A Prospective Study of C-Peptide, Insulin-like Growth Factor-I, Insulin-like Growth Factor Binding Protein-1, and the Risk of Colorectal Cancer in Women

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Abstract

Hyperinsulinemia, hyperglycemia, and elevated insulin-like growth factor (IGF)-1 levels have been implicated in the etiology of colorectal cancer. However, the joint effects of insulin and IGF-I have not been considered, and whether hyperinsulinemia or hyperglycemia is more etiologically relevant is unclear. IGF binding protein-1 (IGFBP-1) has been hypothesized to mediate the effects of insulin, but epidemiologic data on IGFBP-1 are sparse. We conducted a nested case-control study among the 32,826 women of the Nurses' Health Study who provided a blood sample in 1989 to 1990. After excluding diabetics, we confirmed 182 incident colorectal cancer cases over 10 years of follow-up and 350 controls. Cases were matched to two controls on year of birth, date of blood draw, and fasting status. C-peptide levels were weakly associated with risk of colon cancer [top quartile (Q4) versus bottom quartile (Q1): multivariable relative risk (MVRR), 1.76; 95% confidence interval (95% CI), 0.85-3.63]. Fasting IGFBP-1 was inversely associated with risk of colon cancer (MVRR, 0.28; 95% CI, 0.11-0.75). We observed no clear association between glycosylated hemoglobin and risk for colorectal cancer. The IGF-I to IGFBP-3 molar ratio was associated with colon cancer risk (MVRR, 2.82; 95% CI, 1.35-5.88), and women with low levels of both IGF-I/IGFBP-3 and C-peptide (or high IGFBP-1) were at low risk, and elevation of either was sufficient to increase risk. Although altering IGF-I levels may not be practical, the growing burden of obesity and consequently hyperinsulinemia, which seems increasingly important for colon cancer, may be a target for effective prevention.

Introduction

Hyperinsulinemia has been proposed as an underlying biological mechanism for the observed associations between sedentary behavior, obesity, and colorectal cancer (1-11). Some evidence suggests that hyperglycemia may also play a role (12-14). The possible association between diabetes mellitus, diets high in sugar, refined grains, high glycemic load, and colorectal cancer supports these observations (15-21). Conversely, energy restriction and its consequent insulin lowering effects seem to inhibit carcinogenesis in both animal models and humans (10, 22, 23). Although higher concentrations of insulin are directly mitogenic for certain tumor cells *in vitro* (24, 25), the physiologic relevance of this requires further study. Higher insulin levels, however, do lower levels of insulin-like growth factor (IGF) binding protein-1 (IGFBP-1; ref. 26) and thereby may increase the bioavailability of IGFs in certain tissues. Higher IGF-I levels, particularly relative to IGFBP-3, have been associated with increased colorectal cancer risk (27-29).

The association between C-peptide (a marker of insulin production), IGFBP-1, and colorectal cancer has been specifically investigated (29, 30). Others have directly measured plasma insulin or glucose (13, 17, 31) or markers of glucose control (12, 14, 32). However, previous studies have generally focused on either the insulin axis (e.g., C-peptide, IGFBP-1) or the IGF-I axis (e.g., IGF-I, IGF-I/IGFBP-3 ratio), and whereas independent effects have been observed, the joint effects of these axes have not been carefully examined. Because insulin is a major determinant of expression of IGFBP-1, a protein that can modulate the biological effects of IGFs, biological interactions between these axes are plausible. In addition, whether or not risk associated with high insulin levels corresponds to and accounts for risk related to body size or physical activity levels remains unsettled. Finally, little attention has been given to comparing hyperinsulinemia and hyperglycemia, both of which are associated with insulin resistance, in terms of etiologic relevance to colorectal cancer. Thus, we examined prospectively the associations between C-peptide, IGFBP-1, IGF-I, IGFBP-3, glycosylated hemoglobin (HbA1c), and colorectal cancer, as well as the joint effect of IGF-I and both C-peptide and IGFBP-1 on colorectal cancer risk among women in the Nurses' Health Study.

Materials and Methods

Study Population. The Nurses' Health Study began in 1976 when 121,701 female nurses, ages 30 to 55 years, enrolled and completed a questionnaire about lifestyle and medical history. Since then, these women have been mailed self-administered questionnaires biennially to update information on lifestyle, medical history, and diet (every 4 years). In 1989 to 1990, 32,826 women, ages 43 to 69 years, provided a blood sample. Ninety-seven percent of the samples were received via overnight courier within 26 hours of being drawn. Most

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samples (60-70%) were drawn while the nurses were in a fasting state (8 or more hours since their last meal). All samples were separated into plasma, erythrocytes, and buffy coat and have been continuously stored in well-monitored liquid nitrogen freezers. The blood collection methods have been described in more detail previously (33). The women in the blood subcohort are generally similar to the women in the main Nurses' Health Study cohort with respect to lifestyle and diet.

Follow-up for this analysis was between the return of the blood sample and June 1, 2000. Incident cancer cases were self-reported on the biennial questionnaire, then verified by medical records. As of 2000, the follow-up rate in the subcohort who provided a blood sample is 99%. For both cases and controls, we excluded anyone who had been previously diagnosed with cancer (except nonmelanoma skin cancer). Because type II diabetes results initially in hyperinsulinemia, due to insulin resistance, and then over time in hypoinsulinemia as the β-cells of the pancreas fail, C-peptide levels among diabetics likely do not represent their long-term insulin exposure. Therefore, we further excluded anyone who had reported diabetes before 1990. Two controls were matched to most cases on year of birth, fasting status, and month of blood draw. However, for 14 cases, only one appropriate control was available. Information on diet, anthropometry, and lifestyle factors was calculated using responses provided on the biennial questionnaires. For these analyses, we included body mass index (BMI), physical activity, duration of aspirin use (nonuser, <10 years, ≥10 years of use) cigarette smoking (measured in pack-years), alcohol, family history of colon or rectal cancer, history of endoscopy, menopausal status, and postmenopausal hormone use (in 1990). Our ability to validly measure diet, BMI, and physical activity has been documented in several previous studies (34-36).

Laboratory Assays. In the process of insulin secretion, proinsulin is cleaved into equimolar amounts of insulin and C-peptide, which has a longer half-life than insulin. C-peptide is therefore an effective biomarker for insulin secretion (37). Physical activity and obesity have been shown to predict C-peptide levels (38). Plasma C-peptide, as well as IGF-I, IGFBP-1, and IGFBP-3, was assayed using ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory of Dr. Michael Pollak.

HbA1c, formed when a molecule of glucose attaches to the last amino acid of the β chain of hemoglobin A and widely used to determine blood glucose control in diabetics, has been shown to be a more stable indicator of glycemia over the prior 6 to 8 weeks compared with direct measures of plasma glucose (39). We determined levels of HbA1c by turbidometric immunoinhibition in RBC using the Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN) in the lab of Dr. Nader Rifai.

Samples from matched sets were assayed together along with randomly inserted masked quality control samples. All laboratory personnel were blinded with respect to case or control status. The mean intra-assay coefficients of variation from the quality control samples were less than 13% for C-peptide, 15% for IGF-I, 12% for IGFBP-1, 9% for IGFBP-3, and 2.7% for HbA1c.

Statistical Analysis. We log transformed the plasma biomarkers to improve normality and then compared cases and controls using paired t tests, Wilcoxon signed rank, and χ^2 tests. All quantile cutpoints were generated among the controls only and were batch year specific (due to laboratory variation over time). Partial Spearman correlation coefficients were used to examine the relationships among the various biomarkers, BMI, and alcohol.

To compute relative risks (RR) and 95% confidence intervals (95% CI), we used conditional logistic regression. We used the median of the categories in the controls in models (continuous variable) to test for linear trend; the P values from these tests are two sided. We limited the IGFBP-1 main analysis to women with fasting levels only (≥6 hours since last meal) because fasting IGFBP-1 levels are a good indicator of long-term insulin levels (40, 41).

In an alternative analysis, to better classify individuals with respect to their insulin levels, and to minimize measurement error from using one assay, we cross-classified C-peptide and IGFBP-1 levels (the within-person correlation coefficient for C-peptide measured 4 years apart in a similar cohort of men was previously reported to be reasonably high, $r = 0.57$ ref. 30). Because C-peptide levels are influenced by physical activity and BMI, we evaluated whether the risk associated with C-peptide varied by these factors. To evaluate statistical interaction between the IGF-I/IGFBP-3 ratio and C-peptide (or IGFBP-1), we cross-classified the variables and entered the medians of each individual variable along with a term created by multiplying the medians of each variable as a continuous variable in a model; the P value was determined by a Wald test on the interaction term.

We evaluated risk by subsites because previous publications suggest that risk factors may exert their effects differentially by site within the colon (42-45).

Results

There were 193 incident colorectal cancer cases through June 2000 among the women who gave blood and had not reported any noncutaneous cancer previous to the colorectal cancer diagnosis. After excluding women who reported a diagnosis of diabetes, there were 182 colorectal cancer cases (140 colon, 42 rectal) and 350 matched controls eligible for this analysis. Table 1 compares the cases and controls with respect to several lifestyle, medical, and dietary factors. Few statistically significant differences were observed between the colorectal cancer cases and controls; a slightly greater proportion of controls were current postmenopausal hormone users and overall the differences were in the expected direction. Table 2 shows the Spearman correlation between the plasma markers, BMI, alcohol intake, and physical activity among the controls (adjusted for age, assay batch, and time since last meal). An inverse correlation was observed for C-peptide and IGFBP-1 ($r = -0.53$). C-peptide levels were modestly correlated with IGFBP-3, but not with IGF-I and the IGF-I/IGFBP-3 ratio. C-peptide (positively) and IGFBP-1 (inversely) were nearly equally correlated with BMI ($r = 0.44$, $r = -0.43$). IGFBP-1 was inversely correlated with all of the other analytes and with BMI. IGF-I was strongly correlated with the IGF-I/IGFBP-3 ratio and with IGFBP-3, but essentially uncorrelated with BMI, alcohol, and physical activity. HbA1c was slightly correlated with C-peptide, IGF-I, IGFBP-3, and BMI, and inversely with IGFBP-1.

Higher C-peptide levels were only weakly associated with risk for colorectal cancer (Table 3). When we evaluated risk by subsite, the association was slightly stronger for colon cancer and stronger for proximal colon cancer [multivariable RR (MVRR), 2.62; 95% CI, 0.91-7.53, $P_{trend} = 0.17$]. The associations did not appear to be linear; similar RRs observed in the second and fourth quartiles suggested a threshold effect. Among fasting women, the results were attenuated (data not shown). An inverse association between fasting levels of IGFBP-1 and risk of colorectal cancer was observed (Table 3). This association became stronger and statistically significant when we limited the analysis to cases arising in colon (MVRR, 0.28; 95% CI, 0.11-0.75, $P_{trend} = 0.05$) or the proximal colon only (MVRR, 0.05; 95% CI, 0.008-0.30, $P_{trend} = 0.002$).

HbA1c showed a very slight, nonlinear, inverse association with risk for colorectal cancer and colon cancer (Table 3). Results for proximal colon cancer were essentially null (MVRR, 1.00; 95% CI, 0.38-2.61, $P_{trend} = 0.65$).

Table 1. Characteristics of colorectal cancer cases and matched controls nested in the Nurses' Health Study, 1989-2000

	Cases (n = 182)	Controls (n = 350)	P*
Plasma C-peptide (ng/mL) geometric mean interquartile range (25-75%)	1.87 (1.26-2.58)	1.80 (1.15-2.72)	0.42
Plasma IGFBP-1 (ng/mL) geometric mean interquartile range (25-75%)	18.4 (10.2-36.3)	20.0 (12.2-41.7)	0.38
Plasma IGF-I (ng/mL) geometric mean interquartile range (25-75%)	156.7 (123.5-206.1)	147.1 (111.3-192.5)	0.10
Plasma IGFBP-3 (ng/mL) geometric mean interquartile range (25-75%)	4,049 (3,479-4,952)	4,060 (3,489-4,952)	0.42
IGF-I/IGFBP-3 molar ratio mean interquartile range (25-75%)	0.15 (0.12-0.17)	0.14 (0.11-0.16)	0.05
Glycosylated hemoglobin (%; mean ± SD)	5.34 ± 0.32	5.32 ± 0.35	0.86
Age at blood draw (mean ± SD)	60.4 ± 6.5	60.3 ± 6.5	—
BMI (kg/m^2; mean ± SD)	24.9 ± 4.3	24.9 ± 4.1	0.52
Mean physical activity (mean MET-hours/wk ± SD)	14.9 ± 15.0	14.5 ± 14.9	0.45
Alcohol (mean g/d ± SD)	7.9 ± 10.9	6.3 ± 9.6	0.12
Cigarette smoking (mean pack-years ± SD)	19.5 ± 25.1	16.3 ± 20.8	0.67
Current postmenopausal hormone use (%)	24.7	32.6	0.06
Screening before 1990 (%)	35.7	39.7	0.37
Family history of colorectal cancer (%)	18.1	14.0	0.21
Multivitamin use (%)	37.9	35.7	0.62
Aspirin use >10 y (%)	8.8	8.9	0.98

*P value for plasma biomarkers from paired t test, Wilcoxon signed rank for all other continuous variables, and χ^2 test for categorical variables.

After exclusion of the cases in the first 2 years of follow-up, the results were not remarkably different from those presented, suggesting that preclinical disease did not influence our results (data not shown).

To evaluate the joint effect of C-peptide and IGFBP-1, we cross-classified the two variables by tertiles and focused on colon cancer only. The number of cases in some categories was low but we found evidence of a stronger association among women who had both high C-peptide and low IGFBP-1 levels; women in the highest tertile of C-peptide and lowest tertile of IGFBP-1 had an RR of 2.88 (95% CI, 1.20-6.93) compared with those in the lowest tertile of C-peptide and highest tertile of IGFBP-1. Likewise, compared with those in the highest physical activity tertile (>15.4 MET-hours/wk), who are likely more insulin sensitive and lowest C-peptide tertile, the women in the lowest physical activity category (<5.7 MET-hours/wk) and highest C-peptide tertile had an RR of 2.46 (95% CI, 1.03-5.88). The association of C-peptide and colon cancer did not vary by either BMI or fasting status (data not shown).

Previously, we reported a suggestive association between IGF-I and colorectal cancer in this cohort (highest versus lowest tertile of IGF-I: RR, 2.18; 95% CI, 0.94-5.08; ref. 27), but we had too few cases to evaluate colon cancer separately. With additional 6 years of follow-up, we observed similar associations between plasma IGF-I levels and risk for colon cancer and a suggestive but nonstatistically significant inverse association with IGFBP-3 and colon cancer (Table 4). In addition, the molar ratio of IGF-I to IGFBP-3 (IGF-I/IGFBP-3, not included in the previous publication), which may reflect IGF-I that is bioavailable for the tissue, was associated with increased risk (Table 4). The results for total colorectal cancer were similar for the IGF-I/IGFBP-3 ratio (P_{trend} = 0.01), slightly attenuated for IGF-I (P_{trend} = 0.07), and slightly stronger for IGFBP-3 (P_{trend} = 0.09).

To evaluate the joint effects of the insulin and IGF-I axes, we cross-classified both IGFBP-1 and C-peptide with IGF-I/IGFBP-3. We found an increased risk with decreasing IGFBP-1, which was more pronounced for women with a low IGF-I/IGFBP-3 ratio ($P_{interaction}$ = 0.29). In the highest tertile of IGF-I/IGFBP-3 ratio, the risk for the highest and lowest IGFBP-1 tertiles was similar (Fig. 1). Conversely, among those in the highest IGFBP-1 tertile (correlated with low insulin exposure), the effect of increasing IGF-I/IGFBP-3 was much more marked than in those in the lowest IGFBP-1 tertile (highest risk). A similar but slightly weaker pattern (in the opposite direction) was observed when C-peptide was used instead of IGFBP-1 as a marker of insulin resistance and excretion.

Discussion

In this prospective study, we found evidence for a direct association between plasma C-peptide levels and risk of colon cancer and a strong inverse association between fasting levels of IGFBP-1 and colon cancer. In contrast, we did not observe any direct association between glycosylated hemoglobin and

Table 2. Partial Spearman correlation coefficients between C-peptide, IGF-I, IGFBP-1 and -3, glycosylated hemoglobin, and other variables among colorectal cancer controls nested in Nurses' Health Study, 1989-2000

	C-peptide	IGFBP-1	IGF-I	IGFBP-3	Ratio	HbA1c	BMI	Alcohol	Physical activity
C-peptide	1.00								
IGFBP-1	−0.53*	1.00							
IGF-I	0.07	−0.29*	1.00						
IGFBP-3	0.13†	−0.15†	0.58*	1.00					
Ratio	−0.005	−0.27*	0.81*	0.07	1.00				
HbA1c	0.20†	−0.18†	0.15†	0.15†	0.09	1.00			
BMI	0.44*	−0.43*	−0.03	0.03	−0.04	0.14‡	1.00		
Alcohol	−0.07	0.07	−0.09	0.02	−0.11‡	−0.008	−0.16†	1.00	
Physical activity	−0.08	0.05	0.05	0.14‡	−0.08	0.002	−0.12‡	0.14‡	1.00

NOTE: Adjusted for age, assay batch, and time since last meal.
*P < 0.05.
†P < 0.01.
‡P < 0.001.

Table 3. RR and 95% CI of colorectal cancer according to quartiles of plasma levels of C-peptide, IGFBP-1, and HbA1c in the Nurses' Health Study, 1989-2000

	Quartile				P_{trend}
	1	2	3	4	
C-peptide					
Median (ng/mL)	0.9	1.4	2.0	3.6	
Colorectum					
Cases/Controls	35/87	50/86	54/89	43/88	
RR* (95% CI)	1.00	1.46 (0.86-2.45)	1.50 (0.88-2.57)	1.22 (0.70-2.13)	
RR† (95% CI)	1.00	1.46 (0.84-2.53)	1.41 (0.79-2.55)	1.17 (0.63-2.20)	0.94
Colon					
Cases/Controls	23/70	41/65	42/71	34/62	
RR* (95% CI)	1.00	1.88 (1.04-3.42)	1.83 (0.96-3.48)	1.65 (0.88-3.09)	
RR† (95% CI)	1.00	1.88 (1.00-3.56)	1.85 (0.92-3.74)	1.76 (0.85-3.63)	0.38
IGFBP-1‡					
Median (ng/mL)	5.7	19.1	31.8	55.7	
Colorectum					
Cases/Controls	34/43	25/61	41/62	28/70	
RR* (95% CI)	1.00	0.42 (0.21-0.83)	0.81 (0.45-1.44)	0.45 (0.23-0.89)	
RR† (95% CI)	1.00	0.41 (0.19-0.86)	0.82 (0.42-1.62)	0.48 (0.21-1.09)	0.31
Colon					
Cases/Controls	30/33	20/52	28/53	21/51	
RR* (95% CI)	1.00	0.39 (0.18-0.82)	0.61 (0.31-1.17)	0.42 (0.20-0.89)	
RR† (95% CI)	1.00	0.32 (0.14-0.75)	0.45 (0.20-1.01)	0.28 (0.11-0.75)	0.05
HbA1c§					
Median (%)	5.1	5.4	5.6	5.8	
Colorectum					
Cases/Controls	41/73	40/89	48/87	46/83	
RR*	1.00	0.82 (0.48-1.41)	1.01 (0.59-1.73)	0.99 (0.57-1.71)	
RR†	1.00	0.75 (0.43-1.30)	0.94 (0.54-1.65)	0.85 (0.47-1.51)	0.74
Colon					
Cases/Controls	33/55	26/70	41/66	35/64	
RR*	1.00	0.66 (0.35-1.23)	1.08 (0.59-1.98)	0.95 (0.51-1.76)	
RR†	1.00	0.57 (0.29-1.10)	1.02 (0.53-1.95)	0.82 (0.42-1.61)	0.91

*RR from conditional logistic regression.
†RR from conditional logistic regression additionally adjusted for BMI, physical activity, pack-years smoked, and alcohol intake as continuous variables, family history of colorectal cancer, aspirin use, history of screening, menopausal status, and use of postmenopausal hormones.
‡IGFBP-1 reported only among women who reported their blood collection was 6 or more hours since their last meal (128 cases/236 controls).
§The total number of cases and controls for HbA1c is less than that for C-peptide due to missing data.

risk of colorectal cancer. Lowest risk was observed in women with a low IGF-I/IGFBP-3 ratio and low insulin exposure (low C-peptide or high IGFBP-1); being high in either was sufficient to substantially elevate colon cancer risk.

Low IGFBP-1 and high C-peptide levels are indicative of hyperinsulinemia, thus, jointly classifying these variables may improve our ability to correctly classify individuals with high or low insulin levels. Although our estimates were somewhat unstable, the elevated risk among those with high C-peptide and low IGFBP-1 suggests hyperinsulinemia increases risk. Our results are consistent with the results of Schoen et al. (13) who reported a small elevated risk with higher insulin and glucose values 2 hours after a glucose challenge.

HbA1c has been suggestively positively associated with colorectal cancer risk but the evidence is inconclusive (12, 14, 32). Previously we evaluated this association among

Table 4. Relationship between plasma levels of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 and risk of colon cancer among 137 cases and 262 controls nested in the Nurses' Health Study, 1989-2000

	Quartile				P_{trend}
	1	2	3	4	
IGF-I					
Median (ng/mL)	95	133	170	230	
Cases/Controls	26/69	31/62	36/66	44/65	
RR* (95% CI)	1.00	1.14 (0.59-2.20)	1.38 (0.71-2.71)	1.95 (0.97-3.91)	0.09
RR† (95% CI)	1.00	1.19 (0.60-2.37)	1.46 (0.07-3.08)	2.17 (0.96-4.88)	0.03
IGFBP-3					
Median	2,920	3,823	4,602	5,443	
Cases/Controls	30/64	36/71	37/62	34/65	
RR* (95% CI)	1.00	1.08 (0.56-2.12)	1.36 (0.71-2.61)	1.20 (0.62-2.30)	0.62
RR† (95% CI)	1.00	0.85 (0.41-1.78)	1.04 (0.51-2.16)	0.81 (0.38-1.75)	0.12
IGF-I/IGFBP-3					
Median	0.09	0.12	0.14	0.18	
Cases/Controls	24/67	31/67	34/62	48/66	
RR† (95% CI)	1.00	1.88 (0.90-3.94)	2.33 (1.11-4.87)	2.82 (1.35-5.88)	0.01

*Adjusted for BMI, physical activity, pack-years smoked, and alcohol intake as continuous variables, family history of colorectal cancer, aspirin use, history of screening, menopausal status, and use of hormone replacement therapy.
†Adjusted for BMI, physical activity, pack-years smoked, and alcohol intake as continuous variables, family history of colorectal cancer, aspirin use, history of screening, menopausal status, and use of hormone replacement therapy and IGF-I or IGFBP-3.

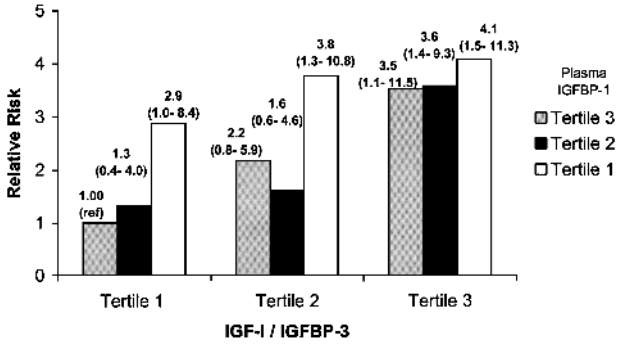

Figure 1. Multivariate relative risk of colon cancer by tertiles of IGFBP-1 and tertiles of the IGF-I/IGFBP-3 ratio in the Nurses' Health Study, 1989-2000.

79 incident colorectal cancer cases from the Nurses' Health Study (1989-1990 to 1994) and reported a modest increased risk (MVRR, 1.2; 95% CI, 0.6-2.7; including women with diabetes; ref. 14). More recently, preliminary results from the EPIC cohort, based on only 67 incident colorectal cancer cases, showed a 34% increased risk of colorectal cancer for each 1% increase in HbA1c (32). Our findings suggest that hyperinsulinemia and high IGF-I may be more etiologically relevant than high glucose with respect to colorectal cancer risk, consistent with earlier findings that the increased risk of colon cancer associated with diabetes weakens with time since diabetes diagnosis: in early adult-onset diabetes, hyperinsulinemia is prominent; later in the disease, insulin levels may decrease and hyperglycemia becomes more marked (15).

Previous prospective studies concerning IGF-I and IGFBP-3 and risk of colorectal cancer are somewhat inconsistent, particularly with respect to IGFBP-3 (46). With additional 6 years of follow-up, we found positive associations between levels of IGF-I and risk for colon cancer and an inverse, nonstatistically significant association for IGFBP-3. We further found that IGF-I/IGFBP-3 was positively associated with risk, similar to results in men previously published (47). Although the binding equilibria between IGFs and IGFBPs are complex (48), it is possible that this simple ratio more closely reflects bioavailable IGF-I than total serum IGF-I.

When we examined the joint effect of IGF-I/IGFBP-3 and hyperinsulinemia (estimated by high C-peptide or low IGFBP-1), hyperinsulinemia was more strongly associated with risk among those with low IGF-I/IGFBP-3. Women with high IGF-I/IGFBP-3 were already at high risk, and the additional influence of C-peptide or IGFBP-1 was minimal. Further, hyperinsulinemia or a high IGF-I/IGFBP-3 ratio was sufficient to convey a higher risk, but being high in both did not confer appreciable additional risk. This finding, and similar results reported in men (49), suggests a plateau of risk from the insulin and IGF-I pathways.

Physical activity has been shown to reduce C-peptide levels and increase IGFBP-1 levels (38). One would expect that the individuals with low physical activity, averaged over a 10-year period, and with high C-peptide levels are likely to have long-term hyperinsulinemia, and thus an increased risk for colon cancer, as observed.

Given that overweight/obesity is associated with hyperinsulinemia, we predicted women with higher BMI would have a higher risk of colon cancer, but in this nested cohort, BMI was not as strongly associated with increased risk as has been previously reported (3). In women, BMI might have opposing effects on colon cancer risk; for example, higher BMI may lead to higher estrogen levels which have been inversely associated with colorectal cancer risk (50-52). The lack of association with BMI and the observed increase risk with high C-peptide or low IGFBP-1 levels suggest that the plasma markers provide additional information related to the underlying mechanism(s) of colorectal cancer risk rather than acting merely as surrogate measures of adiposity. Similar findings have also been observed in men (49).

Strengths of our study are prospectively collected blood, which reduces the possibility of preclinical disease influencing the results, and extensive information on potential confounding factors.

Limitations of our study include having only a one-time blood measure, which reduced our ability to evaluate associations between long-term circulating levels of these exposures and risk. Previous evidence suggests, however, that C-peptide and IGF-I and IGFBP-3 are relatively stable over time. Chan et al. (53) reported a correlation of 0.65 between assays of IGF-I taken 8 weeks apart, and in another study, $r = 0.97$ between two measures taken an average of 5.8 days apart in 10 subjects, and $r = 0.94$ between two measures taken an average of 42 days apart in 24 subjects (54). Also, in a cohort of male health professionals, Platz et al. (55) reported Spearman partial correlation coefficients (adjusting for race) for time 1 versus time 2 (mean, 3.0 ± 0.5 years apart) of 0.70 for IGF-I, 0.68 for IGFBP-3, and 0.59 for their ratio. We also had relatively small sample sizes for the subsites within the colon. Thus, these results require further investigation.

In conclusion, we found that hyperinsulinemia was associated with an increased risk for colon cancer, but a one-time measure of glycemia was not. Further, the associations we observed seemed to be independent of body size. These results suggest that insulin secretion and its sequelae are driving the increased risk associated with factors such as physical activity and western dietary patterns. Plasma IGF-I and IGF-I/IGFBP-3 were also associated with increased risk for colon cancer. However, the biomarkers of hyperinsulinemia were more important among those who had a low IGF-I/IGFBP-3 ratio and, conversely, the IGF-I/IGFBP-3 ratio was associated with increased risk particularly among those without hyperinsulinemia.

The growing epidemic and burden of overweight and obesity (56, 57) suggests that hyperinsulinemia is rapidly becoming more prevalent and thus could become an increasingly important factor for cancer incidence. Although altering IGF-I and IGFBP-3 levels may not be practical or desirable, dietary and lifestyle modifications to reduce hyperinsulinemia could play an important role for colon cancer prevention.

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References

1. Lee IM, Paffenbarger RS Jr, Hsieh C. Physical activity and risk of developing colorectal cancer among college alumni. J Natl Cancer Inst 1991;83:1324–9.
2. Longnecker MP, Gerhardsson le Verdier M, Frumkin H, et al. A case-control study of physical activity in relation to risk of cancer of the right colon and rectum in men. Int J Epidemiol 1995;24:42–50.
3. Martinez ME, Giovannucci E, Spiegelman D, et al. Leisure-time physical activity, body size, and colon cancer in women. Nurses' Health Study Research Group. J Natl Cancer Inst 1997;89:948–55.
4. Colditz GA, Cannuscio CC, Frazier AL. Physical activity and reduced risk of colon cancer: implications for prevention. Cancer Causes Control 1997;8:649–67.
5. Gehardsson de Verdier M, Steineck G, Hagman U. Physical activity and colon cancer: A case-referent study in Stockholm. Int J Cancer 1990;46:985–9.
6. Giovannucci E, Ascherio A, Rimm EB, et al. Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1995;122:327–34.
7. Giovannucci E, Colditz GA, Stampfer MJ, et al. Physical activity, obesity, and risk of colorectal adenoma in women (United States). Cancer Causes Control 1996;7:253–63.

8. Slattery ML, Edwards SL, Boucher KM, et al. Lifestyle and colon cancer: an assessment of factors associated with risk. Am J Epidemiol 1999;150:869–77.
9. Caan BJ, Coates AO, Slattery ML, et al. Body size and the risk of colon cancer in a large case-control study. Int J Obes Relat Metab Disord 1998;22:178–84.
10. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 2001;131:3109–20S.
11. McKeown-Eyssen G. Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994;3:687–95.
12. Saydah SH, Platz EA, Rifai N, et al. Association of markers of insulin and glucose control with subsequent colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2003;12:412–8.
13. Schoen RE, Tangen CM, Kuller LH, et al. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999;91:1147–54.
14. Platz EA, Hankinson SE, Rifai N, et al. Glycosylated hemoglobin and risk of colorectal cancer and adenoma (United States). Cancer Causes Control 1999;10:379–86.
15. Hu FB, Manson JE, Liu S, et al. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J Natl Cancer Inst 1999;91:542–7.
16. Will JC, Galuska DA, Vinicor F, et al. Colorectal cancer: another complication of diabetes mellitus? Am J Epidemiol 1998;147:816–25.
17. Nilsen TI, Vatten LJ. Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyperinsulinaemia hypothesis. Br J Cancer 2001;84:417–22.
18. Fung T, Hu FB, Fuchs C, et al. Major dietary patterns and the risk of colorectal cancer in women. Arch Intern Med 2003;163:309–14.
19. Slattery ML, Benson J, Berry TD, et al. Dietary sugar and colon cancer. Cancer Epidemiol Biomarkers Prev 1997;6:677–85.
20. Higginbotham S, Zhang ZF, Lee IM, et al. Dietary glycemic load and risk of colorectal cancer in the Women's Health Study. J Natl Cancer Inst 2004;96:229–33.
21. Franceschi S, Dal Maso L, Augustin L, et al. Dietary glycemic load and colorectal cancer risk. Ann Oncol 2001;12:173–8.
22. Steinbach G, Heymsfield S, Olansen NE, et al. Effect of caloric restriction on colonic proliferation in obese persons: implications for colon cancer prevention. Cancer Res 1994;54:1194–7.
23. Steinbach G, Kumar SP, Reddy BS, et al. Effects of caloric restriction and dietary fat on epithelial cell proliferation in rat colon. Cancer Res 1993;53:2745–9.
24. Koenuma M, Yamori T, Tsuruo T. Insulin and insulin-like growth factor 1 stimulate proliferation of metastatic variants of colon carcinoma 26. Jpn J Cancer Res 1989;80:51–8.
25. Tran TT, Medline A, Bruce WR. Insulin promotion of colon tumors in rats. Cancer Epidemiol Biomarkers Prev 1996;5:1013–5.
26. Powell DR, Suwanichkul A, Cubbage ML, et al. Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. J Biol Chem 1991;266:18868–76.
27. Giovannucci E, Pollak MN, Platz EA, et al. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 2000; 9:345–9.
28. Ma J, Pollak M, Giovannucci E, et al. A prospective study of plasma levels of insulin-like growth factor I (IGF-I) and IGF-binding protein-3, and colorectal cancer risk among men. Growth Horm IGF Res 2000;10 Suppl A:S28–9.
29. 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 2000;92:1592–600.
30. Ma J, Giovannucci E, Pollak M, et al. A prospective study of plasma C-peptide and colorectal cancer risk in men. J Natl Cancer Inst 2004;96:546–53.
31. Palmqvist R, Stattin P, Rinaldi S, et al. Plasma insulin, IGF-binding proteins-1 and -2 and risk of colorectal cancer: a prospective study in northern Sweden. Int J Cancer 2003;107:89–93.
32. Khaw KT, Wareham N, Bingham S, et al. Preliminary communication: glycated hemoglobin, diabetes, and incident colorectal cancer in men and women: a prospective analysis from the European prospective investigation into cancer-norfolk study. Cancer Epidemiol Biomarkers Prev 2004; 13:915–9.
33. Hankinson SE, Willett WC, Manson JE, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Natl Cancer Inst 1995;87:1297–302.
34. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and Validity of a self-administered physical activity questionnaire. Int J Epidemiol 1994;23: 991–9.
35. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26; discussion 1127–36.
36. Rimm EB, Stampfer MJ, Colditz GA, et al. Validity of self-reported waist and hip circumferences in men and women. Epidemiology 1990;1:466–73.
37. Bonser AM, Garcia-Webb P. C-peptide measurement: methods and clinical utility. Crit Rev Clin Lab Sci 1984;19:297–352.
38. Allen NE, Appleby N, Kaaks R, 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 and Control 2003;14:65–74.
39. Rochman H. Hemoglobin A1c and diabetes mellitus. Ann Clin Lab Sci 1980; 10:111–5.
40. Frystyk J, Grofte T, Skjaerbaek C, et al. The effect of oral glucose on serum free insulin-like growth factor-I and -II in health adults. J Clin Endocrinol Metab 1997;82:3124–7.
41. Nyomba BL, Berard L, Murphy LJ. Free insulin-like growth factor I (IGF-I) in healthy subjects: relationship with IGF-binding proteins and insulin sensitivity. J Clin Endocrinol Metab 1997;82:2177–81.
42. Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer 2002; 101:403–8.
43. Larsson SC, Rafter J, Holmberg L, et al. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: The Swedish Mammography Cohort. Int J Cancer 2004.
44. Lindblom A. Different mechanisms in the tumorigenesis of proximal and distal colon cancers. Curr Opin Oncol 2001;13:63–9.
45. Bufill JA. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. Ann Intern Med 1990;113:779–88.
46. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505–18.
47. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999;91:620–5.
48. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824–54.
49. Ma J, Pollak MN, Gaziano JM, et al. The association between plasma insulin-like growth factor (IGF)-1 and colorectal cancer is modified by BMI or C-peptide. Orlando, Florida: AACR; 2004.
50. Grodstein F, Newcomb A, Stampfer MJ. Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med 1999;106:574–82.
51. Slattery ML, Ballard-Barbash R, Edwards S, et al. Body mass index and colon cancer: an evaluation of the modifying effects of estrogen (United States). Cancer Causes Control 2003;14:75–84.
52. Terry D, Miller AB, Rohan TE. Obesity and colorectal cancer risk in women. Gut 2002;51:191–4.
53. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279: 563–6.
54. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol 1997;145:970–6.
55. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999;8:1107–10.
56. Stein CJ, Colditz GA. The epidemic of obesity. J Clin Endocrinol Metab 2004;89:2522–5.
57. Bray GA. Medical consequences of obesity. J Clin Endocrinol Metab 2004;89:2583–9.