Genetic Variants, Prediagnostic Circulating Levels of Insulin-like Growth Factors, Insulin, and Glucose and the Risk of Colorectal Cancer: The Multiethnic Cohort Study

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Abstract

Background: Increased exposure of colonic and rectal epithelial cells to the promitotic and antiapoptotic effects of insulin and insulin-like growth factors (IGF) is hypothesized to increase colorectal cancer risk.

Methods: In a case–control study nested within the Multiethnic Cohort, we attempted to replicate associations for five genetic variants associated with IGF system biomarkers, insulin, or glucose and to examine their association with the risk of colorectal cancer. In a subset of participants, the association between circulating biomarkers and colorectal cancer risk was examined. Unconditional logistic regression was used to calculate ORs and 95% confidence intervals (CI) for genetic variants (1,954 cases/2,587 controls) and serum biomarkers (258 cases/1,701 controls).

Results: Associations with circulating biomarkers were replicated in the Multiethnic Cohort for *IGF1* rs35767 and for *IGFBP3* rs2854744, rs2854746, and rs3110697 ($P < 0.05$). Homozygous carriers of the glucokinase regulator (*GCKR*) rs780094 variant T-allele were at a decreased risk of colorectal cancer (OR, 0.77; 95% CI, 0.64–0.92). In risk factor–adjusted models, participants with the highest prediagnostic IGF-II levels were at an increased risk [OR (T1 vs. T3), 1.58; 95% CI, 1.09–2.28; $P_{trend} = 0.011$] and participants with the highest prediagnostic IGF-binding protein (IGFBP)-3 levels were at a decreased risk of colorectal cancer (OR, 0.53; 95% CI, 0.34–0.83; $P_{trend} = 0.003$).

Conclusion: These data provide further support for a role of prediagnostic IGF and insulin levels in the etiology of colorectal cancer.

Impact: Future studies attempting to replicate the association between the *GCKR* rs780094 variant and the risk of colorectal cancer are warranted.

Introduction

Colorectal cancer is the third most frequently diagnosed cancer in men and women in the United States, with an estimated 142,570 new cases diagnosed and 51,370 deaths occurring annually (1). Obesity, diabetes, and low levels of physical activity are positively associated with an increased risk of colorectal cancer (2–4) and have been hypothesized to influence the risk of disease by increasing the exposure of colonic and rectal epithelial cells to the promitotic and antiapoptotic effects of insulin and insulin-like growth factors (IGF; ref. 5).

The IGF system regulates cellular growth, differentiation, and apoptosis via a complex system of circulating growth factors (IGF-I and IGF-II), cell surface receptors (IGF receptors 1 and 2), and binding proteins [IGF-binding proteins (IGFBP)-1 to 6); ref. 6]. IGF-I, the IGF found in the circulation, has been shown to be a potent mitogen and to inhibit apoptosis (6, 7), properties that potentially increase the risk of cancer. Conversely, IGFBP-3, the primary binding protein for IGF-I, may reduce cancer risk through its ability to mediate the bioavailability of free IGF-I in circulation and, in an IGF-I–independent manner, reduce cellular proliferation and stimulate apoptosis (8). Hyperinsulinemia may also increase the risk of colorectal cancer, as it has been reported *in vivo* to increase cell proliferation and aberrant crypt foci in the colon (9, 10) and to inhibit the transcription of IGFBP-1 (11). In addition, glucokinase, a hepatic hexokinase and glucose sensor in pancreatic β-cells, is involved in insulin/glucose homeostasis. Therefore, factors influencing glucokinase, or the levels of glucokinase regulatory protein (GKRP) involved in the nucleus–cytosol localization of glucokinase (12),

may influence the risk of cancer through modifications of circulating insulin and glucose.

Despite biologic evidence for a role of the IGF system and hyperinsulinemia in the etiology of colorectal cancer, previous epidemiologic studies, including a recent meta-analysis (13), have reported only modest positive associations between serum levels of IGF-I and colorectal cancer (13–21) with risk estimates generally failing to differ from unity in individual studies (13, 16, 17, 19–21). Associations for circulating levels of IGF-II (17, 22–24), IGFBP-3 (13, 15–18, 20), and insulin (16, 20, 21, 25–32) with the risk of colorectal cancer have been similarly equivocal. Recent genome-wide association studies (GWAS) and candidate gene studies have identified several single-nucleotide polymorphisms (SNP) associated with circulating levels of IGF-I (33–35), IGFBP-3 (33–39), and insulin levels (40, 41). As circulating levels of these hormones have been shown to change with age, diet, and other lifestyle factors (13), polymorphisms in gene regions regulating serum concentrations may better reflect long-term exposure and exhibit an association with colorectal cancer risk.

For this report, we examined whether we could replicate associations identified in previous studies between selected SNPs and circulating IGF-I, IGF-II, IGFBP-1, IGFBP-3, insulin, or glucose levels and whether the genetic variants were associated with the risk of colorectal cancer in a case–control study nested within the Multiethnic Cohort (MEC) Study. In addition, we examined whether associations between IGF/insulin-associated SNPs and colorectal cancer risk may be partially mediated by circulating IGFs, insulin, or glucose by evaluating whether these biomarkers were associated with the risk of colorectal cancer and if genotypic associations could be explained by variations in biomarker concentrations.

Materials and Methods

Study population

The MEC is a longitudinal study designed to investigate associations between dietary, lifestyle, genetic factors, and the incidence of cancer and has been described previously in detail (42). Briefly, from 1993 to 1996, more than 215,000 men and women who were between 45 and 75 years of age at recruitment and residing in Hawaii and California entered the cohort. Potential participants were identified through drivers' license files, voter registration lists, and Medicare files to obtain a multiethnic sample of African-Americans, Japanese-Americans, Latinos, Native Hawaiians, and Whites. At cohort entry, participants completed a self-administered, 26-page baseline questionnaire that included a detailed quantitative food frequency questionnaire and queries on demographic characteristics, anthropometric measures, medical history, family history of cancer, reproductive and menstrual history, cancer screening practices, occupational history, and physical activity. The study protocol was approved by the Institutional Review Boards of the University of Hawaii (Honolulu, HI) and the University of Southern California (Los Angeles, CA).

A biospecimen subcohort of MEC participants was established from 1996 to 2006. First, incident colorectal cancer cases were contacted to provide a blood sample after diagnosis, as well as a random sample of the cohort to serve as controls. Between 2001 and 2006, all surviving cohort members were recontacted and asked to provide biologic specimens (blood and urine) to constitute a prospective biorepository. Blood samples were drawn and processed within 4 hours of collection by centrifugation. Blood components (serum, plasma, buffy coat, red cells) were aliquoted into 0.5-mL cryotubes and stored in the vapor phase of liquid nitrogen ($-150°C$). For 95% of the biorepository participants, ≥ 8-hour fasting blood samples were obtained. In total, 67,594 cohort members contributed to the biorepository from which cases and controls were selected for the present study.

Selection of cases and controls

For the analyses examining the associations of IGF/insulin-associated SNPs with the risk of colorectal cancer, cases were defined as all participants providing a biologic specimen to the MEC biorepository (pre- or postdiagnostic) and identified through regular linkages of the cohort to the Surveillance, Epidemiology, and End Results (SEER) cancer registries for Hawaii and California to have had a diagnosis of invasive colon (ICD-O-3 codes C18.0-C18.9) or rectal (ICD-O-3 code C20.9) cancer ($n = 1,954$ cases and $n = 2,587$ controls) after the cohort baseline. Controls for these analyses were defined as participants free of a diagnosis of colon or rectal cancer and were randomly selected from the biorepository and matched to cases on age, sex, race, and the date of blood draw. For the analyses examining the associations of IGF/insulin-associated SNPs with circulating biomarker concentrations and for the analyses examining the associations between circulating biomarker concentrations and the risk of colorectal cancer, only those cases providing prediagnostic biospecimens were included. For these analyses involving biomarker concentrations, only controls that remained free of colorectal cancer on the last follow-up date (December 31, 2004) were eligible for this analysis and were matched to cases with biomarker measurements on age, sex, race, date of blood draw, and hours of fasting before blood draw. For IGF-I, IGFBP-1, IGFBP-3, insulin, and glucose, data on serum values were available for 258 colorectal cases and 1,701 controls. For IGF-II, data were available for 255 colorectal cases and 1,571 controls. The median time from blood draw to the date of diagnosis for cases providing prediagnostic serum samples was 595 days.

SNP selection and genotyping

Five SNPs previously identified to be associated with circulating levels of IGF-I (33–35), IGFBP-3 (33–39), or insulin (40, 41) were selected for this study. Genotyping was conducted at the University of Hawaii Cancer Center on amplified DNA samples purified from blood buffy coat using QIAamp DNA Blood Kits (Qiagen). The genotyping

of the 5 IGF/insulin-associated SNPs (rs35767, rs780094, rs2854744, rs2854746, and rs3110697) was conducted using the TaqMan Allelic Discrimination Assay from Applied Biosystems. All assays were conducted by laboratory personnel blinded to the case–control status of the samples. Among controls, all SNPs were found to be in Hardy–Weinberg equilibrium ($P > 0.05$) in at least 4 of the racial/ethnic groups included in the analysis. Replicates were genotyped for more than 10% of the samples and, after our quality control filters, were all concordant. The average genotyping call rate was 98.1%.

Biochemical assays

All assays were conducted at McGill University (Montreal, QC, Canada) in the laboratory of M.N. Pollak. Samples for cases and controls were thawed and analyzed together with laboratory personnel blinded to the case–control status of the samples. ELISAs from Diagnostic System Laboratories were used to obtain serum values for IGF system biomarkers. IGF-I and IGF-II assays included an acid–ethanol precipitation to reduce the interference with IGFBPs. The molar ratio of IGF-I and IGFBP-3 was calculated using the conversion factor of 0.13 for IGF-I and 0.035 for IGFBP-3 (as specified by the manufacturer). Insulin concentrations were measured using standard ELISA method with reagents purchased from Millipore Corp. Glucose levels were determined using quantitative colorimetric method with reagents purchased from Bio-Assay Systems. Homeostatic model assessment (HOMA) was calculated as the product of fasting glucose (mg/dL) and insulin (μIU/mL) divided by 405 (43). Blinded duplicates for a randomly selected subset of samples (∼5%) showed an average intrabatch coefficient of variation (CV) of 2.4%, 2.9%, 2.7%, 2.4%, 3.4%, and 2.3% for IGF-I, IGF-II, IGFBP-1, IGFBP-3, insulin, and glucose, respectively. The average interbatch CV was 4.2%, 3.9%, 5.9%, 4.5%, 5.3%, and 2.9% for IGF-I, IGF-II, IGFBP-1, IGFBP-3, insulin, and glucose, respectively.

Statistical analyses

Serum concentrations of IGF system biomarkers, insulin, and glucose were regressed on IGF/insulin-associated SNPs to estimate their association, assuming an additive genetic model. Serum values were natural log-transformed to better meet the linear model assumptions, and geometric means and 95% confidence intervals (CI) calculated for each genotype, adjusting for age, sex, race/ethnicity, and case–control status.

Unconditional logistic regression of colorectal cancer was used to obtain ORs and 95% CIs for IGF/insulin-associated SNPs. Genotypic ORs were calculated assuming a codominant genetic model adjusted for age, sex, and race/ethnicity with the most common homozygote genotype serving as the referent in all models. Linear trends were tested by modeling SNPs as continuous variables in logistic models (log-additive model). The independent effect for each SNP was examined by entering all SNPs into a single multivariable logistic regression model. The

heterogeneity of effect for SNP–colorectal cancer associations across racial/ethnic groups was tested by a Wald test of the cross-product term. Associations for the main SNP effects were also examined in analyses stratified by anatomic subsite (colon/rectum) and by colorectal cancer risk factors. Heterogeneity by anatomic subsite was assessed using polytomous logistic regression and was statistically tested using the Wald test.

To estimate the associations of prediagnostic circulating IGF system biomarkers, insulin, and glucose with the risk of colorectal cancer, unconditional logistic regression was used to obtain ORs and 95% CIs. Tertiles of serum values were examined in all models using cutoff points based on the exposure distribution among controls. The lowest exposure group served as the referent in all models. Crude logistic regression models were adjusted for age, sex, and race/ethnicity. Multivariable models were further adjusted for personal history of colorectal polyps (yes/no), family history of colorectal cancer (yes/no), body mass index (BMI; kg/m^2; continuous), moderate or vigorous physical activity (continuous), processed meat intake (g/1,000 kcal/d; continuous), pack-years of cigarette smoking (continuous), and alcohol consumption (ethanol g/d; continuous) to examine the impact of colorectal cancer risk factors on model estimates. Covariates selected for inclusion were those shown previously to be associated with the risk of colorectal cancer in MEC plasma studies of colorectal cancer. Additional colorectal cancer risk factors were not included in the final models as they were not found alone, or in combination, to change the risk estimates by more than 10% (44). Linear trends were tested by modeling tertile medians as a continuous variable in regression models. The heterogeneity of effect for serum biomarkers across racial/ethnic groups was tested by a Wald test of the cross-product term. As previous analyses in the MEC have found IGF levels to differ by age, sex, race/ethnicity, and BMI (45–47), departures from the assumption of multiplicative joint effects for IGF system biomarkers and age, sex, race/ethnicity, and BMI were tested by a Wald test of the cross-product terms. Associations for the main biomarker effects were also examined in analyses stratified by anatomic subsite (colon/rectum).

For the SNPs found to be associated with the risk of colorectal cancer, statistical mediation analyses were conducted by entering prediagnostic insulin, glucose, and IGF system biomarkers, independently and in combination, as covariates into logistic regression models. Serum values changing the risk of colorectal cancer by more than 10% were considered to partially mediate the SNP–colorectal cancer association. All data analyses were conducted using SAS 9.2 statistical software (SAS Institute Inc.).

Results

Baseline characteristics of colorectal cases and controls

The baseline characteristics of study participants by case–control status are provided in Table 1. The median

Table 1. Characteristics of eligible participants in the IGF/insulin colorectal cancer nested case–control study

	Cases (N = 1,954)	Controls (N = 2,587)
Age at blood draw, y	70 (64–77)	69 (63–74)
Male, n (%)	1,082 (55.4)	1,503 (58.1)
Race/ethnicity, n (%)		
African-American	376 (19.2)	792 (30.6)
Native Hawaiian	106 (5.4)	174 (6.7)
Japanese-American	681 (34.9)	775 (30.0)
Latino	451 (23.1)	418 (16.2)
White	340 (17.4)	428 (16.5)
Positive history of colorectal polyp, n (%)	121 (6.2)	202 (7.8)
Positive first-degree family history of colorectal cancer, n (%)	201 (10.3)	233 (9.0)
BMI, kg/m^2	26.3 (23.8–29.8)	25.9 (23.5–28.9)
Moderate or vigorous physical activity, h/d	0.71 (0.36–1.43)	0.71 (0.36–1.43)
Processed meat intake, g/1,000 kcal/d	7.8 (4.2–12.5)	7.8 (4.1–12.7)
Pack-years of smoking	3.9 (0–19.7)	2.0 (0–19.8)
Alcohol intake (ethanol, g/d)	0.4 (0–10.6)	0.4 (0–8.0)
IGF-I,[a] ng/mL	149.0 (114.0–197.5)	164.5 (125.0–205.3)
IGF-II,[b] ng/mL	996 (776–1,202)	937 (759–1,161)
IGFBP-1,[a] ng/mL	25.5 (13.8–43.1)	24.3 (13.0–41.4)
IGFBP-3,[a] ng/mL	3,449 (2,838–4,296)	3,829 (3,144–4,534)
IGF-I/IGFBP-3,[a] nmol/L	0.16 (0.13–0.21)	0.16 (0.13–0.19)
Insulin,[a] μIU/mL	6.0 (4.0–10.6)	5.7 (3.7–9.4)
Glucose,[a] mg/dL	89.4 (80.3–104.7)	89.9 (81.1–102.1)
HOMA[a]	1.44 (0.86–2.62)	1.30 (0.79–2.25)

NOTE: The values are the median (25th–75th percentile) unless otherwise specified.
[a]Serum values were available for 1,959 participants (258 cases/1,701 controls).
[b]IGF-II serum values were available for 1,478 participants (255 cases/1,223 controls).

ages at blood draw were 70 and 69 years of age for cases and controls, respectively. There was a higher proportion of males than females for both cases (55%) and controls (58%). Japanese-Americans comprised the largest racial/ethnic group (32%), followed by African-Americans, Latinos, Whites, and Native Hawaiians. For colorectal cancer risk factors, median values were similar for cases and controls for hours of moderate or vigorous physical activity, processed meat intake, and alcohol consumption; however, cases were more likely to report a family history of colorectal cancer, a higher BMI, and greater pack-years of cigarette smoking than controls. In our sample, the median levels of IGF-I and IGFBP-3 were lower and the median levels of IGF-II, IGFBP-1, insulin, and HOMA were higher for cases than for controls.

Associations of IGF/insulin-associated SNPs with circulating biomarker concentrations

Associations between IGF/insulin-associated SNPs and circulating biomarker concentrations are given in Table 2. Previously observed associations for the *IGF1* rs35767 polymorphism with circulating IGF-I levels ($P = 0.001$) and the *IGFBP3* rs2854744 (A-202C; $P = 2 \times 10^{-8}$), *IGFBP3* rs2854746 (G-2133C; $P = 1 \times 10^{-12}$), and *IGFBP3*

rs3110697 ($P = 3 \times 10^{-6}$) polymorphisms, with circulating IGFBP-3 levels replicated in additive genetic models adjusted for age, sex, race/ethnicity, and case–control status. However, the glucokinase regulator (*GCKR*) rs780094 polymorphism was not associated with circulating levels of insulin ($P = 0.941$) or glucose ($P = 0.272$) in our sample (data not shown). All 5 SNPs examined were associated with the IGF-I:IGFBP-3 molar ratio ($P \leq 0.002$). Mean IGF-II levels were found to differ by genotype for SNPs *GCKR* rs780094, *IGFBP3* rs2854744, *IGFBP3* rs2854746, and *IGFBP3* rs3110697 ($P < 4 \times 10^4$). In no model, did all SNPs combined or an individual SNP account for more than 4.0% and more than 3.1%, respectively, of the variance in circulating hormone levels.

IGF/insulin-associated SNPs and the risk of colorectal cancer

The ORs and 95% CIs for associations between IGF/insulin-associated SNPs and the risk of colorectal cancer are presented in Table 3. Adjusting for age, sex, and race/ethnicity, the risk of colorectal cancer was lower for carriers of the *GCKR* rs780094 TT genotype (OR, 0.77; 95% CI, 0.64–0.92; $P_{trend} = 0.007$) than for *GCKR* rs780094 CC homozygotes. No statistically significant associations

Table 2. Associations between previously identified IGF/insulin-associated SNPs and circulating biomarker levels

Locus	SNP genotype	IGF-I, ng/mL		IGF-II, ng/mL		IGFBP-1, ng/mL		IGFBP-3, ng/mL		IGF-I/IGFBP-3, nmol/L	
		n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)
IGF1[33, 34, 36, 39]	rs35767										
	GG	942	145.5 (141.1–150.0)	700	998 (969–1,028)	942	21.0 (19.6–22.5)	942	3,571 (3,490–3,653)	942	0.151 (0.148–0.155)
	GA	813	151.5 (146.5–156.7)	617	997 (965–1,030)	813	21.6 (20.0–23.2)	813	3,637 (3,546–3,730)	813	0.155 (0.151–0.158)
	AA	204	157.9 (149.4–166.9)	161	987 (935–1,041)	204	22.4 (19.7–25.3)	204	3,612 (3,464–3,766)	204	0.162 (0.156–0.169)
	P_{trend} [a]		0.001		0.743		0.296		0.296		0.001
GCKR[39, 40]	rs780094										
	CC	758	145.6 (140.8–150.6)	582	958 (928–990)	758	20.6 (19.1–22.3)	758	3,486 (3,399–3,575)	758	0.155 (0.152–0.159)
	CT	854	153.2 (148.4–158.2)	641	1,013 (983–1,045)	854	21.1 (19.6–22.7)	854	3,680 (3,593–3,770)	854	0.155 (0.151–0.158)
	TT	347	144.2 (137.7–151.0)	255	1,057 (1,010–1,105)	347	24.3 (21.9–26.9)	347	3,673 (3,548–3,803)	347	0.146 (0.141–0.151)
	P_{trend} [a]		0.718		4.0×10^{-5}		0.011		0.001		0.002
IGFBP3[32–37]	rs2854744										
	AA	676	145.6 (140.5–150.9)	526	1,052 (1,016–1,088)	676	21.9 (20.2–23.8)	676	3,742 (3,643–3,843)	676	0.145 (0.141–0.148)
	AC	863	149.8 (145.1–154.7)	643	1,005 (975–1,036)	863	21.0 (19.5–22.6)	863	3,629 (3,543–3,717)	863	0.153 (0.150–0.157)
	CC	420	151.5 (145.3–157.9)	309	911 (876–949)	420	21.0 (19.2–23.1)	420	3,356 (3,254–3,462)	420	0.168 (0.163–0.172)
	P_{trend} [a]		0.089		8.7×10^{-9}		0.385		1.6×10^{-8}		1.0×10^{-17}
IGFBP3[33–37]	rs2854746										
	GG	593	151.4 (145.8–157.1)	450	911 (880–944)	593	20.3 (18.6–22.1)	593	3,355 (3,263–3,449)	593	0.168 (0.163–0.172)
	GC	838	149.2 (144.5–154.0)	630	1,008 (978–1,039)	838	21.6 (20.1–23.2)	838	3,646 (3,561–3,734)	838	0.152 (0.149–0.155)
	CC	528	145.2 (139.5–151.2)	398	1,091 (1,050–1,135)	528	22.1 (20.2–24.2)	528	3,828 (3,715–3,945)	528	0.141 (0.137–0.145)
	P_{trend} [a]		0.102		1.1×10^{-13}		0.113		1.1×10^{-12}		6.8×10^{-24}
IGFBP3[33–36]	rs3110697										
	GG	753	146.9 (142.0–152.1)	591	1,050 (1,016–1,086)	753	22.4 (20.7–24.2)	753	3,730 (3,635–3,827)	753	0.146 (0.143–0.150)
	GA	862	147.8 (143.2–152.6)	640	979 (950–1,009)	862	21.0 (19.6–22.6)	862	3,568 (3,485–3,654)	862	0.154 (0.151–0.157)
	AA	343	155.0 (148.3–162.1)	247	938 (898–980)	343	20.1 (18.2–22.2)	343	3,429 (3,317–3,546)	343	0.168 (0.163–0.173)
	P_{trend} [a]		0.058		5.9×10^{-7}		0.038		3.1×10^{-6}		7.5×10^{-15}

NOTE: Mean values are the predicted geometric means from linear regression adjusted for age, sex, race/ethnicity, and case–control status. Superscripts provide citations for previous studies examining SNP–biomarker associations.

Abbreviation: *GCKR*, glucokinase regulator gene.

[a] *P* value from log-additive genetic model adjusted for age, sex, race/ethnicity, and case–control status.

Table 3. ORs and 95% CIs for IGF/insulin-associated SNPs and colorectal cancer

Locus	SNP genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	$P_{\text{for racial/ethnic heterogeneity}}$[b]
IGF1	rs35767				
	GG	1,012 (51.8)	1,233 (47.7)	1.00	
	GA	768 (39.3)	1,053 (40.7)	0.94 (0.83–1.07)	
	AA	173 (8.9)	301 (11.6)	0.81 (0.66–1.01)	
	P_{trend}[a]			0.068	0.025
GCKR	rs780094				
	CC	753 (38.5)	1,028 (39.8)	1.00	
	CT	862 (44.1)	1,090 (42.1)	0.92 (0.80–1.05)	
	TT	339 (17.4)	469 (18.1)	0.77 (0.64–0.92)	
	P_{trend}[a]			0.007	0.025
IGFBP-3	rs2854744				
	AA	668 (34.2)	880 (34.0)	1.00	
	AC	847 (43.3)	1,158 (44.8)	0.98 (0.85–1.13)	
	CC	439 (22.5)	549 (21.2)	0.98 (0.81–1.17)	
	P_{trend}[a]			0.764	0.283
IGFBP-3	rs2854746				
	GG	579 (29.6)	818 (31.6)	1.00	
	GC	817 (41.8)	1,090 (42.1)	1.04 (0.89–1.20)	
	CC	558 (28.6)	679 (26.3)	1.05 (0.87–1.25)	
	P_{trend}[a]			0.621	0.232
IGFBP-3	rs3110697				
	GG	737 (37.7)	1,009 (39.0)	1.00	
	GA	855 (43.8)	1,130 (43.7)	1.02 (0.89–1.17)	
	AA	361 (18.5)	447 (17.3)	1.01 (0.84–1.21)	
	P_{trend}[a]			0.846	0.563

NOTE: ORs were obtained using unconditional logistic regression adjusted for age, sex, and race/ethnicity.
Abbreviation: *GCKR*, glucokinase regulator gene.
[a]P value for trend from log-additive genetic model.
[b]P value for the test of heterogeneity across racial/ethnic groups.

($P < 0.05$) were detected for the other IGF/insulin-associated SNPs examined. The association between the *GCKR* rs780094 variant TT genotype and the risk of colorectal cancer remained unchanged with adjustment for colorectal cancer risk factors or the other IGF-associated SNPs examined in this study but was found to be confined to cancers of the colon in analyses stratified by anatomic subsite (OR_{colon}, 0.72; 95% CI, 0.59–0.89; $n = 224$; OR_{rectum}, 0.94; 95% CI, 0.69–1.27; $n = 88$; $P_{\text{heterogeneity}} < 0.104$). In analyses stratified by colorectal cancer risk factors and diabetes status, the association between the *GCKR* rs780094 variant TT genotype and the risk of colorectal cancer was also found to be confined to participants below the median value for BMI [OR (BMI < 26.0 kg/m^2), 0.68; 95% CI, 0.53–0.87; $n = 484$; OR (BMI $\geq$ 26.0 kg/m^2), 0.85; 95% CI, 0.64–1.12; $n = 320$; $P_{\text{heterogeneity}} = 0.074$] and to participants reporting no history of diabetes [OR (nondiabetic), 0.75; 95% CI, 0.62–0.91; $n = 725$; OR (diabetic), 0.98; 95% CI, 0.55–1.72; $n = 83$; $P_{\text{heterogeneity}} = 0.841$], although the test for heterogeneity was not statistically significant.

Heterogeneity in disease risk by race/ethnicity was detected for SNPs *IGF1* rs35767 and *GCKR* rs780094

($P = 0.025$ for both). In analyses stratified by race/ethnicity, among Latinos, the risk of colorectal cancer for *IGF1* rs35767 was lower for carriers of the AA genotype (OR, 0.41; 95% CI, 0.19–0.86; $P_{\text{trend}} = 0.033$) than for GG homozygotes but did not reach statistical significance in the other racial/ethnic groups (Supplementary Table S1). For *GCKR* rs780094, the risk of colorectal cancer was lower for homozygous carriers of the minor T-allele among African-Americans (OR, 0.42; 95% CI, 0.22–0.88; $P_{\text{trend}} = 0.429$) and for Latinos (OR, 0.54; 95% CI, 0.34–0.86; $P_{\text{trend}} = 0.006$) than for CC homozygotes but not among Japanese-Americans or Whites.

Statistical mediation analyses were conducted for the *GCKR* rs780094 polymorphism in the total study population. In no model were circulating biomarkers, independently or in combination, found to influence the SNP–colorectal cancer association. Statistical mediation analyses for racial/ethnic-specific associations could not be conducted because of the limited data for participants possessing variant homozygote genotypes and circulating hormone levels within racial/ethnic groups.

Table 4. ORs and 95% CIs for tertiles of circulating IGF system biomarkers, insulin, glucose, and colorectal cancer

	Cases/controls	Tertile 1 OR (95% CI)	Tertile 2 OR (95% CI)	Tertile 3 OR (95% CI)	P_{trend}[d]	$P_{for\ racial/ethnic\ heterogeneity}$[e]
IGF-I						
Median, ng/mL		109.81	164.71	224.29		
Model 1[a]	258/1,701	1.00	0.67 (0.48–0.94)	0.84 (0.60–1.17)	0.301	0.788
Model 2[b]	258/1,701	1.00	0.80 (0.56–1.16)	1.18 (0.76–1.82)	0.489	0.764
Model 3[c]	249/1,571	1.00	0.80 (0.55–1.17)	1.18 (0.75–1.84)	0.483	0.869
IGF-II						
Median, ng/mL		687.50	937.02	1,283.45		
Model 1[a]	255/1,223	1.00	1.10 (0.77–1.58)	1.63 (1.14–2.32)	0.005	0.288
Model 3[c]	246/1,133	1.00	1.11 (0.76–1.61)	1.58 (1.09–2.28)	0.011	0.184
IGFBP-1						
Median, ng/mL		9.52	24.31	50.70		
Model 1[a]	258/1,701	1.00	0.86 (0.61–1.21)	0.86 (0.61–1.22)	0.486	0.736
Model 3[c]	249/1,571	1.00	0.86 (0.60–1.23)	1.00 (0.68–1.46)	0.826	0.651
IGFBP-3						
Median, ng/mL		2,880	3,830	4,819		
Model 1[a]	258/1,701	1.00	0.60 (0.43–0.83)	0.63 (0.45–0.88)	0.004	0.480
Model 2[b]	258/1,701	1.00	0.55 (0.39–0.78)	0.53 (0.34–0.82)	0.002	0.459
Model 3[c]	249/1,571	1.00	0.58 (0.40–0.83)	0.53 (0.34–0.83)	0.003	0.546
IGF-I/IGFBP-3, nmol/L						
Median		0.122	0.161	0.206		
Model 1[a]	258/1,701	1.00	0.85 (0.60–1.21)	1.19 (0.83–1.69)	0.252	0.188
Model 3[c]	249/1,571	1.00	0.84 (0.58–1.20)	1.19 (0.83–1.72)	0.257	0.076
Insulin						
Median, μIU/mL		3.04	5.75	11.41		
Model 1[a]	258/1,701	1.00	1.16 (0.83–1.62)	1.47 (1.05–2.05)	0.022	0.955
Model 3[c]	249/1,571	1.00	1.06 (0.75–1.51)	1.21 (0.84–1.75)	0.293	0.633
Glucose						
Median, mg/dL		77.18	89.87	111.89		
Model 1[a]	258/1,701	1.00	0.90 (0.65–1.25)	1.03 (0.75–1.43)	0.738	0.021
Model 3[c]	249/1,571	1.00	0.87 (0.62–1.23)	0.87 (0.62–1.22)	0.472	0.009
HOMA						
Median		0.636	1.296	2.935		
Model 1[a]	258/1,701	1.00	1.06 (0.75–1.48)	1.55 (1.12–2.16)	0.004	0.839
Model 3[c]	249/1,571	1.00	0.94 (0.66–1.34)	1.28 (0.88–1.85)	0.100	0.746

NOTE: ORs obtained from unconditional logistic regression. Tertiles based on the exposure distribution among controls.
[a]Adjusted for age, sex, and race/ethnicity.
[b]Adjusted for age, sex, race/ethnicity, and IGF-I or IGFBP-3, where appropriate.
[c]Adjusted for age, sex, race/ethnicity, history of colorectal polyp, family history of colorectal cancer, BMI (kg/m^2), moderate or vigorous physical activity, processed meat intake, pack-years of smoking, alcohol consumption, and mutual adjustment for IGF-I and IGFBP-3, where appropriate.
[d]Trend based on the median value for each tertile.
[e]P value for the test of heterogeneity across racial/ethnic groups.

Prediagnostic circulating biomarkers and the risk of colorectal cancer

The crude and multivariable adjusted ORs and 95% CIs for associations of prediagnostic circulating biomarkers with the risk of colorectal cancer are presented in Table 4. Adjusting for colorectal cancer risk factors, the risk of colorectal cancer was higher for participants in the third tertile than in the first tertile of IGF-II concentration (OR, 1.58; 95% CI, 1.09–2.28; P_{trend} = 0.011). For IGFBP-3, participants in the second (OR, 0.58; 95% CI, 0.40–0.83) and third (OR, 0.53; 95% CI, 0.34–0.83; P_{trend} = 0.003) tertiles of exposure were at a lower risk of colorectal cancer relative to those in the first. In crude models, the risk of colorectal cancer was higher for participants in the

third tertile of insulin (OR, 1.47; 95% CI, 1.05–2.05; $P_{trend} = 0.022$) and HOMA (OR, 1.55; 95% CI, 1.22–2.16; $P_{trend} = 0.004$) exposure. In multivariable models, risk estimates for insulin and HOMA no longer reached statistical significance; however, this attenuation was entirely explained by the addition of BMI to the statistical model. Racial/ethnic heterogeneity in the risk of colorectal cancer was detected for circulating glucose ($P = 0.009$), although in analyses stratified by race/ethnicity, no clear racial/ethnic differences were observed (data not shown). In analyses of circulating levels stratified on anatomic subsite, findings for colon and rectal cancers were similar to that for colorectal cancer in all models (data not shown).

Discussion

In this case–control study nested within the MEC, we were able to replicate associations for the *IGF1* rs35767 polymorphism with circulating IGF-I levels and for the *IGFBP3* rs2854744, rs2854746, and rs3110697 polymorphisms with circulating IGFBP-3 levels but not for the *GCKR* rs780094 polymorphism with circulating levels of insulin or glucose. We found a lower risk of colorectal cancer among *GCKR* rs780094 TT homozygotes which was strongest for African-Americans and Latinos, but in the direction of decreased risk for all groups included in the MEC. The lower risk of colorectal cancer among *GCKR* rs780094 TT homozygotes also appeared to be confined to participants below the median value for BMI, participants reporting no history of diabetes, and to cancers of the colon. In analyses examining associations of prediagnostic circulating IGF system biomarkers, insulin, and glucose with the risk of colorectal cancer, we found an increased risk of cancer among participants with the highest circulating levels of IGF-II and a decreased risk of cancer among participants with the highest circulating levels of IGFBP-3. These associations remained after controlling for colorectal cancer risk factors and were similar across the racial/ethnic groups included in the MEC.

To the best of our knowledge, ours is the first study to report an inverse association for the *GCKR* rs780094 polymorphism with the risk of colorectal cancer. In previous studies, the *GCKR* rs780094 variant allele has been found to be associated with decreased levels of insulin (40, 48–50), glucose (40, 48–50), and the risk of type II diabetes (40, 48–50). Thus, the association of this variant with colorectal cancer risk provides some support for the role of insulin and glucose in this cancer; however, while circulating levels of insulin and glucose were lower for *GCKR* rs780094 homozygotes, these associations did not reach statistical significance. The lack of statistical significance for the SNP and biomarker association may largely reflect our limited sample size and power for these analyses. In contrast, we found a strong association of *GCKR* rs780094 with serum IGF-II and IGFBP-3, and to a lesser extent serum IGFBP-1. Furthermore, as the *GCKR* rs780094 variant has shown pleiotropic associations with

several disease traits hypothesized to influence colorectal cancer risk, it remains plausible that if causal, the inverse association for *GCKR* rs780094 may be operating via pathways other than those examined. It is also noteworthy, that the inverse association for carriers of the *GCKR* rs780094 TT genotype was confined to participants below the median value for BMI in our sample. Thus, any effect that the variant allele may have on biologic factors influencing disease risk may be is modest and is possibly mitigated by obesity-related metabolic sequelae. Furthermore, as no association between the *GCKR* rs780094 polymorphism and the risk of colorectal cancer has been reported in GWAS to date, our results may represent a chance finding and require replication. Once public GWAS data become available, we will better be able to see how this SNP ranks in relation to the risk of disease.

We hypothesized the intronic *GCKR* polymorphism rs780094 may influence the risk of colorectal cancer via long-term modulation of circulating insulin and glucose levels based on previous reports; however, the results from recent fine mapping (51) and functional studies (52) suggest that the nonsynonymous *GCKR* rs1260326-Pro446Leu polymorphism may be the functional variant. The rs1260326 is in high linkage disequilibrium with rs780094 in several of the HapMap populations (41) and it has been shown that the variant results in the reduced ability of fructose-6-phosphate to regulate GKRP, indirectly resulting in impaired glucokinase activity and glycolytic flux (52); potentially increasing the risk of colorectal cancer. As the *GCKR* rs780094 polymorphism has been found to be correlated with various metabolic traits associated with chronic disease risk (41, 51, 53, 54), future studies should also test rs1260326 and additional related metabolic pathways, including the IGF pathway.

Our overall findings for the *IGF1* rs35767 polymorphism and colorectal cancer are consistent with 2 previous null reports (55, 56). Interestingly, despite a positive association with circulating IGF-I levels, risk estimates for colorectal cancer were in the direction of a decreased risk for carriers of the rs35767 minor A-allele, perhaps reflecting that the influence of this variant on plasma levels may be too small to affect risk. Our findings for *IGFBP-3* rs2854744 (A-202C; refs. 55–58) and rs2854746 (G-2133C; refs. 55, 56) are also consistent with several previous reports that have not detected an association between polymorphisms in this gene region and the risk of colorectal cancer. We were unable to confirm our previous report of a positive association of colorectal cancer with the rs2854746 variant C-allele in the MEC (39) with this much larger sample size.

Our findings for prediagnostic circulating levels of IGF-II and IGFBP-3 are consistent with a model by which increased colonic and rectal exposure to biologically active IGF hormones may increase the risk of cancer. In a meta-analysis of prospective studies examining associations of IGF-II with the risk of colorectal cancer, Morris

and colleagues (24) reported an increased risk of 1.95 (1.26–3.00) for individuals in the fourth versus first quartile of IGF-II exposure. For IGFBP-3, our findings are also consistent with several previous studies reporting an inverse association with the risk of colorectal cancer (14, 15). However, in a recent large study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC), Rinaldi and colleagues (13) found no association between prediagnostic IGFBP-3 levels and the risk of colorectal cancer, with several other studies also reporting null results (17, 20). Positive associations for IGFBP-3 and colorectal cancer have also been reported (16, 18), although these discrepancies may be due to differences in the assay specificities used between studies (13, 59).

For IGF-I and the IGF-I:IGFBP-3 molar ratio, we found a nonstatistically significant increased risk of colorectal cancer among participants with the highest prediagnostic circulating levels. These findings are consistent with a recent meta-analysis suggesting a modest increased risk of colorectal cancer for a one SD increase in circulating IGF-I (13), with risk estimates in individual studies generally failing to differ from unity (14, 17, 18, 20–22). Previous reports examining associations of insulin exposure with the risk of colorectal cancer have also been equivocal. A recent meta-analysis of prospective studies reported a 35% increased risk of colorectal cancer for greater prediagnostic insulin exposure (60); however, risk estimates in several studies failed to reach statistical significance (20, 26, 27, 31, 32). In our sample, the increased risk of colorectal cancer for higher prediagnostic insulin and HOMA levels in crude models was found to be attenuated upon adjustment for BMI. As insulin is a hypothesized intermediary of the established association between BMI and the risk of colorectal cancer, and as the risk estimates were similar in crude models and multivariable models that did not include adjustment for BMI, we feel these data provide additional support that obesity may operate, in part, through an insulin-mediated pathway in the etiology of colorectal cancer. In addition, at least one previous study has reported that risk estimates for IGF-I and hyperinsulinemia were attenuated in statistical models that allowed for mutual adjustment, leading the authors to conclude that confounding by insulin may account for the observed IGF-I association (28). However, in our study, mutual adjustment for insulin, IGF-I, and IGF-II had little effect on model estimates. The limited number of colorectal cancer cases across cross-classified tertiles for circulating IGF-I and insulin precluded our ability to examine the joint effects of these hormones on the risk of colorectal cancer.

There are several strengths to the current study, including the prospective design allowing for the prediagnostic assessment of exposures and covariates, the use of multiple biomarkers for IGFs and IGFBPs, the increased variation in exposure due to the diversity of the study population, and the population-based sampling frame used by the MEC allowing for the generalizability of the study results. There were also limitations. First, prediagnostic serum values for IGF system biomarkers, insulin, and glucose were only available for a subsample of study participants reducing the power to detect associations and our ability to conduct certain sub-group analyses. Second, similar to other studies in this area, only a single prediagnostic serum measurement for insulin, glucose, or IGF system biomarkers was available. Despite the potential for misclassification error and the resulting attenuation of risk estimates obtained from a single exposure measure, for IGF-I and IGFBP-3 (61–63), a single measurement has been shown to adequately rank individuals on their long-term exposure. Third, the test of statistical mediation used may not adequately capture biologic mediation (64) and assumes no confounding of the exposure–mediator or mediator–outcome associations or interaction between the exposure and the mediator.

In conclusion, our findings provide additional support to the hypothesis that greater exposure to circulating levels of IGFs and insulin may increase the risk of colorectal cancer. In addition, we detected an association between the rs780094 polymorphism in *GCKR* and risk of colorectal cancer; however, this finding requires replication. Future studies should also test rs1260326 in *GCKR* as it may represent the functional variant associated with circulating biomarker concentrations.

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