C-Peptide, Insulin-like Growth Factor Binding Protein-1, Glycosylated Hemoglobin, and the Risk of Distal Colorectal Adenoma in Women

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Abstract

Background: **Determinants of insulin secretion and insulin-like growth factors (IGF) have been directly associated with risk for colorectal cancer. However, few studies have evaluated whether these factors are also associated with risk of colorectal adenoma, the main precursor lesion to colorectal cancer.**

Methods: **We identified 380 distal colorectal adenoma cases diagnosed between 1989 and 1998 and 380 controls among nondiabetic women from the cohort of 32,826 women, nested in the Nurses' Health Study, who provided blood samples in 1989 to 1990. Cases and controls were individually matched on year of birth, time period of and indication(s) for endoscopy, and date of blood draw.**

Results: **High concentrations of C-peptide, an indicator of insulin secretion, were statistically significantly associated with risk of distal colorectal adenoma [multivariable relative risk (MVRR) top versus bottom quartile, 1.63; 95% confidence interval (95% CI), 1.01-2.66; P = 0.01], even after including body mass index and physical activity in the statistical model. Fasting IGF binding protein-1 (IGFBP-1) concentrations did not show any clear association with risk for adenoma (MVRR top versus bottom quartile, 1.08; 95% CI, 0.56-2.07). These associations did not differ significantly by size/stage of adenoma. Glycosylated hemoglobin (HbA1c) was associated with a nonstatistically significant increased risk of colorectal adenoma (MVRR top versus bottom quartile, 1.47; 95% CI, 0.89-2.44).**

Conclusions: **High HbA1c and low IGFBP-1 were not clearly associated with increased risk of distal colorectal adenoma. However, our current results and previous associations between C-peptide and colorectal cancer suggest that hyperinsulinemia may play a role throughout the development of colorectal neoplasia.**

Introduction

Determinants of hyperinsulinemia, such as a sedentary lifestyle, being overweight/obese, and consuming a diet high in processed grains and refined sugar, have been associated with increased risk for colorectal carcinogenesis (1). Direct markers of hyperinsulinemia (e.g., insulin and C-peptide) are also fairly consistently associated with colorectal cancer (2-7). At physiologic concentrations, high insulin levels may lower some insulin-like growth factor binding proteins (IGFBP), such as IGFBP-1, which may lead to higher bioavailable IGF-I at the tissue level (8); high levels of IGF-I have been associated with neoplastic progression and reduced apoptosis (8). Studies have reported that high levels of circulating IGF-I are associated with an increased risk of both colorectal cancer (2, 3, 9-12) and ''high-risk'' adenoma (those most likely to progress to cancer; ref. 9). Lifestyle factors associated with hyperinsulinemia, such as overweight and physical inactivity, have also been implicated in the adenoma-carcinoma sequence as promoters of adenoma to carcinoma rather than initiators of neoplasia (13-15). The specific associations between direct measures of hyperinsulinemia, such as C-peptide and IGFBP-1, and incident adenoma have not been as well studied. In addition, whether hyperglycemia or hyperinsulinemia is more directly related to colorectal carcinogenesis remains unresolved. Glycosylated hemoglobin (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 (16). Therefore, we examined prospectively the association between plasma levels of C-peptide, IGFBP-1, and HbA1c, a marker of hyperglycemic control, and subsequent risk of distal colorectal adenoma among women in the Nurses' Health Study.

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Materials and Methods

Study Population. The Nurses' Health Study began in 1976 when 121,700 female nurses, ages 30 to 55 years, were enrolled and completed a questionnaire about lifestyle and medical history. Since then, these women have been mailed follow-up self-administered questionnaires biennially to update information on lifestyle and medical history as well as diet (every 2-4 years). In 1989, a subset of these women was asked to provide a blood sample; 32,826 women, ages 43 to 69 years, returned a blood sample at this time via overnight courier using a kit that was provided (including a cold pack). Ninety-seven percent of the samples were received within 26 hours of being drawn. Most samples (~62% in this study population) were drawn while the women were in a fasting state (≥8 hours since their last meal). All samples were chilled and 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 elsewhere (17).

Identification of Cases and Controls. Cases and controls were selected from the women who provided a blood sample in 1989 to 1990. To avoid selection bias that could arise due to only a proportion of women having an endoscopic procedure, both cases and controls must have had a sigmoidoscopy or colonoscopy after providing a blood sample. Cases and controls also could not have been previously diagnosed with cancer (other than nonmelanoma skin cancer) or adenoma. Newly diagnosed polyps during follow-up (through 1998) were reported by the nurse on the biennial questionnaire and confirmed by reviewing medical records. Using histopathology records, polyps were classified as adenomatous, hyperplastic, or other nonadenomatous, and information was obtained regarding the location and size. Only adenomatous polyps were included in the case definition. Because a substantial proportion of women in this cohort underwent sigmoidoscopy, we may have had limited accuracy in determining the presence of polyps in the proximal colon. Therefore, we limited our analysis to only adenomas in the distal colon and rectum. We excluded any women who reported a diagnosis of diabetes on their biennial questionnaire before 1990; in addition, we restricted the analysis of IGFBP-1 to women who were fasting at the time of blood draw (18, 19).

Each case that occurred during follow-up was individually matched to one control on year of birth, month of blood draw, fasting status (<8 hours since last meal or ≥8 hours since last meal), time period of endoscopy (within 2 years), and routine screening, gastrointestinal symptoms, or family history of colorectal cancer as indication(s) for endoscopy.

Plasma C-Peptide and IGFBP-1 Assays. Plasma C-peptide and IGFBP-1 were assayed using ELISAs with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory of Dr. Michael Pollak. We determined levels of HbA1c by turbidometric immunoinhibition in RBC using the Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN) in the laboratory of Dr. Nader Rifai.

Samples from the matched sets were assayed together, with their order random, along with randomly inserted masked quality control samples. All laboratory personnel were blinded with respect to case or control status. The C-peptide and HbA1c samples were assayed in two batches (C-peptide batch 1: cases and controls from baseline through May 1996; C-peptide batch 2: June 1996 to May 1998; HbA1c batch 1: baseline to May 1994; HbA1c batch 2: June 1994-1998). The mean intra-assay coefficient of variation from the quality control samples was <10.2% for C-peptide, <7.3% for IGFBP-1, and <2.2% for HbA1c.

Assessment of Other Factors. Mean values for diet, anthropometry and lifestyle factors were calculated using the information provided on the biennial questionnaires, including body mass index (BMI; weight in kilograms/square of height in meters), physical activity (MET-hours/wk), aspirin use (duration of use in years), pack-years of cigarette smoking, alcohol intake (g/d), family history of colon or rectal cancer, and use of hormone replacement therapy by women who were postmenopausal in 1990. For BMI, physical activity, and alcohol intake, we averaged the responses on the four questionnaires between 1980 and 1990.

Statistical Analysis. We used log-transformed C-peptide values to improve normality and then compared the cases and controls with respect to geometric means of C-peptide and fasting IGFBP-1 as well as various other factors using paired *t* tests. We used Wilcoxon signed rank tests for nonbiomarker continuous variables and χ^2 tests for categorical variables. Category cut points were generated based on distributions among the controls only, and the cut points were batch specific for C-peptide and HbA1c. Partial Spearman correlation coefficients were used to examine the relationships among the various biomarkers, BMI, and alcohol.

We computed odds ratios and 95% confidence intervals (95% CI) using conditional logistic regression. We used the median of the categories in the controls as a continuous variable in models to test for linear trend across the quantiles. We also examined the association between C-peptide, IGFBP-1, and HbA1c and adenoma stage (early-stage adenoma were small and tubular, and advanced adenoma were ≥1 cm, villous, or tubulovillous).

Results

Table 1 compares several lifestyle and dietary factors of the 380 distal colorectal adenoma cases that occurred in nondiabetic women between 1989 to 1990 and 1998 and their 380 nondiabetic matched controls. Compared with controls, adenoma cases had higher C-peptide levels, a slightly higher percentage of HbA1c, and were less likely to report using postmenopausal hormones and more likely to have a family history of colon or rectal cancer.

Table 1. Characteristics of distal colorectal adenoma cases and matched controls nested in the Nurses' Health Study, 1989-1998

	Cases (n = 380)	Controls (n = 380)	P*
Plasma C-Peptide (ng/mL), geometric mean (25-75%)	1.91 (1.19-2.80)	1.71 (1.12-2.49)	0.006
Plasma IGFBP-1† (ng/mL), geometric mean (25-75%)	22.1 (14.4-40.1)	23.9 (14.9-43.8)	0.25
HbA1c‡ (mean % ± SD)	5.41 ± 0.30	5.38 ± 0.3	0.06
Age at blood draw (mean ± SD)	58.2 ± 6.6	58.2 ± 6.5	0.68§
BMI (kg/m^2) (mean ± SD)	24.9 ± 3.7	24.7 ± 3.8	0.54
Physical activity (average 1980-1990; mean MET-h/wk ± SD)	14.5 ± 18.5	15.7 ± 18.0	0.34
Alcohol (average intake 1980-1990; mean g/d ± SD)	7.3 ± 10.5	6.8 ± 9.7	0.57
Cigarette smoking‖ (mean pack-years ± SD)	23.9 ± 18.0	21.5 ± 18.6	0.71
Postmenopausal hormone use in 1990 (%)	32.6	39.5	0.05
Endoscopy before 1990 (%)	31.6	33.7	0.32
Family history of colorectal cancer (%)	24.0	17.6	0.03
Multivitamin use in 1990 (%)	37.1	39.7	0.46
Regular aspirin use ≥10 y before 1990 (%)	6.8	9.0	0.28

*P from paired t test for geometric means (plasma biomarkers), Wilcoxon signed rank for all other continuous variables, and χ^2 test for categorical variables.
†Among fasting women (cases, n = 234; controls, n = 234).
‡HbA1c available for 371 cases and 371 controls.
§Matching factor.
‖Among cigarette smokers.

Table 2. Partial Spearman correlation coefficients between C-peptide, IGFBP-1, HbA1c, BMI, alcohol, and physical activity among adenoma controls (n = 371) nested in Nurses' Health Study, 1989-1998

	C-peptide	IGFBP-1*	HbA1c	BMI	Alcohol	Physical activity
C-peptide	1.00					
IGFBP-1*	−0.55 †	1.00				
HbA1c	0.17 ‡	−0.14 §	1.00			
BMI	0.44 †	−0.45 †	0.09	1.00		
Alcohol	−0.13 §	−0.02	−0.06	−0.14 ‡	1.00	
Physical activity	−0.05	0.05	−0.09	−0.03	0.13 §	1.00

NOTE: Adjusted for age, assay batch, and time since last meal.
*Correlations with IGFBP-1 only among fasting controls (n = 234).
†$P < 0.001$.
‡$P < 0.01$.
§$P < 0.05$.

Table 2 shows the correlation between the various blood markers and other factors. C-peptide levels were inversely correlated with IGFBP-1. C-peptide was positively and IGFBP-1 was inversely correlated with BMI, and alcohol intake was weakly and inversely correlated with both C-peptide and BMI. Physical activity was not correlated with C-peptide, IGFBP-1, and HbA1c.

C-Peptide, IGFBP-1, HbA1c, and Risk of Distal Colorectal Adenoma. C-peptide was directly associated with risk for colorectal adenoma in a univariate model, conditioned on the matching factors. Adjusting for several potential confounding variables, including BMI and physical activity, had little effect, and in fact, the top versus bottom quartile relative risk (RR) became slightly stronger [multivariable RR (MVRR), 1.63; 95% CI, 1.01-2.66; P_{trend} = 0.01; Table 3]. When we restricted the analysis to adenoma in the sigmoid colon, the point estimates and overall trend were similar to the main results (MVRR, 1.64; 95% CI, 0.93-2.88; P_{trend} = 0.01). The association in the rectum also suggested a direct association, but the estimates were imprecise due to the limited number of cases (n = 74 cases; MVRR, 2.03; 95% CI, 0.62-6.67).

Large adenoma (≥1 cm) and those with a villous component may be more likely than small adenoma to develop into carcinomas. Thus, we evaluated whether risk varied by size and type of adenoma. The associations with advanced adenoma were slightly weaker than the main results (MVRR Q4 versus Q1, 1.46; 95% CI, 0.64-3.32). We found that the association was mainly for tubular adenoma (tubular adenoma, n = 283 cases: MVRR for Q4 versus Q1, 1.78, 95% CI, 1.02-3.11; P_{trend} = 0.01; villous adenoma, n = 96 cases: MVRR for Q4 versus Q1, 1.00, 95% CI, 0.30-3.31; P_{trend} = 0.89).

We found little evidence for an association between fasting plasma levels of IGFBP-1 and risk for adenoma (MVRR, 1.08; 95% CI, 0.56-2.07; P_{trend} = 0.63; Table 3). The association for adenoma in the sigmoid colon only was essentially the same as for all distal colorectal adenoma (MVRR, 1.08, 95% CI, 0.52-2.26; P_{trend} = 0.62). For advanced adenoma, the results were more suggestive of an inverse association in the univariate analysis but not in the multivariable analysis (MVRR, 0.87; 95% CI, 0.31-2.45; P_{trend} = 0.49).

HbA1c was suggestively but nonstatistically significantly positively associated with risk of distal colorectal adenoma (Table 3). When we restricted the analysis to the sigmoid colon, the RRs were slightly attenuated (MVRR, 1.31; 95% CI, 0.74-2.34; P_{trend} = 0.46). The results for HbA1c levels and risk for high-risk adenoma (large or with a villous component) were essentially the same as when all adenoma were considered together (early-stage adenoma: 191 cases and 191 controls; MVRR, 1.38; 95% CI, 0.70-2.75). In a model that included both HbA1c and C-peptide, the RR for HbA1c was nonstatistically significantly elevated (MVRR, 1.39; 95% CI, 0.84-2.31; P_{trend} = 0.24); however, the RR for the top versus bottom quartile of C-peptide remained statistically significantly elevated (C-peptide MVRR, 1.65; 95% CI, 1.00-2.72; P_{trend} = 0.02).

BMI, Physical Activity, Alcohol, and C-Peptide. The association between C-peptide and adenoma did not vary by BMI or physical activity (BMI, $P_{interaction}$ = 0.93; physical activity, $P_{interaction}$ = 0.73). When we cross-classified alcohol intake and C-peptide levels, the highest RR was observed among those in both the highest alcohol tertile and the highest C-peptide tertile ($P_{interaction}$ = 0.27). The RRs (95% CI) for tertiles of C-peptide among those with lowest alcohol intake (in tertiles) were 1.00 (reference group: 24 cases and 38 controls), 1.82 (0.87-3.81), and 1.82 (0.89-3.73). The RRs for C-peptide among women in the top tertile of alcohol intake were 1.94 (0.97-3.88), 0.90 (0.43-1.87), and 3.35 (1.57-7.14). There were 57 cases and 33 controls in the top tertile of both C-peptide and alcohol. When we restricted this analysis to sigmoid colon adenoma cases only (306 cases/306 controls), the estimates were imprecise, but the suggestion of a stronger association of C-peptide and risk of adenoma among those in the highest tertile of alcohol intake remained (RR for those in top tertile of both alcohol and C-peptide versus those low in both, 4.42, 95% CI, 1.83-10.7; $P_{interaction}$ = 0.14).

HbA1c, C-Peptide, and Risk of Distal Colorectal Adenoma. To further evaluate the association between HbA1c and C-peptide, we cross-classified HbA1c and C-peptide quartiles. Although the test for statistical interaction between the two variables was not significant, ($P_{interaction}$ = 0.86), women who were in the top quartiles of HbA1c and C-peptide had a borderline significantly elevated RR compared with women who were in the lowest quartile of both (MVRR, 2.33; 95% CI, 0.96-5.63; data not shown). Women in the highest quartile of C-peptide had a consistently higher RR regardless of which quartile of HbA1c they were in, and similarly, being in the highest quartile of HbA1c generally resulted in a higher RR regardless of C-peptide quartile.

Discussion

In this prospective study, we observed a positive association between C-peptide and risk of distal colorectal adenoma, which was independent of physical activity and BMI. This association was similar for the sigmoid colon and rectum when evaluated separately (although we had limited numbers of rectal adenoma cases) and slightly stronger for tubular adenoma. The association was most marked when comparing the top to bottom quintiles of the various plasma markers.

We observed no appreciable association between concentrations of IGFBP-1 or HbA1c, a marker of plasma glucose levels, and risk for distal colorectal adenoma. However, women who were high in both HbA1c and C-peptide had a borderline significantly elevated RR compared with those low

in both. Circulating IGFBP-1 and IGF-1 levels may not directly reflect tissue levels of these compounds (8). Therefore, our null finding for circulating IGFBP-1 does not exclude the possibility that increased insulin leads to lower IGFBP-1 levels and increased bioavailable IGF-I at the level of the colon tissue.

Although lifestyle factors associated with high insulin secretion have been associated with adenoma progression and colorectal cancer, few studies have addressed the association between more direct measures of hyperinsulinemia (measured by C-peptide and IGFBP-1) and risk of colorectal adenoma. The associations we observed were largely independent of body size and physical activity, strongly suggesting that biomarkers of insulin secretion, such as C-peptide, are not solely surrogate markers for lifestyle or anthropometric characteristics. Rather, insulin secretion itself is likely an etiologic factor in colorectal carcinogenesis.

Our results support the previously observed positive association between low physical activity and obesity, both associated with hyperinsulinemia, and an increased risk of large or advanced adenomas (13, 14). However, our results did not vary significantly for advanced adenoma versus early adenoma.

One main hypothesis for the role of insulin in colorectal neoplasia is based on the observation that short-term increases in insulin reduce IGFBP-1, a major binding protein of IGF-I (20, 21). Lower IGFBP-1 levels may then result in more bioavailable IGF-I, which has been associated with an increased risk of colorectal cancer (9, 12). Insulin is also involved in regulating IGF-I levels by regulating the concentration of hepatic growth hormone receptors (22, 23). Animal studies suggest that insulin may also have direct mitogenic effects (24), influence tumor promotion (25, 26), or exert a direct effect via insulin receptors in the colon (27). In addition, insulin may be related to risk for adenoma independent of IGFBPs via its role in the farnesylation of ras, a proto-oncogene. Higher insulin levels result in larger pools of farnesylated ras protein, which may contribute to carcinogenesis (28, 29).

We observed some variation in the association between C-peptide and adenoma when we stratified by alcohol intake. The highest risk for colon adenomas occurred among those in the highest categories of both alcohol and C-peptide. However, these factors could be acting as independent risk factors. Alcohol intake has been associated with an increased risk of both adenoma and cancer (30, 31). Furthermore, alcohol consumption has been associated with reduced C-peptide and improved insulin sensitivity, although whether the reduction in C-peptide levels is substantial enough to appreciably influence risk is unclear (32-34). In a recent study of C-peptide and colorectal cancer, Ma et al. reported a significant interaction between C-peptide and alcohol that is consistent with our results: individuals who were high alcohol consumers and had high C-peptide levels were at the highest risk for colorectal cancer when compared with low alcohol consumers who were also in the lowest C-peptide category (3). In contrast, in a previous study evaluating colorectal cancer and C-peptide among participants in the Nurses' Health Study (2), we found no interaction between C-peptide and alcohol. Therefore, further studies are required to clarify the relationship between alcohol and C-peptide and the risk of colorectal neoplasia.

We observed a suggestively positive association between plasma HbA1c and risk of colorectal adenoma; however, this association was moderate and not statistically significant. HbA1c levels are determined by diet, degree of insulin resistance, and pancreatic β-cell activity. The reference range of HbA1c is 4.3% to 5.8% (35). Preliminary results from a recent

Table 3. Relationship between plasma levels of C-peptide and risk of distal colorectal adenoma among 380 cases and 380 controls nested in the Nurses' Health Study, 1989-1998

	Quartile				P_{trend}
	1	2	3	4	
C-Peptide					
Median (ng/mL)	0.9	1.4	2.0	3.4	
Distal colon and rectum					
Cases/controls	84/94	75/96	94/94	127/96	
RR* (95% CI)	1.00	0.88 (0.57-1.35)	1.14 (0.74-1.76)	1.53 (1.01-2.32)	0.007
MVRR† (95% CI)	1.00	0.91 (0.58-1.43)	1.12 (0.70-1.81)	1.63 (1.01-2.66)	0.01
Advanced adenoma					
Cases/controls	37/38	37/37	39/51	55/42	
RR* (95% CI)	1.00	1.02 (0.55-1.91)	0.77 (0.40 -1.49)	1.40 (0.73-2.69)	0.22
MVRR† (95% CI)	1.00	1.12 (0.56-2.24)	0.69 (0.33-1.45)	1.46 (0.64-3.32)	0.29
IGFBP-1‡					
Median (ng/mL)	5.9	16.5	31.6	52.1	
Distal colon and /Rectum					
Cases/controls	54/56	82/58	50/63	48/57	
RR* (95% CI)	1.00	1.41 (0.85-2.33)	0.82 (0.47-1.42)	0.86 (0.49-1.50)	0.18
MVRR† (95% CI)	1.00	1.56 (0.90-2.70)	0.97 (0.51-1.82)	1.08 (0.56-2.07)	0.63
Advanced adenoma					
Cases/controls	27/29	48/24	19/33	18/26	
RR* (95% CI)	1.00	2.24 (1.07-4.70)	0.53 (0.23-1.23)	0.62 (0.27-1.43)	0.06
MVRR† (95% CI)	1.00	2.76 (1.20-6.31)	0.71 (0.25-1.99)	0.87 (0.31-2.45)	0.49
HbA1c					
Distal colon and rectum					
Cases/controls	71/85	85/76	96/110	119/100	
RR*	1.00	1.39 (0.87-2.21)	1.11 (0.71-1.72)	1.56 (0.98-2.49)	0.07
RR† (95% CI)	1.00	1.49 (0.91-2.43)	1.18 (0.74-1.89)	1.47 (0.89-2.44)	0.16
Advanced adenoma					
Cases/controls	32/32	38/32	40/54	54/46	
RR*	1.00	1.18 (0.56-2.50)	0.79 (0.40-1.56)	1.25 (0.60-2.60)	0.58
RR† (95% CI)	1.00	1.43 (0.62 -1.84)	0.87 (0.41-1.84)	1.22 (0.54-2.77)	0.80

*RR from conditional logistic regression.

†RR from conditional logistic regression adjusted for: BMI, physical activity, pack-years smoked, and alcohol intake as continuous variables, family history of colorectal cancer, aspirin use (<10 and ≥10 years), and menopausal status, and use of hormone replacement therapy.

‡IGFBP-1 results only among 234 fasting cases and 234 fasting controls (≥8 hours since last meal).

study support a linear association between HbA1c levels and colorectal cancer risk that was independent of known diabetes (36), but that analysis was limited to 67 cases.

Diets high in processed starch and sugars or high glycemic load lead to high glucose levels that are counteracted by increased pancreatic insulin secretion. In the long term, elevated insulin levels due to insulin resistance and diet eventually lead to β cell depletion and a reduced ability to secrete adequate insulin. Therefore, although insulin resistance may underlie both, elevated C-peptide levels reflect hyperinsulinemia, whereas elevated HbA1c reflects β cell depletion and hyperglycemia. The association we observed between insulin and adenoma was stronger than the association we observed between HbA1c and adenoma. Moreover, when mutually adjusting for both factors, C-peptide remained significantly associated with adenoma risk; these results support the hypothesis that hyperinsulinemia may play a more direct role than hyperglycemia in colorectal carcinogenesis.

Although HbA1c has been generally considered the gold standard for assessing glycemic control, it may have several limitations in the study of insulin, glucose, and colorectal neoplasia. In one study, HbA1c did not reflect a high glycemic index diet given to healthy individuals over 6 weeks, whereas serum fructosamine levels, a proposed alternate biomarker for glycemic control, were increased (37). Furthermore, because HbA1c is not glycated rapidly (38), it may reflect longer-term glycemia rather than post-prandial insulin surges. The previous direct associations reported for nonfasting C-peptide or post-prandial insulin (3-5) and colorectal cancer and weaker (or inverse) associations between fasting insulin and colorectal cancer (5, 7, 39) all support the hypothesis that hyperinsulinemia, especially in the context of a sedentary lifestyle and obesity, may be a major factor in increasing risk of colorectal neoplasia.

One of the strengths of this study is that we had prospectively collected blood, which reduces the possibility of preclinical disease exerting an effect on our results as well as reducing the possibility of misclassification of the exposure variables. We had extensive information on other potential confounding factors and were able to evaluate whether they modified or obscured the association between our main variables of interest and the outcome. We were also able to reduce possible bias due to differential screening behaviors because all cases and controls were screened within the same 2-year time period. Finally, we had detailed information on adenoma size, which allowed us to evaluate in detail where in the course of the adenoma-carcinoma sequence these factors could be acting.

One limitation of our study is that we had only one blood measure. Multiple blood samples over time would improve our ability to evaluate associations between long-term circulating levels of these exposures and risk. However, C-peptide has previously been shown to be relatively stable; 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. 3). Despite compelling evidence that risk factors may vary in their association with cancers within the large bowel by anatomic location, we had limited power to evaluate associations separately for adenoma in the distal colon and rectum. Matching on screening allowed us to better control for unknown but screening-related factors. However, whether the associations we observed are similar for proximal colon adenoma requires further study. Finally, our analysis did not address the possible association between insulin-related factors and other types of polyps (such as serrated polyps), which are proposed to progress in a pathway different from the traditional adenoma-carcinoma sequence. Future studies should evaluate in detail whether these associations are similar for other types of precursor lesions.

In conclusion, we observed a moderate positive association between elevated plasma C-peptide levels and colorectal adenoma. We did not, however, observe any significant associations with plasma levels of IGFBP-1 or HbA1c and adenoma risk. Combined with our previous findings regarding C-peptide and IGFBP-1 and risk for colorectal cancer (2), our results suggest that insulin is involved in both the early and later stages of colon carcinogenesis. In addition to having a positive influence on a myriad of other chronic diseases, increasing physical activity, reducing overweight and obesity, and making dietary changes may lead to reductions in the incidence of colorectal neoplasia due to effects on insulin secretion.

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