patients	35	30	45	48	.06
Total vitamin D intake, U/d					.04
Median	261.1	240.0	321.3	347.5	
SD	268.2	226.6	268.8	245.6	
Tumor location, % of patients					.51
Colon	81	74	72	74	
Rectum	19	26	28	26	
Tumor differentiation, % of patients					.64
Well	11	13	12	12	
Moderate	54	59	61	60	
Poor	13	5	13	12	
Unknown	22	23	14	16	
Stage of disease, % of patients					.04
I	23	34	34	37	
II	27	36	28	19	
III	26	16	24	32	
Unknown	24	14	14	12	
Chemotherapy received, % of patients†					.93
Yes	16	12	13	13	
No	14	12	10	10	
Unknown	70	76	77	77	
No. of years between plasma collection and cancer diagnosis					.92
Median	6.4	7.1	6.7	5.9	
SD	3.3	3.3	3.7	4.1	

Abbreviations: IGFBP-1, insulin-like growth factor binding protein-1; SD, standard deviation; MET, metabolic equivalent.
*Covariates were analyzed using analysis of variance for continuous variables and χ^2 tests for categorical variables.
†Data are available for Nurses' Health Study participants only.

Table 3. Overall and Colorectal Cancer–Specific Mortality by Quartile of Prediagnosis Plasma C-Peptide and IGFBP-1

Plasma Marker	Quartiles of Plasma Marker				*P* for Trend
	1	2	3	4	
C-peptide					
Median, ng/mL	1.04	1.67	2.42	4.45	
Overall mortality					
No. of patients	88	86	89	87	
No. of deaths	18	24	26	30	
Age-adjusted analysis*					.03
HR	1.0	1.44	1.58	1.87	
95% CI		0.78 to 2.67	0.87 to 2.90	1.04 to 3.36	
Multivariable-adjusted analysis†					.08
HR	1.0	1.69	1.79	2.11	
95% CI		0.89 to 3.22	0.93 to 3.44	1.06 to 4.21	
Colorectal cancer–specific mortality					
No. of patients	88	86	89	87	
No. of deaths	13	18	17	16	
Age-adjusted analysis*					.71
HR	1.0	1.45	1.37	1.31	
95% CI		0.71 to 2.97	0.66 to 2.82	0.63 to 2.72	
Multivariable-adjusted analysis†					.33
HR	1.0	1.65	1.72	1.51	
95% CI		0.79 to 3.45	0.72 to 3.74	0.65 to 3.47	
IGFBP-1					
Median, ng/mL	5.0	13.8	27.5	53.7	
Overall mortality					
No. of patients	94	92	92	90	
No. of deaths	33	24	29	21	
Age-adjusted analysis*					.02
HR	1.0	0.72	0.76	0.48	
95% CI		0.42 to 1.22	0.46 to 1.25	0.28 to 0.84	
Multivariable-adjusted analysis†					.004
HR	1.0	0.86	0.75	0.44	
95% CI		0.48 to 1.55	0.43 to 1.31	0.24 to 0.81	
Colorectal cancer–specific mortality					
No. of patients	94	92	92	90	
No. of deaths	23	16	15	14	
Age-adjusted analysis*					.13
HR	1.0	0.69	0.63	0.57	
95% CI		0.36 to 1.31	0.33 to 1.22	0.29 to 1.11	
Multivariable-adjusted analysis†					.006
HR	1.0	0.80	0.60	0.43	
95% CI		0.40 to 1.60	0.30 to 1.21	0.21 to 0.89	

Abbreviations: IGFBP-1, insulin-like growth factor binding protein-1; HR, hazard ratio.
*Adjusted for age at diagnosis.
†Adjusted for age at diagnosis, cohort (sex), stage of disease, histologic differentiation, tumor location (colon or rectum), time period of diagnosis (1990-1995, 1996-1999, or 2000-2004), time between last meal and plasma collection, receipt of chemotherapy, and patient characteristics from the most recent questionnaire before blood draw, including smoking status (current, past, or never), aspirin use (< or ≥ 2 times a week), alcohol consumption (g/d), total vitamin D intake (U/d), and postmenopausal hormone use.

bottom quartile, those in the top quartile of C-peptide level had an age-adjusted HR for death of 1.87 (95% CI, 1.04 to 3.36; $P = .03$ for trend). Little change in the HRs were noted after adjusting for other covariates known or suspected to influence mortality; participants in the top versus the bottom quartile of plasma C-peptide had an HR of 2.11 (95% CI, 1.06 to 4.21). The HRs for colorectal cancer–specific mortality did not seem to increase monotonically by quartile of C-peptide, and the tests for trend were not statistically significant in the age-adjusted or multivariable-adjusted analyses.

Higher IGFBP-1 levels were associated with a reduction in the risk of overall and colorectal cancer–specific mortality, before and after adjusting for other known or suspected predictors of patient outcome. Compared with patients in the bottom quartile, those in the top quartile of IGFBP-1 experienced a multivariable-adjusted HR of 0.44 (95% CI, 0.24 to 0.81; $P = .004$ for trend) for death and of 0.43 (95% CI, 0.21 to 0.89; $P = .006$ for trend) for colorectal cancer–specific death. Cumulative incidence curves for all-cause mortality by quartile of C-peptide and IGFBP-1 are shown in Figure 1.

We further examined the associations of C-peptide and IGFBP-1 with overall mortality after including prediagnosis BMI and physical activity in the multivariable models. The HRs for C-peptide and IGFBP-1 were 2.14 (95% CI, 1.01 to 4.50) and 0.49 (95% CI, 0.25 to

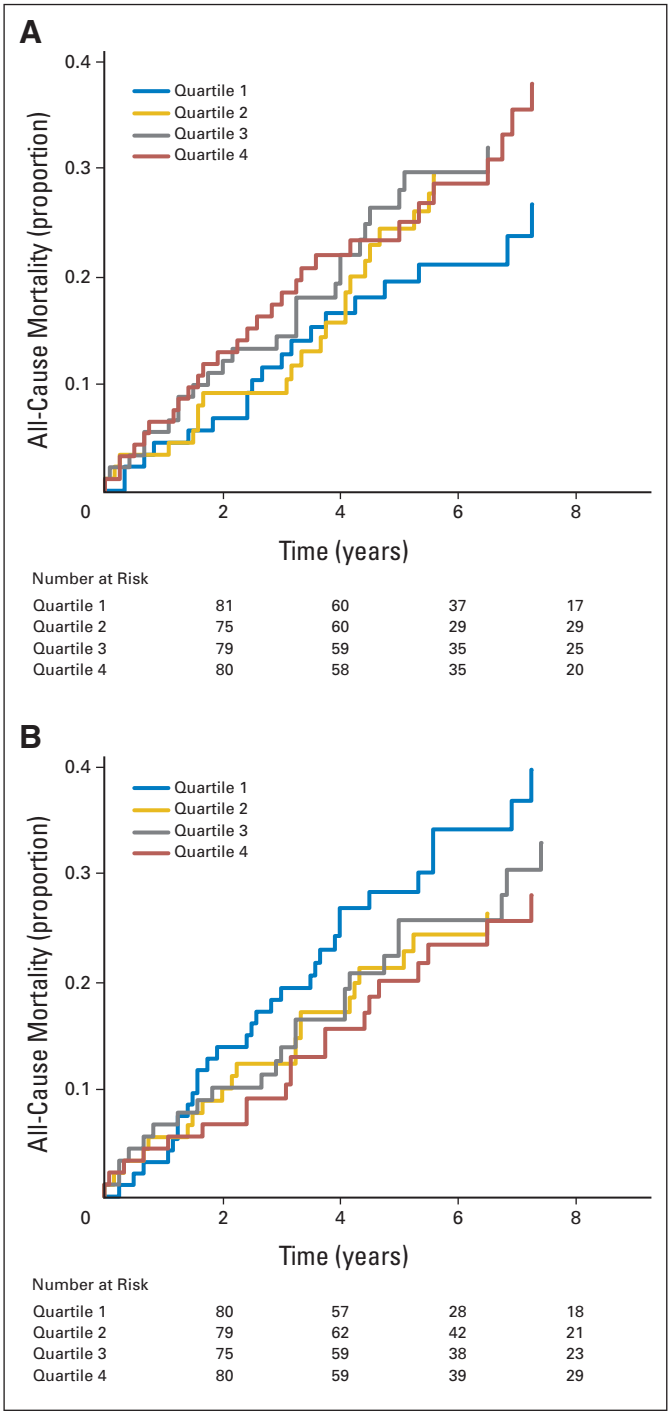

Fig 1. Cumulative incidence curves of overall mortality by quartiles of circulating (A) C-peptide and (B) insulin-like growth factor binding protein-1.

0.95), respectively, comparing the top versus the bottom quartiles. Similarly, inclusion of postdiagnosis BMI and physical activity in our multivariable models resulted in HRs for C-peptide and IGFBP-1 of 2.43 (95% CI, 1.18 to 5.01) and 0.41 (95% CI, 0.22 to 0.78), respectively, comparing the top versus the bottom quartiles. To determine whether one plasma marker was of primary importance, we evaluated models that included C-peptide and IGFBP-1 simultaneously. The HRs were attenuated slightly for both plasma markers; HRs comparing the top versus bottom quartiles for C-peptide and IGFBP-1 were 1.89 (95% CI, 0.92 to 3.87) and 0.53 (95% CI, 0.27 to 1.04), respectively.

We also assessed the influence of prediagnosis plasma IGF-I and IGFBP-3 on patient survival (Table 4). In contrast to C-peptide and IGFBP-1, neither prediagnosis circulating IGF-I nor IGFBP-3 had an apparent influence on overall or colorectal cancer–specific mortality. Simultaneous inclusion of plasma IGF-I and IGFBP-3 in our model did not alter these results.

Stratified Analyses by Potential Effect Modifiers

To examine whether the influence of plasma C-peptide and IGFBP-1 levels on mortality was more pronounced among patients who are overweight or more sedentary, we stratified our analyses by these factors (Table 5). The influence of plasma levels of IGFBP-1 on mortality seemed more pronounced among patients with a prediagnosis BMI greater than the cohort median. The effects of plasma C-peptide and IGFBP-1 on mortality were not significantly modified by age, sex (which also stratifies by cohort), stage of disease, fasting status, tumor location, or plasma level of IGF-I.

Cooperative Effects of Plasma Markers

Finally, we examined the joint effects of prediagnosis C-peptide and IGFBP-1 on mortality. Patients with both high IGFBP-1 levels (> the median) and low C-peptide levels (< the median) experienced a multivariable-adjusted HR for mortality of 0.47 (95% CI, 0.26 to 0.84) when compared with patients with low IGFBP-1 and high C-peptide. The two intermediate categories (ie, low IGFBP-1/low C-peptide and high IGFBP-1/high C-peptide) had multivariable-adjusted HRs of 0.89 (95% CI, 0.48 to 1.68) and 0.81 (95% CI, 0.44 to 1.48), respectively, when compared with low IGFBP-1 and high C-peptide.

DISCUSSION

Among patients with surgically resected colorectal cancer, high prediagnosis plasma levels of C-peptide were associated with an approximate doubling of the risk for death, whereas elevated levels of IGFBP-1 were associated with an approximate 50% reduction in mortality. Although no longer statistically significant after adjustment for one another in a single statistical model, the magnitude of C-peptide and IGFBP-1 effects remained largely unchanged, suggesting that it was mainly the greater *df* used in such a model that explained the change in *P* values. We also noted a stronger association of plasma C-peptide with overall mortality, involving a clear monotonic relationship, than with colorectal cancer–specific mortality, which showed no biologic gradient. Although the reason for this cannot be determined from the current data, it is possible that the relationship of C-peptide with other causes of death (eg, cardiovascular disease) contributed to the strength of association or that the variability in results was simply an aberration related to the smaller number of events in the cause-specific analysis. Plasma IGFBP-1 was strongly associated with both overall and colorectal cancer–specific death. In contrast, no associations with overall or colorectal cancer–specific mortality were noted for circulating IGF-I or IGFBP-3, which are two components of the IGF axis that are poorly correlated with energy balance and lifestyle factors, such as weight, physical activity, and dietary pattern.

Table 4. Overall and Colorectal Cancer–Specific Mortality by Quartile of Prediagnosis Plasma IGF-I and IGFBP-3

Plasma Marker	Quartiles of Plasma Marker 1	2	3	4	*P* for Trend
IGF-I					
Median, ng/mL	113.4	164.5	206.6	279.5	
Overall mortality					
No. of patients	94	91	94	91	
No. of deaths	33	28	27	20	
Age-adjusted analysis*					.54
HR	1.0	1.02	1.15	0.91	
95% CI		0.61 to 1.69	0.69 to 1.93	0.52 to 1.61	
Multivariable-adjusted analysis†					.93
HR	1.0	1.17	1.43	1.08	
95% CI		0.68 to 2.01	0.82 to 2.50	0.57 to 2.01	
Colorectal cancer–specific mortality					
No. of patients	94	91	94	91	
No. of deaths	21	17	17	14	
Age-adjusted analysis*					.18
HR	1.0	0.89	0.94	0.80	
95% CI		0.47 to 1.69	0.49 to 1.79	0.40 to 1.58	
Multivariable-adjusted analysis†					.98
HR	1.0	1.39	1.58	1.31	
95% CI		0.57 to 2.26	0.77 to 3.22	0.61 to 2.82	
IGFBP-3					
Median, ng/mL	3,225.7	3,961.5	4,621.6	5,411.8	
Overall mortality					
No. of patients	94	91	91	94	
No. of deaths	32	27	25	24	
Age-adjusted analysis*					.88
HR	1.0	1.11	1.15	1.02	
95% CI		0.66 to 1.85	0.67 to 1.96	0.59 to 1.75	
Multivariable-adjusted analysis†					.51
HR	1.0	1.07	1.35	1.28	
95% CI		0.62 to 1.85	0.75 to 2.43	0.69 to 2.36	
Colorectal cancer–specific mortality					
No. of patients	94	91	91	94	
No. of deaths	21	18	15	15	
Age-adjusted analysis*					.17
HR	1.0	0.99	0.83	0.76	
95% CI		0.53 to 1.86	0.43 to 1.62	0.39 to 1.49	
Multivariable-adjusted analysis†					.98
HR	1.0	0.97	1.11	1.06	
95% CI		0.49 to 1.91	0.53 to 2.31	0.50 to 2.26	

Abbreviations: IGF-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein-3; HR, hazard ratio.
*Adjusted for age at diagnosis.
†Adjusted for age at diagnosis, cohort (sex), stage of disease, histologic differentiation, tumor location (colon or rectum), time period of diagnosis (1990-1995, 1996-1999, or 2000-2004), time between last meal and plasma collection, receipt of chemotherapy, and patient characteristics from the most recent questionnaire before blood draw, including smoking status (current, past, or never), aspirin use (< or ≥ 2 times a week), alcohol consumption (g/d), total vitamin D intake (U/d), and postmenopausal hormone use.

Weight, physical activity, and diet are well-established risk factors for several types of incident cancer, including colorectal cancer.[4,47-49] More recently, obesity, sedentary lifestyle, and Western dietary pattern have been associated with an increased risk for cancer recurrence and death among patients who have undergone curative surgical resection for colorectal cancer.[20-25] Although the mediators for this increased risk of recurrence and death are poorly defined, hyperinsulinemia and perturbations in the IGF axis have been proposed as underlying biologic mechanisms for these observations.[32,50-52] This hypothesis is supported by data from patients with early-stage breast cancer, in whom an increased risk for tumor recurrence and mortality has been associated with elevated levels of plasma insulin and C-peptide or the presence of metabolic syndrome.[41-43]

In preclinical studies of intestinal epithelial cells and colon cancer cell lines, insulin binds to the insulin receptor on the cell surface and stimulates cell growth, while inhibiting apoptosis,[31,53-57] suggesting that it may act directly as a mitogen for colon cancer cells. In the current study, we measured circulating C-peptide and IGFBP-1 as surrogates for plasma insulin because of the known rapid fluctuations in circulating insulin levels over time. Proinsulin is synthesized in pancreatic β-cells and is enzymatically cleaved to create insulin and C-peptide, which are secreted into the portal circulation in equimolar

Table 5. Overall Mortality by Tertile of Prediagnosis Plasma C-Peptide and IGFBP-1 Stratified by Body Mass Index and Physical Activity

Characteristic*	No. of Patients at Risk	No. of Deaths	Tertile 1	Tertile 2		Tertile 3		*P* (interaction)
			HR†	HR†	95% CI	HR†	95% CI	
C-peptide								
Body mass index, kg/m^2								.91
< 25.1	189	53	1.00	1.29	0.62 to 2.67	1.47	0.65 to 3.33	
≥ 25.1	161	45	1.00	0.93	0.38 to 2.28	2.19	0.91 to 5.26	
Physical activity, MET-h/wk								.55
< 15.2	175	53	1.00	1.37	0.63 to 3.00	1.73	0.79 to 3.80	
≥ 15.2	175	45	1.00	0.91	0.41 to 2.02	1.48	0.63 to 3.45	
IGFBP-1								
Body mass index, kg/m^2								.047
< 25.1	189	53	1.00	1.61	0.69 to 3.73	1.00	0.43 to 2.36	
≥ 25.1	179	54	1.00	0.32	0.16 to 0.66	0.13	0.05 to 0.33	
Physical activity, MET-h/wk								.58
< 15.2	184	61	1.00	0.57	0.30 to 1.07	0.37	0.18 to 0.77	
≥ 15.2	184	46	1.00	1.45	0.59 to 3.51	0.81	0.34 to 1.94	

Abbreviations: IGFBP-1, insulin-like growth factor binding protein-1; HR, hazard ratio; MET, metabolic equivalent.
*Cut points were chosen based on median values for body mass index and physical activity.
†Adjusted for age at diagnosis, cohort (sex), stage of disease, histologic differentiation, tumor location (colon or rectum), time period of diagnosis (1990-1995, 1996-1999, or 2000-2004), time between last meal and plasma collection, receipt of chemotherapy, and patient characteristics from the most recent questionnaire before blood draw, including smoking status (current, past, or never), aspirin use (< or ≥ 2 times a week), alcohol consumption (g/d), total vitamin D intake (U/d), and postmenopausal hormone use.

amounts. The half-life of C-peptide in the circulation is between two and five times longer than that of insulin and better reflects mean levels of circulating insulin, particularly when blood samples have not been uniformly collected under fasting conditions.[58,59] In addition, circulating C-peptide has successfully predicted the risk of incident colorectal cancer in several prospective studies.[36-40]

Plasma levels of IGFBP-1 are closely associated with lifestyle factors, such as weight, physical activity, and diet, and are regulated by hormones outside of the growth hormone axis, including insulin, glucagon, and cortisol.[60-62] In particular, increases in plasma insulin reduce transcription of IGFBP-1 in the liver, so that plasma levels of IGFBP-1 are thought to robustly reflect end organ stimulation by insulin.[60] In addition to reflecting target tissue insulin exposure, IGFBP-1 has independent inhibitory effects on cancer cell growth and migration in preclinical studies, both directly and through local modulation of other components of the IGF axis.[30,33-35,63] Consequently, reductions in circulating IGFBP-1 as a result of lifestyle factors and/or hyperinsulinemia may remove the inhibitory action of IGFBP-1 and allow for greater cellular proliferation and spread.

The current study has several strengths, including the prospective and longitudinal updating of covariate information, high follow-up rates in both cohorts, strict quality control for measurements of plasma markers, and significant preclinical data linking insulin and the IGF axis to colorectal cancer cell growth. Additionally, plasma markers were measured prospectively, before cancer diagnosis. All patients with plasma collected less than 12 months before diagnosis were excluded to reduce the effects of subclinical cancer on our results and limit the likelihood of bias caused by reverse causation.

Our work has several limitations. In the current study, we have only a single prediagnostic measurement of plasma markers. Cancer recurrences are thought to occur as a result of the subsequent growth of micrometastatic disease present at the time of surgical resection. Therefore, an environment promoting tumor growth either before or directly after tumor resection could theoretically lead to poorer outcomes among patients with early-stage colorectal cancer. Because we had only a single prediagnosis measurement of these markers, we were unable to investigate whether the effects of high C-peptide level or low IGFBP-1 level were more influential before or after surgical resection. Additionally, although previous work has demonstrated that these plasma markers are relatively stable over time,[36,37,64,65] these studies did not include measurements before and after cancer diagnosis. Further studies are necessary to better define temporal associations between these plasma factors and mortality after surgical resection of colorectal cancer.

In the current cohort, data on receipt of postoperative chemotherapy are limited. Yet, among those participants with available information, no significant differences were noted in the percentage of patients receiving chemotherapy by quartile of plasma C-peptide or IGFBP-1, and receipt of chemotherapy was included in our multivariable analyses to control for this variable to the extent that was possible. In addition, approximately 60% of patients had stage I or II disease, for which surgery alone is generally considered the standard of care.[66]

Finally, we cannot completely exclude the possibility that plasma levels of C-peptide and IGFBP-1 are reflective of other occult predictors of poor prognosis. However, adjustment for other covariates thought to influence mortality did not materially alter our results. In addition, sicker patients at greater risk for death commonly experience weight loss, which would result in lower plasma C-peptide and higher IGFBP-1 levels, biasing our results toward the null.

Our findings suggest that prediagnosis plasma levels of C-peptide and IGFBP-1 are associated with mortality in patients with nonmetastatic colorectal cancer. Although this study does not provide definitive evidence for causality, alterations in circulating insulin and related hormones are a plausible mechanism by which excess energy balance may adversely affect survival after curative resection of colorectal cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Glossary Terms

Apoptosis: Also called programmed cell death, it is a signaling pathway that leads to cellular suicide in an organized manner. Several factors and receptors are specific to the apoptotic pathway. The net result is that cells shrink, develop blebs on their surface, and their DNA undergoes fragmentation.

IGF (insulin-like growth factor): Proteins with sequences similar to insulin, insulin-like growth factors trigger similar cellular responses as insulin, including mitogenesis. IGF-I (secreted by the liver) and IGF-II (secreted by brain, kidney, pancreas, and muscle) function through cell surface receptors.

IGFBPs (IGF-binding proteins): The IGFBPs represent a family of six conserved proteins that share the ability to bind the insulin-like growth factors IGF-I and IGF-II. They are secreted proteins and are found in serum, all biologic fluids, and tissue extracts. The amino and carboxy termini of the members belonging to this family show sequence similarity, with variability present in the central region of the molecules. IGFBPs function by binding to IGFs and inhibit interactions of IGFs with their receptors, IGF-IR and IGF-IIR. The roles of different IGFBPs may differ depending on their tissue expression, regulation by hormones and growth factors, proteolytic degradation, and association with cell membranes or cell membrane receptors. Some IGFBPs have nuclear localizations signals and may be found within the nucleus.

C-peptide: C-peptide is a protein fragment produced during the enzymatic cleavage of proinsulin to create insulin. It is secreted by pancreatic β-cells at equimolar concentrations to insulin but has a two to five times longer half-life in the circulation. As a result of its greater stability in the peripheral circulation, C-peptide has been measured in research studies as a marker of pancreatic β-cell secretory activity.

Energy balance: Energy balance is the state at which the number of calories eaten equals the number of calories used. Energy balance is affected by physical activity, body size, amount of body fat and muscle, and genetics. Over time, excess energy balance (ie, greater energy intake compared with energy expenditure) can lead to obesity and numerous metabolic consequences, including hyperinsulinemia.

Insulin-like growth factor (IGF) axis: The IGF axis denotes a hierarchy of proteins that work together to regulate the amount of free, biologically active IGF available to interact with target cells. This hierarchy includes growth hormone, insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), six IGF binding proteins (IGFBP), and IGFBP proteases.

Enzyme-linked immunosorbent assay (ELISA): An ELISA is a sensitive, quantitative immunochemical test that involves an enzyme linked to an antibody or antigen to allow detection of a specific protein. Generally, the specimen is added to a surface, on which are immobilized antibodies specific to the protein of interest. If the protein is present, it will bind to the attached antibody layer. The presence of the bound protein is then verified with antibodies that have been tagged with an enzyme, which causes the specimen to change color corresponding to the concentration of the target protein.

Metabolic syndrome: The metabolic syndrome is characterized by a group of metabolic risk factors occurring together in one person. These risk factors include abdominal obesity manifested as excessive fat tissue in and around the abdomen, elevated blood levels of triglycerides, decreased blood levels of HDL cholesterol, elevated blood pressure, and elevated fasting blood glucose, which is a measure of glucose intolerance and is also known as insulin resistance.

Mitogen: A mitogen is a substance able to induce cellular mitosis or cell division.

Western pattern diet: Western pattern diet is characterized by high intakes of red and processed meats, fat, refined grains, and dessert.