

# Association of Markers of Insulin and Glucose Control with Subsequent Colorectal Cancer Risk<sup>1</sup>

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## Abstract

We evaluated the association of plasma insulin and other markers of insulin and glucose control with subsequent colorectal cancer. Incident colon ( $n = 132$ ) and rectal ( $n = 41$ ) cancer cases and matched controls ( $n = 346$ ) were identified between baseline in 1989 and 2000 among participants in a community-based cohort in Washington County, Maryland. Circulating markers of insulin and glucose control were measured in baseline blood samples. Body mass index (BMI) and use of medications to treat diabetes mellitus were self-reported at baseline. Conditional logistic regression was used to estimate matched odds ratios (ORs). Compared with the lowest fourth, participants with insulin concentrations in the highest fourth were not at an increased risk of colorectal cancer [OR, 0.78; 95% confidence interval (CI), 0.45–1.35;  $P_{\text{trend}} = 0.24$ ]. Similarly, no associations were observed for the ratio of total cholesterol:HDL-cholesterol, triglycerides, and insulin-like growth factor binding protein 1. However, those in the highest fourth of glycosylated hemoglobin (HbA<sub>1c</sub>) level had a slightly increased risk of colorectal cancer (OR, 1.57; 95% CI, 0.94–2.60;  $P_{\text{trend}} = 0.02$ ). The OR of colorectal cancer was 1.70 (95% CI, 1.01–2.86;  $P_{\text{trend}} = 0.08$ ) comparing BMI  $\geq 30$  kg/m<sup>2</sup> to  $< 25$  kg/m<sup>2</sup>. The OR of colorectal cancer was 2.43 (95% CI, 1.10–5.38) for the use of medications to treat diabetes. The associations of higher HbA<sub>1c</sub>, higher BMI, and the use of medications to treat diabetes, with colorectal cancer lend support to the hypothesis that perturbations in insulin and glucose

control may influence colorectal carcinogenesis. It is possible that HbA<sub>1c</sub>, BMI, and the use of medications to treat diabetes, as a surrogate for protracted or severe type 2 diabetes mellitus, may have been better time-averaged indicators of hyperinsulinemia and hyperglycemia than the plasma markers that were measured once prediagnostically in a nonfasting population.

## Introduction

McKeown-Eyssen (1) and Giovannucci (2) have hypothesized that insulin and glucose might be risk factors for colorectal carcinogenesis. This hypothesis grew from trying to explain the link between colorectal cancer, diets high in fat and low in vegetables, and obesity (2–4) and from the observations that insulin promotes the growth of colon cells *in vitro* and colon tumors *in vivo* (5, 6). Hyperinsulinemia and hyperglycemia result from the dysregulation of the interrelationship between insulin secretion and insulin action, which can eventually lead to the development of type 2 diabetes mellitus (7). Hyperinsulinemia and hyperglycemia are key components of the metabolic syndrome that includes increased serum triglycerides and lower serum HDL<sup>4</sup>-cholesterol levels (8–10).

Studies over the past decade support the observation that higher circulating concentrations of insulin, glucose, or their markers are associated with an increased risk of colorectal cancer or precursor adenomatous polyps (11–19). Two of the adenoma studies measured plasma levels concurrently with disease, making it difficult to determine the causal direction of the association (12, 13). Typically, studies have measured only one marker or have not simultaneously considered multiple markers. It is unclear whether the positive association for any given marker would be explained by another correlated marker (14).

To evaluate the association of markers of insulin and glucose control with incident colorectal cancer, we conducted a case-control study nested in the CLUE II cohort of Washington County, Maryland, which was established in 1989. We examined plasma insulin, the ratio of total cholesterol:HDL-cholesterol, triglycerides, and IGFBP-1, and RBC-HbA<sub>1c</sub> as circulating markers. We also examined BMI and the use of medications to treat diabetes mellitus, as a surrogate for protracted or severe type 2 diabetes, as possible long-term indicators of perturbed insulin and glucose control.

## Materials and Methods

**Study Population.** Colorectal cancer cases and controls were identified among members of the prospective CLUE II cohort.

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<sup>4</sup> The abbreviations used are: HDL, high-density lipoprotein; IGF, insulin-like growth factor; IGFBP-1, IGF binding protein 1; BMI, body mass index; OR, odds ratio; CI, confidence interval; HbA<sub>1c</sub>, glycosylated hemoglobin.

The CLUE II cohort was established in 1989 to investigate potential blood precursors of cancer. The cohort consisted of 22,888 adult (18+ years old) residents of Washington County, Maryland in 1989, 98.3% were white, reflecting the population of this county, and 59% were female. Participants are sent follow-up questionnaires and newsletters periodically with forwarding addresses requested from the United States Postal Service. Deaths are ascertained by searches of Maryland death certificates, reviews of obituaries of the local newspaper, and reports by next-of-kin. Loss-to-follow up is less than 5% among cohort members who were 45+ years old at baseline; the age range of the majority of cases at baseline and the pool of cohort members eligible to be selected as age-matched controls.

Participants provided a blood sample and completed a brief medical history at baseline. Blood was drawn into 20-ml heparinized Vacutainers (Becton-Dickinson, Rutherford, NJ), kept at 4°C until the plasma was separated, usually within 2–6 h, and was divided into aliquots of plasma, buffy coat, and RBCs. Plasma samples were kept frozen at –70°C until aliquotted, shipped to the laboratory, and assayed.

**Selection of Colorectal Cancer Cases and Controls.** Cases were identified through linkage with the Washington County Cancer Registry and, since 1992, with the Maryland Cancer Registry. The Maryland Cancer Registry is certified by the North American Association of Central Cancer Registries as being more than 95% complete.<sup>5</sup> Compared with the Maryland Cancer Registry, the Washington County Cancer Registry captured 98% of the colorectal cancer cases diagnosed in Washington County residents in 1998. We ascertained 173 men and women in whom colorectal cancers were diagnosed after the date of blood draw through December 2000 and who did not have a prior cancer diagnosis (except for nonmelanoma skin cancer or cervix *in situ*). Of these, 132 were cancers of the colon (ICD 9 153) and 41 were cancers of the rectum (ICD 9 154). Ninety-two % of cases were confirmed by pathology report. Information on the stage of diagnosis was available on 133 of the 173 cases. The majority was diagnosed at Stage 2 ( $n = 44$ ) or 3 ( $n = 38$ ), followed by Stage 1 ( $n = 29$ ) and Stage 4 ( $n = 22$ ). Thirty-three (80.5%) of the rectal cancer cases were adenocarcinomas, 7.3% ( $n = 3$ ) were squamous, and the remainder had an unknown histology.

Controls were selected from among CLUE II participants who did not have a diagnosis of cancer through December 2000 and who were known to be alive at the time the case was diagnosed. Two controls were matched to each case on potentially confounding factors: age ( $\pm 1$  year), sex, race, date of blood draw ( $\pm 2$  weeks), and time since last meal (0–1 h, 2–3 h, 4–5 h, 6–7 h, and  $\geq 8$  h).

**Laboratory Assays.** We measured circulating levels of markers of hyperinsulinemia and hyperglycemia in archived blood. Plasma insulin concentration was determined using an ultrasensitive ELISA kit (ALPCO Diagnostics, Windham, NH). Plasma HDL-cholesterol and triglycerides concentrations were measured simultaneously on the Hitachi 911 analyzer using reagents and calibrations from Roche Diagnostics (Indianapolis, IN). Plasma IGFBP-1 was determined with well-coated ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX). HbA<sub>1c</sub> was determined by turbidometric immunoinhibition in RBCs using the Hitachi 911 analyzer (Boehringer Mannheim, Indianapolis, IN). Total cholesterol was previously measured enzymatically (20). Insulin, HDL-cholesterol, triglycerides, and

HbA<sub>1c</sub> assays were run in the laboratory (Harvard) of Dr. Rifai. The IGFBP-1 assay was run in the laboratory of Dr. Pollak (McGill). For the plasma markers, 27 quality control samples (equal to 5% of total sample) aliquotted from pooled plasma were arranged in triplets among the cases and controls. Quality control samples ( $n = 27$ ) for the RBC assay were derived from nine separate individuals, which were not pooled. The mean intrapair coefficients of variation for the quality control samples were: insulin, 5.1%; HDL-cholesterol, 12.3%; triglycerides, 6.0%; IGFBP-1, 6.1%; and HbA<sub>1c</sub>, 0.66%. The coefficients of variation were similar to those obtained by the laboratories for their internal controls. Measurements were unavailable for some participants for insulin ( $n = 1$ ), HDL-cholesterol ( $n = 12$ ), triglycerides ( $n = 9$ ), and IGFBP-1 ( $n = 7$ ) because of insufficient plasma volume. Cholesterol levels from an earlier analysis were also missing for two participants.

**Assessment of Other Factors.** Self-reported weight, height, use of medications for diabetes mellitus, attained education, and cigarette smoking status were recorded for each participant at baseline. BMI was calculated as weight in kilograms divided by height in meters squared. Participants who reported at baseline currently taking medications to treat diabetes mellitus were classified as having diabetes. BMI and use of medications to treat diabetes may also be correlates of hyperinsulinemia. Education was classified as less than high school or high school graduate and beyond. Participants were classified as ever smokers if they reported ever smoking either cigarettes or cigars.

**Statistical Analysis.** Matched sets containing the case and at least one control with information for any given marker were included in the analysis. Mean circulating concentrations of the markers and other continuous variables were compared between cases and controls by ANOVA to take into account the matching. We used McNemar's test to compare distributions of categorical data between matched cases and controls. To assess the correlation between any two of the markers, we used sets with complete data for the case and at least 1 control (167 cases, 321 controls), and we calculated partial Pearson correlation coefficients adjusting for the other circulating markers.

Matched ORs for colorectal cancer, calculated as an estimate of the relative risk, and the corresponding 95% CI were estimated using conditional logistic regression. The circulating markers were entered separately and together into the model using a series of indicator variables. Quartile cut points for the distribution of concentration among controls were used. For insulin, triglycerides, and IGFBP-1, cut points were also defined within strata of time since last meal. BMIs were entered into the model as indicator variables using standard guidelines for overweight (25–29.9 kg/m<sup>2</sup>) and obese ( $\geq 30$  kg/m<sup>2</sup>) compared with normal weight ( $< 25$  kg/m<sup>2</sup>; Ref. 3). The use of medications to treat diabetes was entered as a binary variable. To examine these associations by site within the large bowel and stage at diagnosis, we broke the matched sets and included all controls in logistic regression models along with the matching factors to preserve power. We tested for trend by entering a single ordinal variable, the coefficient for which was evaluated by the Wald test (21). The analysis for the circulating markers was repeated, stratified by BMI ( $< 25$  kg/m<sup>2</sup>,  $\geq 25$  kg/m<sup>2</sup>) using logistic regression adjusting for the matching factors. Also, the analysis for all of the markers was repeated stratified by sex using conditional logistic regression. All of the analyses were performed using STATA version 6.0 (Stata Corporation, College Station, TX) or SAS version 8.1 (Cary, NC). Reported *P*s are for two-sided statistical tests.

<sup>5</sup> Internet address: <http://www.naacr.org/Certification/1999Certification.html>.

Table 1 Distribution of markers<sup>a</sup> of insulin and glucose control in colorectal cases and matched controls nested in the CLUE II cohort of Washington County, Maryland, 1989

Marker	Colorectal cancer cases ( <i>n</i> = 173)	Controls ( <i>n</i> = 346)	<i>P</i> <sup>b</sup>
Insulin, <sup>c</sup> milliunits/liter (mean ± SD)	20.8 ± 23.3	23.3 ± 30.0	0.24
HDL-cholesterol, mg/dl (mean ± SD)	45.6 ± 13.6	44.6 ± 13.7	0.44
Total cholesterol, mg/dl (mean ± SD)	216.0 ± 41.4	218.4 ± 38.9	0.45
Ratio of total cholesterol:HDL-cholesterol (mean ± SD)	5.1 ± 1.8	5.5 ± 4.1	0.10
Triglycerides, <sup>c</sup> mg/dl (mean ± SD)	168.7 ± 98.8	173.5 ± 90.8	0.60
IGFBP-1, <sup>c</sup> ng/ml (mean ± SD)	26.3 ± 32.2	26.9 ± 32.4	0.84
HbA <sub>1c</sub> , % (mean ± SD)	5.9 ± 1.1	5.8 ± 0.9	0.05
BMI, kg/m <sup>2</sup> (%)			
<25	39.9	44.2	
25–29.9	38.2	41.3	0.10
≥30	22.0	14.5	
Use of medications to treat diabetes (%)	8.1	3.5	0.04

<sup>a</sup> Measurements were unavailable for some participants because of insufficient plasma volume. Included in the analysis were sets for which the case and at least one control had information available. The counts are insulin 172 cases, 344 controls; HDL-cholesterol 170 cases, 331 controls; total cholesterol 173 cases, 344 controls; ratio of cholesterol:HDL-cholesterol 170 cases and 329 controls; triglycerides 170 cases, 334 controls; and IGF1P-1 169 cases, 335 controls.

<sup>b</sup> *P* reported for the test of no difference in means (ANOVA) or proportions (McNemar's test) between cases and controls matched on age, sex, race, time since last meal, and date of blood draw.

<sup>c</sup> 79.8% of participants reported last meal <6 h before blood draw (*i.e.*, nonfasting samples).

## Results

Cases and controls did not differ on the matching factors, education attained (*P* = 0.30), or cigarette smoking status (*P* = 0.19). Plasma insulin, triglycerides, and IGF1P-1 concentrations varied by time since last meal category, but their concentrations did not differ significantly between cases and controls, who were matched on time since last meal (*P* = 0.24, 0.60, and 0.84, respectively). Mean concentration of the ratio of total cholesterol:HDL-cholesterol in cases also did not differ significantly from controls, but cases did have a slightly higher mean HbA<sub>1c</sub> level compared with controls (Table 1). The proportion of cases that was obese (BMI ≥30 kg/m<sup>2</sup>) was slightly greater than the proportion of controls. Cases were also more likely to report using medications to treat diabetes at baseline (Table 1).

Because insulin and glucose may influence colorectal cancer through shared and also independent pathways, we assessed the correlation of the markers pairwise controlling for the other markers among the cases and controls combined. Similar results were obtained using only the controls (not shown). Insulin concentration was weakly negatively correlated with IGF1P-1 (partial Pearson correlation *r*, -0.15; *P* = 0.0009), and positively correlated with triglycerides (*r*, 0.25; *P* < 0.0001), HbA<sub>1c</sub> (*r*, 0.11; *P* = 0.02), and BMI (*r*, 0.17; *P* < 0.0001). The ratio of total cholesterol:HDL-cholesterol was not clearly correlated with insulin (*r*, 0.03; *P* = 0.6), although it was positively correlated with triglycerides (*r*, 0.28; *P* < 0.0001). HbA<sub>1c</sub> level was modestly positively correlated with triglycerides (*r*, 0.17; *P* = 0.0002). IGF1P-1 was negatively correlated with triglycerides (*r*, -0.15; *P* = 0.0009) and was negatively correlated with BMI (*r*, -0.26, *P* < 0.0001).

The risk of colorectal cancer did not change with increasing concentrations of insulin, the ratio of total cholesterol:HDL-cholesterol, triglycerides, or IGF1P-1 when assessed univariately (Table 2); mutually controlling for the markers produced similar results (not shown). Although not statistically significant, risk of colorectal cancer was 57% higher in the top fourth of the distribution of HbA<sub>1c</sub> compared with the bottom fourth (Table 2). After excluding participants who reported using medications for diabetes, the associations for HbA<sub>1c</sub> (comparing highest and lowest fourths: OR, 1.33) and for BMI (comparing BMI ≥30 kg/m<sup>2</sup> with BMI <25 kg/m<sup>2</sup>: OR, 1.33) were

attenuated. Again, no associations were observed for the other markers (not shown).

We also examined possible longer-term indicators of insulin and glucose. The OR for colorectal cancer was 1.70 comparing participants with BMI ≥30 kg/m<sup>2</sup> with those with BMI <25 kg/m<sup>2</sup> (Table 3). Risk was not increased for those with a BMI of 25.0–29.9 kg/m<sup>2</sup>. The OR for colorectal cancer was 2.43 for individuals who reported currently taking medications to treat diabetes at baseline compared with those who did not so report (Table 3).

Stratifying by gender or by BMI (<25 kg/m<sup>2</sup>, ≥25 kg/m<sup>2</sup>), no consistent patterns were observed for the circulating markers (not shown). Although the association of BMI with colorectal cancer was more apparent for men (≥30 kg/m<sup>2</sup> versus <25 kg/m<sup>2</sup>; OR, 2.36; 95% CI, 1.12–4.95) than for women (OR, 1.22; 95% CI, 0.59–2.55), the interaction between sex and BMI was not statistically significant (*P*<sub>interaction</sub> = 0.22).

Because it is unknown how the presence of clinically occult cancer at the time of blood donation might affect markers of hyperinsulinemia and hyperglycemia, the primary analysis (included 87 cases, 174 controls) was repeated excluding cases diagnosed within 5 years after baseline. These results were generally consistent with the overall analysis. In addition, because many of the participants were not fasting at the time of blood draw and because eating influences some of the markers, we also repeated the analysis for insulin, triglycerides, and IGF1P-1 (included 35 cases and 70 controls), excluding cases and controls who reported less than 6 h since their last meal. The hypothesized associations were still not detected for insulin, triglycerides, and IGF1P-1 (not shown), although these analyses were based on small sample sizes.

Colon and rectal cancers may have different etiologies; therefore, we repeated the primary analysis separately for the colon (*n* = 132) and rectum (*n* = 41). In addition, we also repeated the analysis separately for the proximal colon (*n* = 67) and distal colon (*n* = 55). Ten cases were not included in the proximal or distal colon analyses because they were of contiguous sites or had an unknown site in the colon. HbA<sub>1c</sub> was positively associated with colon cancer overall, and with proximal and distal colon cancer, but not with rectal cancer (Table 4). There was no association of between HbA<sub>1c</sub> levels

Table 2 OR<sup>a</sup> and 95% CI of colorectal cancer according to fourths of markers<sup>b</sup> of insulin and glucose control, CLUE II cohort of Washington County, Maryland, 1989–2000

Marker	Fourth <sup>c</sup>				<i>P</i> <sub>trend</sub>
	1	2	3	4	
<b>Insulin</b>					
Cases/controls	43/84	53/86	42/89	34/85	
OR <sup>a</sup>	1.00	1.20	0.92	0.78	0.24
(95% CI)	Reference	(0.72–2.00)	(0.55–1.53)	(0.45–1.35)	
<b>Ratio of total cholesterol:HDL-cholesterol</b>					
Cases/controls	48/82	44/82	43/83	35/82	
OR <sup>a</sup>	1.00	0.94	0.89	0.74	0.28
(95% CI)	Reference	(0.54–1.65)	(0.53–1.50)	(0.42–1.29)	
<b>Triglycerides</b>					
Cases/controls	53/82	38/82	42/86	37/84	
OR <sup>a</sup>	1.00	0.73	0.72	0.69	0.17
(95% CI)	Reference	(0.43–1.23)	(0.42–1.24)	(0.41–1.16)	
<b>IGFBP-1</b>					
Cases/controls	46/82	46/84	33/86	44/83	
OR <sup>a</sup>	1.00	0.97	0.68	0.93	0.53
(95% CI)	Reference	(0.57–1.66)	(0.39–1.18)	(0.55–1.58)	
<b>HbA<sub>1c</sub></b>					
Cases/controls	39/87	29/86	43/86	62/87	
OR <sup>a</sup>	1.00	0.77	1.12	1.57	0.02
(95% CI)	Reference	(0.43–1.36)	(0.65–1.91)	(0.94–2.60)	

<sup>a</sup> ORs estimated from conditional logistic regression. Cases and controls were matched on age, sex, race, time since last meal, and date of blood draw.

<sup>b</sup> Measurements were unavailable for some participants because of insufficient plasma volume. Included in the analysis were sets for which the case and at least one control had information available. The counts are insulin 172 cases, 344 controls; ratio of cholesterol: HDL-cholesterol 170 cases and 329 controls; triglycerides 170 cases, 334 controls; and IGFBP-1 169 cases, 335 controls.

<sup>c</sup> Cut points based on the distribution of concentrations among the controls. For insulin, triglycerides, and IGFBP-1 cut points for the fourths of the distribution of concentrations among the controls were determined separately within strata of time since last meal. The quartile cut points were as follows: insulin (milliunits/liter): 0–1 h: 21.19, 33.01, 49.71; 2–3 h: 6.36, 16.41, 29.48; 4–5 h: 4.92, 8.24, 18.86; 6–7 h: 3.89, 8.14, 12.20; 8+ h: 4.94, 8.15, 17.36. Ratio of total cholesterol (mg/dl): HDL cholesterol (mg/dl): 4.04, 5.07, 6.20; triglycerides (mg/dl): 0–1 h: 121.5, 170.5, 226.5; 2–3 h: 122.5, 165.5, 218.5; 4–5 h: 105, 144.5, 230; 6–7 h: 87.5, 129.0, 219.5; 8+ h: 94, 131, 182.5. IGFBP-1 (ng/ml): 0–1 h: 7.12, 19.64, 47.28; 2–3 h: 4.10, 10.92, 21.71; 4–5 h: 2.75, 14.97, 35.15; 6–7 h: 5.38, 43.16, 88.74; 8+ h: 10.85, 30.09, 60.23; HbA<sub>1c</sub> (%): 5.38, 5.54, 5.78.

Table 3 ORs<sup>a</sup> and 95% CI of colorectal cancer by categories of BMI and use of medications to treat diabetes, CLUE II cohort of Washington County, Maryland, 1989–2000

	Cases/Controls	OR <sup>b</sup>	(95% CI)
<b>BMI at baseline<sup>a</sup></b>			
Normal (<25 kg/m <sup>2</sup> )	69/153	1.00	Reference
Overweight (25–29.9 kg/m <sup>2</sup> )	66/143	1.02	0.66–1.57
Obese (≥30 kg/m <sup>2</sup> )	38/50	1.70	1.01–2.86
<i>P</i> <sub>trend</sub>			0.08
<b>Use of medications to treat diabetes at baseline</b>			
No	159/334	1.00	Reference
Yes	14/12	2.43	1.10–5.38

<sup>a</sup> Cut points from Ref. 3.

<sup>b</sup> ORs estimated from conditional logistic regression. Cases and controls were matched on age, sex, race, time since last meal, and date of blood draw.

and the risk of advanced stage ( $n = 60$ ) colorectal cancer ( $P_{\text{trend}} = 0.32$ ). The positive association of BMI persisted for colon cancer overall and, in particular, for distal colon cancer (Table 4). The OR for use of medications to treat diabetes was greater than 2 for all sites, although the association was statistically significant only for the colon (Table 4). Consistent with the overall findings, there were no associations between insulin, the ratio of total cholesterol:HDL-cholesterol, triglycerides, and IGFBP-1, and colon cancer overall or by colon subsite (not shown). For rectal cancer, inverse associations were observed for the ratio of total cholesterol:HDL-cholesterol (comparing highest to lowest fourths, OR, 0.19;  $P_{\text{trend}} = 0.01$ ) and triglycerides (OR, 0.25;  $P_{\text{trend}} = 0.03$ ).

## Discussion

In this prospective study, we observed that higher prediagnostic levels of HbA<sub>1c</sub> were associated with a higher risk of colorectal cancer diagnosed a mean of 5 years later, in particular for cancer of the colon. Higher BMI and use of medications to treat diabetes were also positively associated with colorectal cancer risk. Higher plasma insulin, the ratio, of total cholesterol:HDL-cholesterol, triglycerides, and lower IGFBP-1 concentrations were not associated with colorectal cancer. Unlike the plasma markers that we measured, which vary pre- and postprandially and with meal quality, HbA<sub>1c</sub> is a stable, time-averaged indicator of glycemic control, and consequently time-integrated circulating insulin levels, over the past 60 days. BMI and the use of medications to treat diabetes may also be better time-integrated indicators of circulating insulin and glucose levels. Thus, despite the null findings for the plasma markers, we believe that our findings of positive associations of HbA<sub>1c</sub>, BMI, and the use of medications to treat diabetes, support the hypothesis that perturbations in insulin and glucose control contribute to the etiology of colorectal cancer.

Other prospective studies have evaluated possible longer-term indicators of insulin and glucose control, such as HbA<sub>1c</sub>, BMI, and type 2 diabetes mellitus. An association between higher levels of HbA<sub>1c</sub> and advanced colorectal cancer, but not colorectal cancer overall, has been suggested (14). However, because of the relatively short time between blood draw and the diagnosis of advanced cases in that study, it is possible that clinically occult disease at blood draw might have influenced HbA<sub>1c</sub> level (14). In our study, we observed an increased risk of colorectal cancer in the top fourth of the distribution of

Table 4 ORs<sup>a</sup> and 95% CI by site within the colorectum comparing the highest to lowest categories of markers of insulin and glucose control, CLUE II cohort of Washington County, Maryland, 1989–2000

Marker	Comparing highest to lowest categories <sup>b</sup>			
	Colon	Proximal colon	Distal colon	Rectum
HbA <sub>1c</sub>				
OR	1.97	2.10	1.61	0.91
(95% CI)	(1.11–3.48)	(1.00–4.40)	(0.72–3.58)	(0.36–2.26)
<i>P</i> <sub>trend</sub>	0.003	0.02	0.13	0.69
BMI				
OR	1.79	1.46	2.60	1.64
(95% CI)	(1.02–3.13)	(0.71–2.98)	(1.18–5.70)	(0.68–3.94)
<i>P</i> <sub>trend</sub>	0.06	0.52	0.02	0.41
Use of medications to treat diabetes				
OR	2.46	2.60	2.11	2.96
(95% CI)	(1.05–5.75)	(0.93–7.32)	(0.65–6.89)	(0.77–11.4)

<sup>a</sup>ORs estimated from unconditional logistic regression models (adjusting for the matching factors) breaking the matching and including all controls to preserve power. Numbers of cases of each site were: colon 132, proximal colon (cecum to splenic flexure) 67; distal colon (below splenic flexure to rectosigmoid junction) 55, and rectum 41.

<sup>b</sup>For HbA<sub>1c</sub>, presented are ORs comparing the highest to lowest fourth of the distribution with cut points based on the distribution among the controls. The quartile cut points for HbA<sub>1c</sub> (%) were: 5.38, 5.54, 5.78. For BMI, presented are ORs comparing  $\geq 30$  (obese) to BMI  $< 25$  kg/m<sup>2</sup> (normal) based on Ref. 3. For use of medications to treat diabetes, presented are ORs comparing use to nonuse.

HbA<sub>1c</sub> compared with the lowest fourth, but not for advanced stage colorectal cancer ( $n = 60$ ). HbA<sub>1c</sub> is formed when a glucose molecule binds to the last amino acids of the  $\beta$  chain of hemoglobin A and is an indicator of average glycemia for the past 6–8 weeks (22). Because high blood glucose levels correspond to high blood insulin levels, HbA<sub>1c</sub> also may be a good marker of chronic hyperinsulinemia. Another advantage of HbA<sub>1c</sub> is that it is not affected by postprandial time or time of day of blood draw.

Obesity is associated with hyperinsulinemia and the development of type 2 diabetes mellitus. Thus, the observation of a higher risk of colorectal cancer with higher BMI in our study as well as in other studies (23–26) supports the role of hyperinsulinemia in the pathogenesis of colorectal cancer. Similar to results of Murphy and colleagues (23), in our study a weaker association for higher levels of BMI was seen in women (BMI  $\geq 30$  kg/m<sup>2</sup> versus  $< 25$  kg/m<sup>2</sup>: OR, 1.22) than in men (OR, 2.36). Because physical activity improves insulin sensitivity, we would have predicted that men and women who were more physically active would have lower insulin and HbA<sub>1c</sub> concentrations and, thus, would have a lower risk of colon cancer. Indeed, physical activity has been shown to be inversely associated with colorectal cancer risk in epidemiological studies (26). We could not examine the relation of physical activity and colorectal cancer because information on physical activity was not collected at baseline in the CLUE II study.

Other studies have observed a positive association between type 2 diabetes mellitus and colorectal cancer (27–32) or adenomatous polyps (12, 33). Consistent with those findings, in our study, we observed a moderate association between the use of medications to treat diabetes at baseline and subsequent colorectal cancer. At baseline, we did not ask participants to report whether they had a diagnosis of diabetes. We did ask them to indicate the medications that they were currently taking, and of those included in this nested case-control study, 26 reported taking a drug used to treat diabetes. Because this nested case-control study consisted of middle-aged and elderly individuals, the majority of these cases are likely type 2 diabetics. The variable, use of medications to treat diabetes, does not capture all of the participants who had a diagnosis of diabetes at baseline or subsequently. Nevertheless, those who require pharmacological intervention for diabetes are most

likely those who had protracted hyperinsulinemia at some point before baseline. In addition, the analysis for the circulating markers measured in blood collected at baseline would have captured perturbations in insulin and glucose control for individuals who currently had poorly regulated diabetes or prediabetic states.

Our results are not consistent with the findings for plasma markers of insulin and glucose control observed in other studies. In the prospective Cardiovascular Health Study cohort, Schoen *et al.* (15) found that there was an increased risk of colorectal cancer with increasing levels of fasting and 2-h postchallenge insulin. In a nested case-control study among the New York University Women's Health Study cohort, increasing C-peptide concentrations, a marker for pancreatic insulin production, was associated with an increased risk of colorectal cancer (16). Trevisan *et al.* (18) examined fasting serum triglycerides, glucose, and HDL-cholesterol in a series of pooled Italian prospective studies and found that there was an increased risk of death from colorectal cancer for participants with a cluster of metabolic abnormalities linked to insulin resistance.

Hyperinsulinemia is also associated with dyslipidemia; and through this pathway, high total cholesterol, low HDL-cholesterol, and high triglycerides may influence the risk of colorectal cancer. Several prospective studies have suggested an inverse association between total cholesterol and colorectal cancer (34–36), which may be attributable to the presence of undetected disease at blood draw for cases diagnosed at the beginning of cohort follow-up (34, 37). Other studies have observed either no association (38) or a positive association (39). Concentration of serum triglycerides has not been consistently associated with colorectal cancer in prospective studies (15, 18, 36). Our study does not support associations for the ratio of total cholesterol:HDL-cholesterol or triglycerides with colorectal cancer.

Insulin also may influence colorectal carcinogenesis through its links with the IGF pathway. We measured one component of the IGF pathway, IGFBP-1, whose circulating concentration is lower with higher insulin concentrations (40, 41). IGFBP-1 is a binding protein that binds IGF-1 in the extracellular space (42). IGFs and their binding proteins are overexpressed in many tumors (42–44). Epidemiological stud-

ies have suggested a positive association of high IGF-1 or high IGF-1 relative to IGF-1 with colorectal cancer or precursor adenoma (16, 45–47). Thus, an insulin-associated decrease in IGF-1 and resultant increase in free IGF-1 might be predicted to increase the risk of colorectal neoplasia. Higher plasma concentration of IGF-1 was associated with a decreased risk of colorectal cancer in the Women's Health Study cohort (16). In our study, IGF-1 was negatively correlated with insulin and BMI as expected. However, IGF-1 was not inversely associated with colorectal cancer.

There are several possible explanations for our null results for insulin, the ratio of total cholesterol:HDL-cholesterol, triglycerides, and IGF-1. Because most participants were not fasting at the time of blood draw, measurement error attributable to imperfect control of time since last meal or glycemic load of last meal is likely. However, when we restricted the analysis to the stratum of individuals that had not eaten for 6 or more h, still no association was present for the plasma markers, albeit the analyses were based on small numbers. The single measurement of plasma concentration may not have correlated well with the time-integrated level, the maximum level, or the average level of exposure to the circulating markers. A single measurement of insulin concentration in individuals with a long duration of type 2 diabetes mellitus may not represent past concentrations because of waning insulin production. When we excluded participants who were using medications to treat diabetes ( $n = 26$ ), the associations for HbA<sub>1c</sub> and BMI were attenuated and no associations were detected for the other markers. We do not have information on diagnosis of diabetes or on duration of diabetes at baseline in this cohort. The effect of including long-term diabetics in the analysis would be to attenuate the association between insulin and colorectal cancer. However, the other studies finding positive associations also relied on a single measurement. The time point at which we measured levels (<1–11 years before diagnosis) may not have corresponded to the etiologically relevant moment in the past for carcinogenesis. Adenomatous polyps typically precede adenocarcinoma by 10 years (48). Thus, measuring plasma concentrations an average of 5 years before diagnosis likely means that the precursor adenoma was already present for some cases. However, we believe that hyperinsulinemia and hyperglycemia would most likely act as growth promoters or progressors, rather than initiators. Thus, levels measured during the adenoma-to-carcinoma sequence may be relevant. However, the results remained null after restricting the analysis to cases that were diagnosed more than 5 years after blood draw.

Participants in this study were drawn from a community cohort, rather than from a clinical setting, increasing the generalizability of the results to the general population. The mean insulin concentrations for individuals who had not eaten for 8 h or more were within normal ranges, for both cases and controls. It is possible that we did not detect associations for the plasma markers because risk is elevated only at very high circulating concentrations, levels that would only be found in those with overt diabetes or prediabetic states.

We cannot rule out that HbA<sub>1c</sub>, BMI, and the use of medications to treat diabetes mellitus might be surrogates for pathways that play a role in the development of colorectal cancer and that are independent of insulin and glucose control. Additional studies are needed to confirm and uncover the mechanism underlying these observations.

In conclusion, our findings for the possible longer-term indicators, HbA<sub>1c</sub>, BMI, and the use of medications for diabetes mellitus, suggest that perturbations in insulin and glucose control may be associated with subsequent risk of colorectal can-

cer. If hyperinsulinemia and hyperglycemia were associated with an increased risk of colorectal cancer, then this area would offer a new approach to the targeted prevention and treatment of colorectal cancer.

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## Association of Markers of Insulin and Glucose Control with Subsequent Colorectal Cancer Risk

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