A Prospective Study of Plasma C-Peptide and Colorectal Cancer Risk in Men

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Background: **Colorectal cancer and type 2 diabetes share many risk factors, and hyperinsulinemia appears to be associated with an increased risk of colorectal cancer. We used the concentration of plasma C-peptide (an indicator of insulin production) to determine whether insulin and insulin resistance are associated with the risk of developing colorectal cancer.** ***Methods:*** **We conducted a nested case–control study in the Physicians' Health Study. Plasma samples were collected from 14 916 cancer-free men from August 1982 through December 1984. Plasma C-peptide concentration, measured with an enzyme-linked immunosorbent assay, was available for 176 case patients who developed incident colorectal cancer through December 31, 1995, and 294 age- and smoking status–matched control subjects. Information on four other insulin resistance–related factors at baseline was obtained. We used conditional logistic regression models to investigate associations. All statistical tests were two-sided.** ***Results:*** **Plasma C-peptide concentration was positively associated with age, body mass index (BMI), and number of insulin resistance–related factors, and it was inversely associated with fasting time before the blood draw, alcohol consumption, and vigorous exercise. An increased concentration of plasma C-peptide was statistically significantly associated with an increased risk of colorectal cancer (relative risk [RR] for the highest versus lowest quintile of plasma C-peptide = 2.7, 95% confidence interval [CI] = 1.2 to 6.2; P_{trend} = .047), after adjusting for age, smoking status, fasting, BMI, alcohol consumption, vigorous exercise, and aspirin assignment in the Physicians' Health Study. The association became even stronger when the analysis was further adjusted for factors related to insulin resistance other than insulin levels (RR for the highest versus lowest quintile = 3.4, 95% CI = 1.4 to 8.3; P_{trend} = .02) or when data from case patients who were diagnosed during the first 5 years of follow-up were excluded (RR for the highest versus the lowest quintile = 3.4, 95% CI = 1.3 to 8.8; P_{trend} = .03). Adjusting for plasma levels of insulin-like growth factor I (IGF-I) and its binding protein 3 (IGFBP-3) did not materially change the results. There was no apparent modification of risk by BMI, insulin resistance–related factors, or vigorous exercise.** ***Conclusion:*** **Elevated insulin production, as reflected by elevated concentrations of plasma C-peptide, may predict the risk of developing colorectal cancer, independently of BMI, factors related to insulin resistance, or levels of IGF-I and IGFBP-3.**

Over the past two decades, epidemiologic studies have identified a cluster of Western dietary and lifestyle risk factors for both type 2 diabetes and colorectal cancer *(1–3)*. These shared risk factors (such as a diet high in calories, animal fat, and refined carbohydrates and low in fiber; sedentary lifestyle; high body mass index [BMI]; and central deposition of adipose tissue) are all associated with hyperinsulinemia and insulin resistance *(4,5)*. In addition, individuals with type 2 diabetes have an increased risk of colorectal cancer *(6,7)*. These observations led to the hypothesis that hyperinsulinemia *(8)* or factors associated with insulin resistance, such as hypertriglyceridemia or hyperglycemia *(9)*, might be an underlying factor for increasing the risk of developing colorectal cancer.

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See "Notes" following "References."

Recently, several prospective studies have evaluated this hypothesis. Some *(6,10,11)* but not all *(12)* studies observed a positive association between fasting or non-fasting glucose levels and colorectal cancer incidence or mortality. A cluster of metabolic abnormalities related to insulin resistance has also been positively associated with colorectal cancer mortality *(11,13)*. Only two studies have directly measured either serum fasting insulin and insulin levels 2 hours after an oral glucose challenge among elderly individuals (≥65 years old) *(10)* or serum levels of C-peptide among women *(14)*. Both studies reported a twofold to threefold increased risk of colorectal cancer when the top and bottom categories were compared.

Circulating C-peptide is a valid indicator of insulin production *(15)*. Proinsulin is synthesized in the β cells of the pancreas and then cleaved to form insulin and C-peptide, both of which are secreted in equimolar amounts into the circulatory system *(15)*. Circulating C-peptide measurement has been used as an effective marker of β-cell function to distinguish type 1 and type 2 diabetes *(16)*. In addition, statistically significantly higher plasma C-peptide concentrations have been observed among individuals who follow a Western dietary pattern, characterized by high intakes of red meat, high-fat dairy products, and refined grains, compared with those who follow a non-Western dietary pattern *(4)*.

We previously reported a statistically significant positive association between pre-diagnostic levels of plasma insulin-like growth factor I (IGF-I) and colorectal cancer in a nested case–control analysis among middle-aged U.S. men in the Physicians' Health Study *(17)*. In the current analysis, we investigated whether the risk of colorectal cancer was associated with plasma C-peptide concentration, BMI, and a cluster of factors related to the insulin resistance syndrome.

Patients and Methods

Study Population

This study was a nested case–control study in the Physicians' Health Study. The methods of the Physicians' Health Study have been described in detail elsewhere *(18)*. Briefly, 22 071 U.S. male physicians, aged 40–84 years in 1982, with no history of cardiovascular disease or cancer, were assigned randomly to receive aspirin, β-carotene, both active drugs, or both placebos. Written informed consent was obtained from all participants, and the research protocol was approved by the Institutional Review Board at Brigham and Women's Hospital in Boston, MA.

Of the 22 071 randomly assigned study participants, 14 916 (68%) provided baseline blood samples in EDTA tubes and sent them on ice to the study investigators between August 1, 1982, and December 31, 1984. Among these blood samples, 44% were drawn 4 hours or more after eating, and 26% were drawn after an 8-hour fast. More than 70% of the samples were received between September 1, 1982, and November 30, 1982. The samples were processed for long-term storage at −80 °C, and no specimen thawed or warmed substantially.

Case Patients With Colorectal Cancer and Selection of Control Subjects

Major end points of the study, including colorectal cancer, were reported annually by the participants through a mailed questionnaire that included items on lifestyle and medical history. Nonresponders to the questionnaires were telephoned. Medical records were obtained and reviewed by the end-point committee for the Physicians' Health Study. Deaths were usually reported by family members or postal authorities. Morbidity and mortality follow-up was more than 99% complete. Cases of colorectal cancer were confirmed by reviewing medical records, including pathology reports. By December 31, 1995, among those who provided adequate baseline plasma samples, we had confirmed 196 diagnoses of colorectal cancer. For each case patient, we attempted to select two control subjects who had provided a blood sample and had not reported a diagnosis of colorectal cancer at the time the diagnosis was reported by the case patient. Control subjects were individually matched with case patients for age (±1 year or ±5 years for elderly case patients) and smoking status (never, past, or current) at baseline. We could identify a second control subject for only 127 case patients and, thus, a total of 323 men formed the control group.

Measurements of Exposures

Plasma levels of C-peptide, IGF-I, and IGF binding protein 3 (IGFBP-3) were assayed by corresponding enzyme-linked immunosorbent assays with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory by Dr. Michael Pollak. Samples from case patients and their matched control subjects were assayed in the same sets and same batch to minimize inter-assay variability, and aliquots from a pool of quality-control plasma were inserted randomly. Laboratory personnel were unable to distinguish among case, control, and quality-control samples. The median intra-assay coefficient of variation for C-peptide from the blinded quality-control samples was 16.5%. Total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were measured with reagents from Roche Diagnostics (Indianapolis, IN) and Genzyme (Cambridge, MA) in a laboratory certified for lipid testing by the Centers for Disease Control and Prevention.

At baseline, each study participant reported his history of diabetes, hypertension, alcohol consumption, vigorous exercise, weight, and height. BMI was calculated, and a cluster of four factors (other than insulin) related to the insulin resistance syndrome *(19)* was defined according to the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III Guidelines *(20)*]: BMI of more than 25 kg/m^2, high-density lipoprotein concentration of less than 40 mg/mL, and systolic blood pressure of more than 130 mmHg or diastolic blood pressure of more than 85 mmHg or use of antihypertensive medication, and elevated triglyceride levels. Because the majority of men in this study had non-fasting blood samples, we defined elevated triglyceride levels as the upper 20% of the distribution for control subjects within the following fasting strata: less than 2 hours, 2–3.9 hours, 4–7.9 hours, 8 or more hours, and missing.

Statistical Analysis

Among a total of 196 case patients with colorectal cancer and 323 control subjects, C-peptide data were available for 182 case patients and 309 control subjects. After excluding case patients and control subjects (and their matched pairs) who had diabetes at baseline, 176 case patients and 294 age- and smoking status–

matched control subjects were included in the analysis. Because plasma C-peptide levels were not normally distributed, we used natural log-transformed values to compute the geometric mean of plasma C-peptide levels according to the various baseline characteristics. We first computed the geometric means and 95% confidence intervals (CIs) with the analysis of covariance, according to age (10-year groups), smoking status (never, past, and current), and fasting time (hours between last meal and blood draw) by mutually controlling for each of the other two in the models. We then computed geometric mean levels of plasma C-peptide according to each of the baseline factors (BMI, number of factors related to insulin resistance, vigorous exercise, and alcohol consumption), controlling for all three basic factors (age, smoking status, and fasting status).

We first categorized men into quintiles according to the distribution of plasma C-peptide levels in control subjects. We calculated odds ratios from conditional logistic regression models to estimate the relative risks (RRs) and 95% confidence intervals of colorectal cancer according to plasma levels of C-peptide that were based on case–control matching and controlling for fasting time (<4 hours, ≥4 hours, and missing). Adjusted estimates of risk were obtained with multivariable models controlling for potential confounding factors including BMI, vigorous exercise, alcohol consumption, and aspirin assignment. In addition, we adjusted for a cluster of factors related to insulin resistance or levels of IGF-I and IGFBP-3 to evaluate whether the association between levels of C-peptide and risk of colorectal cancer was independent of these factors. Tests for trend were performed by entering a variable that represented the median values of plasma C-peptide for the quintiles of the distribution among the control subjects. To eliminate potential influence of undiagnosed cancer on C-peptide levels, these conditional logistic regression analyses were also performed after excluding case patients (with their matched control subjects) diagnosed during the first 5 years after blood was drawn. To eliminate the potential influence of non-fasting on the results, we conducted a subgroup analysis among 59 case patients and 115 control subjects who reported at least 4 hours between their last meal and the time blood was drawn. Because case patients and control subjects were not matched on fasting status and such a restriction requires breaking the matching, the analysis was done by unconditional logistic regression controlling for age, smoking status, and fasting time (4–7.9 hours or ≥8 hours). We also conducted a conditional logistic regression analysis stratified by tumor site (colon or rectum) to estimate the relative risk and the 95% confidence intervals associated with one standard deviation increment increases in C-peptide levels.

We assessed potential effect modification by estimating the relative risks from conditional logistic regression models cross-tabulating C-peptide (in tertiles) with BMI (in tertiles), factors related to insulin resistance (in two categories, 0–1 factor or 2–4 factors), three categories of alcohol consumption (1 drink per week, 2–6 drinks per week, or 1 drink daily), or two categories of vigorous exercise (≤1 time per week or >1 time per week). To test for multiplicative interactions, we fit the conditional logistic regression models with multiplicative interaction terms involving the median level of each tertile of C-peptide and ordinal categories of those variables. We had more than 99% power to detect a 2.5-fold higher relative risk but limited power for the stratified analyses. All statistical tests were two-sided, and all the analyses used the SAS program package, version 8.12 (SAS Institute, Cary, NC) with a statistical significance level of .05.

Results

Table 1 shows the geometric mean levels of plasma C-peptide according to the baseline characteristics. As expected, participants who had blood samples drawn within 4 hours after a meal had statistically significantly higher plasma C-peptide levels than those who had blood drawn at least 4 hours after eating. Older men and smokers tended to have higher, but not statistically significant, levels of C-peptide. After controlling for age, smoking status, and fasting status, plasma C-peptide levels were positively associated with BMI and the number of factors related to insulin resistance and inversely associated with alcohol consumption and vigorous exercise, mainly among control subjects.

Baseline plasma C-peptide levels were statistically significantly higher among case patients (geometric mean = 0.43 pmol/mL, 95% CI = 0.39 to 0.47 pmol/mL) than control subjects (geometric mean = 0.37 pmol/mL, 95% CI = 0.35 to 0.40 pmol/mL) (P = .03), controlling for age, smoking status, and fasting status (Table 1). In the multivariable analysis controlling for age, smoking status, fasting status, BMI, alcohol consumption, vigorous exercise, and aspirin assignment, we observed a positive monotonic association between plasma C-peptide levels and the risk of colorectal cancer, comparing the upper quintile of plasma C-peptide level with the lowest quintile (RR = 2.7, 95% CI = 1.2 to 6.2; P_{trend} = .047) (Table 2). The association became stronger (RR for the highest versus the lowest quintile = 3.4, 95% CI = 1.4 to 8.3; P_{trend} = .02) after controlling for a cluster of factors associated with insulin resistance. Controlling for IGF-I and IGFBP-3 did not materially change the association (Table 2).

To eliminate the potential influence of an undiagnosed tumor on plasma C-peptide levels, we repeated the analysis after excluding data from case patients diagnosed within 5 years of the date blood was drawn and found that the positive association was slightly stronger (RR for the highest compared with the lowest quintile = 3.4, 95% CI = 1.3 to 8.8; P_{trend} = .03) (Table 2). The positive trend was essentially the same for both colon and rectal cancer (multivariable-adjusted RR associated with one unit of incremental increase in natural log-transformed concentration of C-peptide for colon cancer = 1.5, 95% CI = 1.0 to 2.2 [P_{trend} = .05], and for rectal cancer = 1.5, 95% CI = 0.8 to 2.8 [P_{trend} = .19]).

As noted earlier, we adjusted for fasting status (<4 hours, ≥4 hours, or missing) in all analyses of C-peptide. To assess the possible influence of fasting status on the observed association, we conducted an analysis limited to the 59 case patients and 115 control subjects whose blood samples were collected at least 4 hours after a meal. Because this required breaking the case–control matched pairs, we used unmatched logistic regression analysis comparing tertiles of C-peptide levels and adjusted for matching factors (age and smoking status), as well as fasting time (4–7.9 hours or ≥8 hours). Increased risks of colorectal cancer were associated with increasing tertiles of C-peptide level (RR = 1.0, referent; RR = 1.6, 95% CI = 0.8 to 3.3; and RR = 2.4, 95% CI = 0.9 to 6.6; P_{trend} = .08). Additional control for other risk factors had little influence on the results. Although the confidence interval widened in this subgroup analysis restricted

Table 1. Adjusted geometric mean concentration of plasma C-peptide according to baseline characteristics*

Category	Case patients		Control subjects		*P* value†
	No.	C-peptide, pmol/mL (95% CI)	No.	C-peptide, pmol/mL (95% CI)	
Overall	176	0.43 (0.39 to 0.47)	294	0.37 (0.35 to 0.40)	.03
Fasting time					
<2 h	35	0.73 (0.59 to 0.89)[a]	56	0.70 (0.59 to 0.82)[a]	.74
2–3.9 h	41	0.46 (0.38 to 0.55)[a]	81	0.45 (0.40 to 0.52)[a]	.91
4–7.9 h	24	0.32 (0.25 to 0.40)	50	0.26 (0.22 to 0.31)	.18
≥8 h	35	0.31 (0.26 to 0.38)	65	0.26 (0.22 to 0.30)	.15
Missing	41	0.42 (0.35 to 0.51)	42	0.34 (0.28 to 0.41)	.10
Age					
40–49 y	28	0.39 (0.31 to 0.49)	54	0.36 (0.30 to 0.42)	.51
50–59 y	76	0.41 (0.36 to 0.47)	161	0.37 (0.34 to 0.41)	.22
60–69 y	58	0.47 (0.40 to 0.55)	63	0.40 (0.34 to 0.46)	.14
70–84 y	14	0.54 (0.39 to 0.74)	16	0.47 (0.35 to 0.63)	.54
Cigarette smoking status					
Never	68	0.39 (0.34 to 0.45)	119	0.36 (0.32 to 0.40)	.37
Past	90	0.47 (0.41 to 0.53)	145	0.39 (0.35 to 0.43)	.02
Current	18	0.42 (0.32 to 0.55)	30	0.45 (0.36 to 0.55)	.71
BMI					
<23 kg/m^2	32	0.40 (0.33 to 0.50)	77	0.35 (0.30 to 0.40)[b]	.24
23–24.9 kg/m^2	60	0.39 (0.34 to 0.45)	91	0.36 (0.32 to 0.41)[b]	.49
25–26.9 kg/m^2	52	0.50 (0.43 to 0.59)	80	0.38 (0.33 to 0.43)[b]	.01
27–29.9 kg/m^2	22	0.48 (0.37 to 0.62)	35	0.48 (0.39 to 0.58)	.97
≥30 kg/m^2	10	0.33 (0.23 to 0.48)	11	0.56 (0.39 to 0.80)	.05
No. of markers for insulin resistance‡					
0	42	0.37 (0.31 to 0.44)[c]	79	0.35 (0.31 to 0.40)[c]	.55
1	65	0.39 (0.34 to 0.45)[c]	102	0.32 (0.28 to 0.35)[c]	.03
2	40	0.48 (0.40 to 0.57)	67	0.43 (0.38 to 0.49)	.35
3	23	0.55 (0.43 to 0.69)	31	0.50 (0.41 to 0.61)	.58
4	6	0.72 (0.45 to 1.14)	15	0.73 (0.54 to 0.97)	.97
Vigorous exercise					
<1 time per week	39	0.39 (0.33 to 0.48)	71	0.43 (0.37 to 0.49)[d]	.50
1 time per week	34	0.50 (0.41 to 0.61)	44	0.36 (0.30 to 0.43)	.02
2–4 times per week	70	0.44 (0.38 to 0.51)	130	0.38 (0.34 to 0.42)	.11
≥5 times per week	33	0.39 (0.32 to 0.48)	49	0.34 (0.29 to 0.40)	.28
No. of alcoholic drinks					
≤1 per week	50	0.47 (0.40 to 0.56)	106	0.44 (0.39 to 0.49)[e]	.49
2–6 per week	69	0.42 (0.36 to 0.48)	95	0.38 (0.34 to 0.43)[e]	.40
≥1 daily	57	0.41 (0.35 to 0.48)	93	0.32 (0.29 to 0.36)	.02

The following *P* values are comparisons within columns of case patients and/or control subjects: [a]$P<.01$ for those fasting <4 hours compared with those fasting ≥4 hours; [b]$P<.05$ for those who have a BMI <27 kg/m^2 compared with those who have a BMI ≥27 kg/m^2; [c]$P<.05$ for men with 0–1 markers of insulin resistance compared with those with 2–4 markers; [d]$P<.05$ for those who exercise <1 time per week compared with those who exercise ≥5 times per week; [e]$P<.05$ for those who consume <1 alcoholic drink daily compared with those who consume ≥1 alcoholic drink daily.

*Adjusted for age, smoking status, and fasting time. The number of case patients and control subjects may not always add up because of missing information for some factors. CI = confidence interval; BMI = body mass index.

†*P* value for case–control differences overall and in each subgroup strata. All statistical tests were two-sided.

‡The number of markers for insulin resistance is defined as the number of any of the following four factors: BMI of >25 kg/m^2, high-density lipoprotein of <40 mg/mL, triglycerides in the upper 20% category, and blood pressure of >130/85 mmHg or use of antihypertensive drugs.

to participants fasting 4 or more hours, the strength of the association and the positive trend were consistent with the overall results.

We also assessed the associations of BMI and the number of markers related to insulin resistance with risk of colorectal cancer. BMI tended to be positively associated with colorectal cancer risk (the age- and smoking status–adjusted RRs for BMI categories of 23–24.9, 25–26.9, 27–29.9, and ≥30 kg/m^2, respectively, compared with a BMI of <23 kg/m^2, were 1.5 [95% CI = 0.9 to 2.6], 1.5 [95% CI = 0.9 to 2.7], 1.5 [95% CI = 0.7 to 2.9], and 2.4 [95% CI = 0.9 to 6.1]; $P_{trend} = .03$). The risk of colorectal cancer associated with BMI did not change in the multivariable analysis but was attenuated after further controlling for plasma C-peptide levels (for BMI ≥30 kg/m^2 versus <23 kg/m^2, RR = 2.0, 95% CI = 0.7 to 5.4; $P_{trend} = .11$). The number of markers for insulin resistance factors was not associated with the risk of colorectal cancer (the age- and smoking status–adjusted RRs for carrying 0–4 markers = 1.0 [referent], 1.1 [95% CI = 0.7 to 1.8], 1.0 [95% CI = 0.6 to 1.8], 1.3 [95% CI = 0.7 to 2.6], and 0.7 [95% CI = 0.3 to 2.1], respectively; $P_{trend} = .92$).

We conducted stratified analyses to evaluate whether the positive association between C-peptide levels and risk of colorectal cancer was modified by BMI status or by carrying 0–1 or 2–4 of the factors related to insulin resistance (i.e., BMI of >25 kg/m^2, high-density lipoprotein cholesterol of <40 mg/mL, elevated triglycerides, and blood pressure of >130/85 mmHg or use of antihypertensive drugs) (Table 3). Because there were

Table 2. Association between the risk for colorectal cancer and quintiles of plasma C-peptide concentration

	Plasma C-peptide quintile					
	Q1	Q2	Q3	Q4	Q5	P_{trend}
C-peptide median concentration, pmol/mL (range)	0.14 (0.06–0.20)	0.24 (0.20–0.30)	0.38 (0.30–0.44)	0.54 (0.44–0.73)	0.97 (0.74–2.02)	
Overall follow-up						
No. of case patients/No. of control subjects	19/58	29/59	46/59	39/59	43/59	
RR* (95% CI)	1.0 (referent)	1.4 (0.7 to 3.0)	2.5 (1.2 to 5.2)	2.3 (1.1 to 4.7)	2.5 (1.1 to 5.6)	.06
RR* adjusted for BMI (95% CI)	1.0 (referent)	1.4 (0.7 to 2.9)	2.2 (1.1 to 4.7)	2.0 (0.9 to 4.2)	2.3 (1.0 to 5.2)	.09
Multivariable RR (95% CI)†	1.0 (referent)	1.4 (0.7 to 3.0)	2.4 (1.1 to 5.0)	2.2 (1.0 to 4.6)	2.7 (1.2 to 6.2)	.047
Multivariable RR (95% CI)‡	1.0 (referent)	1.5 (0.7 to 3.2)	2.8 (1.3 to 6.1)	2.5 (1.1 to 5.5)	3.4 (1.4 to 8.3)	.02
Multivariable RR (95% CI)§	1.0 (referent)	1.5 (0.7 to 3.3)	2.2 (1.0 to 4.8)	1.6 (0.7 to 3.5)	2.5 (1.2 to 5.6)	.04
Excluding first 5 years of follow-up						
No. of case patients/No. of control subjects	16/50	22/42	40/50	24/43	35/48	
RR* (95% CI)	1.0 (referent)	1.6 (0.7 to 3.8)	2.8 (1.2 to 6.3)	2.1 (0.9 to 4.8)	3.3 (1.4 to 8.2)	.03
Multivariable RR (95% CI)†	1.0 (referent)	1.6 (0.7 to 3.8)	2.6 (1.1 to 6.0)	2.0 (0.8 to 4.7)	3.4 (1.3 to 8.8)	.03

*Matched by age and smoking status and adjusted for fasting status. RR = relative risk; CI = confidence interval; BMI = body mass index.

†Further adjusted for BMI, alcohol consumption, vigorous exercise, and aspirin assignment.

‡Further adjusted for the number of markers for insulin resistance defined as the number of any of the following four factors: BMI of >25 kg/m^2, high-density lipoprotein of <40 mg/mL, triglycerides in the upper 20% category, and blood pressure of >130/85 mmHg or use of antihypertensive drugs.

§Further adjusted for insulin-like growth factor I (IGF-I) and IGF binding protein 3 (IGFBP-3); 158 case patients and 261 control subjects had complete data of IGF-I, IGFBP-3, and C-peptide.

small numbers of subjects in each category, we used tertiles of C-peptide level and BMI. The increased risk associated with C-peptide level persisted across all three BMI groups, with a more than twofold higher risk in the top tertile of C-peptide levels versus the bottom tertile among participants with normal BMI (23.5–25.5 kg/m^2) and among overweight participants (BMI >25.5 kg/m^2). We observed similar positive associations between C-peptide and the risk of colorectal cancer among men with 0–1 or with 2–4 factors related to insulin resistance. Overall, there was no apparent modification of the positive association between the level of C-peptide and the risk of colorectal cancer by BMI or insulin resistance–related factors.

Because moderate alcohol consumption or vigorous exercise may increase insulin sensitivity and thus reduce insulin production (Table 1), we assessed whether the association between C-peptide and colorectal cancer varied by alcohol consumption or vigorous exercise. As shown in Table 3, we observed a possible interaction between alcohol consumption and C-peptide

Table 3. Association of the risk of colorectal cancer with plasma C-peptide and body mass index (BMI), number of markers for insulin resistance, alcohol consumption, or vigorous exercise*

	Plasma C-peptide tertiles					
	T1		T2		T3	
	No. of case patients/No. of control subjects	RR (95% CI)	No. of case patients/No. of control subjects	RR (95% CI)	No. of case patients/No. of control subjects	RR (95% CI)
Median BMI (cutoff point)						
22.3 (<23.5) kg/m^2	16/39	1.0 (referent)	11/27	1.0 (0.4 to 2.6)	17/32	1.7 (0.7 to 4.1)
24.4 (23.5–25.5) kg/m^2	13/29	1.1 (0.4 to 2.6)	28/36	1.8 (0.8 to 4.2)	26/32	2.5 (1.1 to 6.0)
26.8 (>25.5) kg/m^2	8/30	0.8 (0.3 to 2.1)	29/35	2.3 (1.0 to 5.1)	28/34	2.2 (1.0 to 5.2)
			$P_{interaction}$ = .84			
No. of markers for insulin resistance†						
0–1	30/77	1.0 (referent)	39/54	1.8 (1.0 to 3.5)	38/50	2.4 (1.2 to 4.9)
2–4	7/21	0.7 (0.3 to 1.8)	29/44	1.8 (0.9 to 3.5)	33/48	1.9 (1.0 to 3.9)
			$P_{interaction}$ = .78			
No. of alcoholic drinks						
≤1 per week	9/31	1.0 (referent)	25/34	2.2 (0.8 to 5.8)	16/41	1.5 (0.5 to 4.1)
2–6 per week	20/28	2.1 (0.8 to 5.8)	16/33	1.7 (0.6 to 4.9)	33/34	3.4 (1.3 to 9.3)
≥1 daily	8/39	0.7 (0.2 to 2.2)	27/31	2.8 (1.1 to 7.2)	22/23	3.9 (1.3 to 11.7)
			$P_{interaction}$ = .05			
Vigorous exercise						
≤1 time per week	15/37	1.0 (referent)	24/33	1.8 (0.8 to 4.1)	34/45	2.2 (1.0 to 4.9)
>1 time per week	22/61	0.8 (0.4 to 1.9)	44/65	1.7 (0.8 to 3.5)	37/53	2.0 (0.9 to 4.3)
			$P_{interaction}$ = .93			

*Case patients and control subjects were matched by age and smoking status, and data were adjusted for fasting status. RR = relative risk; CI = confidence interval.

†The number of markers for insulin resistance is defined as the number of any of the following four factors: BMI of >25 kg/m^2, high-density lipoprotein of <40 mg/mL, triglycerides in the upper 20% category, and blood pressure of >130/85 mmHg or use of antihypertensive drugs.

with cancer risk ($P_{interaction}$ = .05); there was a trend toward a stronger association of C-peptide with risk of colorectal cancer among those who drank one or more drinks daily than among those who drank less. We observed no apparent modification by vigorous exercise.

Discussion

In this prospective study of nondiabetic U.S. male physicians, baseline plasma levels of C-peptide were positively associated with the risk of developing colorectal cancer. The association was not confounded by known colorectal cancer risk factors including age, smoking status, vigorous exercise, and alcohol consumption. The association was also independent of BMI, a cluster of factors related to insulin resistance syndrome, and levels of IGF-I and IGFBP-3. Over 13 years of follow-up, a C-peptide level in the top quintile was associated with an approximately 2.7-fold higher risk of colorectal cancer compared with that in the bottom quintile. Moreover, after excluding data from case patients diagnosed with colorectal cancer within 5 years of the date of blood collection, the association was stronger, suggesting that elevated plasma C-peptide levels preceded the colorectal cancer rather than being caused by an undiagnosed cancer.

Although the fasting serum insulin level has been widely used as an indicator of pancreatic β-cell function, circulating C-peptide levels may be a more stable indicator of pancreatic β-cell function. After being synthesized in the β cell as proinsulin and cleaved into insulin and C-peptide, both peptides are secreted separately at equimolar concentrations and pass through the portal venous bed of the liver before reaching the systemic circulation *(15).* Approximately half the secreted insulin is extracted during one passage through the liver *(21),* but between 85% and 90% of the secreted C-peptide reaches peripheral circulation *(22),* where it has a much slower metabolic clearance rate than insulin and lacks cross-reactivity with antibodies to proinsulin *(15).* Cross-reaction with antibodies to proinsulin has been a problem for some of the assays of insulin measurement. For these reasons, measurement of circulating C-peptide provides a useful and relatively stable indicator of pancreatic β-cell function and has been used to distinguish type 1 and type 2 diabetes *(15,16).* In a study of healthy men in the Health Professionals Follow-up Study who had an age range similar to that of our study participants, plasma C-peptide levels were strongly correlated with insulin levels in both fasting (correlation coefficient r = .83; n = 249) and non-fasting (r = .87; n = 183) status (Rimm E; personal communication). In the same study, the within-person correlation coefficient for C-peptide levels measured 4 years apart was reasonably good (r = .57). The relatively high average intra-assay coefficient of variation, which is based on the blinded quality-control samples of 16.5% in our analysis, and the imperfect long-term within-person correlation would tend to attenuate the association and suggest that the true underlying association between C-peptide and colorectal cancer risk may be stronger than what we observe.

Several prospective studies have linked being overweight or obese (the major cause of insulin resistance) *(6,23–25),* having type 2 diabetes (the major consequence of insulin resistance) *(6,7,26,27),* and the insulin resistance syndrome *(11,13)* to the risk of colorectal cancer. Insulin resistance refers to a decreased peripheral response to insulin that compensatorily leads the pancreas to increase insulin secretion, with consequent hyperinsulinemia, to control glucose levels *(19,28).* Individuals with newly diagnosed type 2 diabetes typically have a functional compensatory response, whereas in individuals with a long history of diabetes, hyperglycemia may eventually become uncontrollable as this response fails *(29).* In the Nurses' Health Study, a higher risk of colon cancer was associated with a recent diagnosis of type 2 diabetes but was not associated with long-standing diabetes *(7),* suggesting that hyperinsulinemia during the early stage of diabetes might promote tumor growth.

Our results for plasma C-peptide are remarkably consistent with findings from two previous prospective studies. Schoen et al. *(10)* found a twofold increased risk of colorectal cancer associated with elevated serum levels of insulin or glucose (the highest versus the lowest quartile) 2 hours after standard glucose challenge among nondiabetic elderly men, and Kaaks et al. *(30)* observed a threefold increased risk associated with elevated non-fasting plasma C-peptide levels in women (the highest versus the lowest quintile). Both studies excluded subjects with diabetes *(10)* or diabetes treated with insulin *(30)* at baseline. Interestingly, Schoen et al. found that fasting insulin and glucose levels were less strongly linked to colon cancer risk than were insulin and glucose levels measured 2 hours after a glucose challenge. This result, coupled with our findings and those of Kaaks et al., both of which used non-fasting C-peptide levels, suggests that elevated postprandial insulin production might be associated with the risk for colorectal cancer. The close correlation between plasma C-peptide and insulin levels in plasma samples from both fasting and non-fasting individuals, as well as the similarity in our overall results controlling for fasting time and the subgroup analysis restricted to men fasting for 4 or more hours, strongly supports the plasma C-peptide level as a valid biomarker for assessing the association between insulin and the risk of colorectal cancer. Thus, findings from these three prospective studies with measurement of baseline circulating levels of insulin 2 hours after a glucose challenge or of non-fasting C-peptide, in both men and women, provide strong evidence that long-term exposure to a high level of insulin was associated with an increased risk of developing colorectal cancer, independent of BMI, diabetes, and other insulin resistance–related factors, such as lipids and hypertension.

Insulin and IGF-I belong to the same IGF family and share the same insulin receptor substrates IRS-1 and IRS-2 and phosphatidylinositol 3′-kinase/p85 signaling pathway via the insulin receptor and the IGF-I receptor, respectively *(31).* High circulating levels of IGF-I and low circulating levels of IGFBP-3 have been associated with an increased risk for cancers of the colon and/or rectum *(17,32),* prostate *(33),* and breast *(34).* The positive association between C-peptide levels and the risk of colorectal cancer was independent of circulating levels of IGF-I and IGFBP-3 in the current analyses. Insulin may stimulate tumor growth by lowering IGFBP-1 production *(35),* increasing tissue bioavailability of IGF-I, or inhibiting apoptosis via binding to the IGF-I receptor *(31).* Insulin may also directly stimulate *in vitro* mitogenesis of cultured normal colorectal epithelial cells and tumor angiogenesis *(36,37).* Animals fed high-fat, high-calorie diets had increased insulin levels, developed insulin resistance, and experienced greater colon tumor growth *(38–41);* whereas animals fed a calorie-restricted diet had reduced insulin levels, reduced insulin resistance, and reduced tumor promotion *(40,42–44).* Caloric restriction in animals also re-

duced levels of IGF-I and insulin and decreased angiogenesis and tumor growth *(45)*. Although C-peptide binds to specific G protein–coupled receptors on human cell membranes, which provides a molecular basis for its biologic effects *(46)*, it is unclear whether circulating levels of C-peptide can directly affect tumor growth.

Several dietary and lifestyle factors have been associated with circulating C-peptide levels. In our study, statistically significantly higher plasma C-peptide levels were detected in blood drawn within 4 hours after a meal than in blood drawn after a longer fast, consistent with the postprandial insulin secretion pattern. In addition, elevated C-peptide levels were associated with older age, high BMI, and multiple factors related to the insulin resistance syndrome, consistent with previous reports that these factors are associated with increased peripheral insulin resistance and hyperinsulinemia *(19,47)*. In contrast, moderate and frequent alcohol consumption and vigorous exercise were associated with lower levels of plasma C-peptide, also consistent with reports linking these factors with enhanced insulin sensitivity, leading to decreased insulin production *(48–50)*. In a group of healthy middle-aged men of the Health Professionals Follow-up Study, plasma C-peptide level was also statistically significantly correlated with the Western dietary pattern characterized by high intakes of red meat, high-fat dairy products, and refined grains *(4)*.

In stratified analyses, the positive association between C-peptide levels and risk of colorectal cancer persisted regardless of BMI, vigorous exercise, or the presence of the insulin resistance syndrome. However, there was evidence of a stronger association of C-peptide levels with risk of colorectal cancer among those who drink alcohol frequently than among those who drink it less frequently ($P_{\text{interaction}} = .05$). Although higher alcohol consumption in our study was associated with lower C-peptide levels, our results also suggest that high C-peptide levels might be associated with an even higher risk of colorectal cancer in the presence of high levels of alcohol, perhaps due to alcohol-enhanced insulin sensitivity of colorectal epithelial tissue. The number of case patients in some of the stratified analyses was relatively small, so our findings in the stratified analyses should be further investigated in larger studies with more detailed information about these factors (i.e., physical activity, insulin resistance syndrome, and alcohol consumption).

In conclusion, our results in men and a previous study in women *(14)* suggest that elevated levels of C-peptide were associated with the risk of developing colorectal cancer, independent of BMI, factors related to insulin resistance, or levels of IGF-I and IGFBP-3. Our data not only support the hypothesis that elevated long-term insulin production is one underlying mechanism to link dietary and lifestyle risk factors with colorectal cancer risk but also provide a strong biologic argument that avoiding or reducing the modifiable risk factors, such as being overweight, being physically inactive, and following the Western dietary pattern, could effectively decrease the risk of colorectal cancer *(51)* and the risk of type 2 diabetes and cardiovascular disease, especially when the global prevalence of obesity is increasing rapidly.

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NOTES

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