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## Associations Between Glycemic Traits and Colorectal Cancer: A Mendelian Randomization Analysis

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### Abstract

**Background:** Glycemic traits—such as hyperinsulinemia, hyperglycemia, and type 2 diabetes—have been associated with higher colorectal cancer risk in observational studies; however, causality of these associations is uncertain. We used Mendelian randomization (MR) to estimate the causal effects of fasting insulin, 2-hour glucose, fasting glucose, glycosylated hemoglobin (HbA1c), and type 2 diabetes with colorectal cancer. **Methods:** Genome-wide association study summary data were used to identify genetic variants associated with circulating levels of fasting insulin ( $n = 34$ ), 2-hour glucose ( $n = 13$ ), fasting glucose ( $n = 70$ ), HbA1c ( $n = 221$ ), and type 2 diabetes ( $n = 268$ ). Using 2-sample MR, we examined these variants in relation to colorectal cancer risk (48 214 case patient and 64 159 control patients). **Results:** In inverse-variance models, higher fasting insulin levels increased colorectal cancer risk (odds ratio [OR] per 1-SD = 1.65, 95% confidence interval [CI] = 1.15 to 2.36). We found no evidence of any effect of 2-hour glucose (OR per 1-SD = 1.02, 95% CI = 0.86 to 1.21) or fasting glucose (OR per 1-SD = 1.04, 95% CI = 0.88 to 1.23) concentrations on colorectal cancer risk. Genetic liability to type 2 diabetes (OR per 1-

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unit increase in log odds = 1.04, 95% CI = 1.01 to 1.07) and higher HbA1c levels (OR per 1-SD = 1.09, 95% CI = 1.00 to 1.19) increased colorectal cancer risk, although these findings may have been biased by pleiotropy. Higher HbA1c concentrations increased rectal cancer risk in men (OR per 1-SD = 1.21, 95% CI = 1.05 to 1.40), but not in women. **Conclusions:** Our results support a causal effect of higher fasting insulin, but not glucose traits or type 2 diabetes, on increased colorectal cancer risk. This suggests that pharmacological or lifestyle interventions that lower circulating insulin levels may be beneficial in preventing colorectal tumorigenesis.

Obesity is an established risk factor for colorectal cancer development (1–3) and is invariably characterized by dysregulated metabolism, such as insulin resistance, hyperinsulinemia, hyperglycemia, and type 2 diabetes (4). Extensive epidemiological research has shown that patients with type 2 diabetes are at higher colorectal cancer risk than those without diabetes (5,6). However, recent findings from 2 relatively small Mendelian randomization (MR) studies (both including fewer than 7000 colorectal cancer case patients) did not support a causal relationship between genetic liability to type 2 diabetes and colorectal cancer (7,8). Prior epidemiologic studies examining how prediagnostic concentrations of fasting glucose, glucose tolerance (the measurement of circulating glucose levels 2 hours after an oral glucose challenge), and glycated hemoglobin (HbA1c) relate to colorectal cancer risk have reported conflicting results (9–15). Numerous epidemiological studies have examined the associations between circulating levels of insulin and colorectal cancer risk, with positive associations generally found in studies that measured circulating levels of C-peptide (a marker of insulin secretion) (16–18), but inconsistent results reported in studies that directly measured insulin levels (19–24). Possible explanations for the conflicting results to date include the use of nonfasting blood samples in some studies, differences in laboratory assays used, and the vulnerability of prior investigations to the inherent biases of observational studies, such as residual confounding and reverse causality.

MR uses germline genetic variants as instrumental variables to allow causal effects of an exposure and outcome relationship to be estimated. Due to the random assortment of alleles during meiosis and germline genetic variants being fixed at conception, MR analyses are less susceptible to conventional confounding and reverse causality. To date, a large-scale MR study examining the associations between multiple glycemic traits and colorectal cancer has not been reported.

We used 2-sample MR to examine potential causal effects of glycemic traits on colorectal cancer risk. This involved combining genetic variants robustly associated with circulating concentrations of fasting insulin, 2-hour glucose, fasting glucose and HbA1c, and type 2 diabetes in genome-wide association studies (GWAS) and then assessing the association of these variants with colorectal cancer risk in a large consortium including up to 48 214 colorectal cancer case patients and 64 159 control patients (25).

## Methods

### Genetic Determinants of Glycemic Traits

Genetic instrumental variables comprised single nucleotide polymorphism (SNPs) identified as being robustly associated with each glycemic trait (at  $P < 5 \times 10^{-8}$ ) from the largest GWAS of that trait to date (26–29). For circulating concentrations of 2-hour glucose, fasting glucose, and fasting insulin, the Meta-Analyses of Glucose and Insulin-related traits Consortium

(MAGIC) GWAS included 63 396, 200 622, and 151 013 participants, respectively (28). Each glycemic trait was regressed with body mass index (BMI), study-specific covariates, and principal components (28). For HbA1c, the GWAS conducted by the Neale laboratory included 361 194 UK Biobank participants (27) and used least-squares linear models with sex and the first 10 principal components from the UK Biobank sample quality control (QC) file as covariates. For type 2 diabetes, the GWAS included 74 124 type 2 diabetes cases and 824 006 controls without type 2 diabetes (26). Within each contributing study, all variants were tested for the association with type 2 diabetes using regression models, with and without adjustment for BMI, and additionally adjusted for study-specific covariates as well as principal components. Participants were of European ancestry, approximately 55% were women, and aged a mean of more than 50 years. From the genome-wide significant variants identified in these GWAS for each glycemic trait, we excluded correlated SNPs based on a linkage disequilibrium level of  $R^2$  less than 0.01 using genotype data from European individuals from phase 3 (version 5) enrolled in the 1000 Genomes Project as a reference panel. The proportion of variance explained by the genetic instruments for the glycemic traits ranged from 0.6% to 5.7% (Table 1). We also estimated the F-statistic, a formal test of whether the proportion of variance explained is sufficiently high for a trait given the sample size used. In our study, the estimated F-statistic values were greater than 516 for all genetic instruments. Summary information on the genetic instruments, and the effect estimates for each individual SNP with concentrations of fasting insulin ( $n = 34$  SNPs), 2-hour glucose ( $n = 13$  SNPs), fasting glucose ( $n = 70$  SNPs), HbA1c ( $n = 221$  SNPs), and type 2 diabetes ( $n = 268$  SNPs), are presented in Table 1 and Supplementary Tables 1 and 2 (available online).

### Data on Colorectal Cancer

Summary data for associations of the glycemic traits with colorectal cancer were obtained from a GWAS of 112 373 participants (48 214 colorectal cancer cases and 64 159 controls). For HbA1c, summary data were sourced from a smaller colorectal cancer GWAS of 85 638 participants (42 886 colorectal cancer cases and 42 752 controls) that excluded UK Biobank to avoid sample overlap. The GWAS data were from a meta-analysis that combined the ColoRectal Transdisciplinary Study (CORECT), the Colon Cancer Family Registry (CCFR), and studies within the Genetics and Epidemiology of Colorectal Cancer (GECCO) consortium (30). Imputation was performed using the Haplotype Reference Consortium r1.1 reference panel. Logistic regression models were adjusted for age, sex, and study or genotyping project to specific covariates, including principal components (of all genetic variants that surpassed quality control filtering) to adjust for population structure (25). Participants were of European ancestry, approximately 55% were women, and aged a mean of more than 50 years. All participants provided written informed consent, and each study was approved by the relevant

**Table 1.** Summary of the glycemic trait instrument variables used in this study<sup>a</sup>

Glycemic trait	No. of SNPs	Variance explained, %
Fasting insulin (29)	34	0.6
2-hour glucose (29)	13	2.4
Fasting glucose (29)	70	1.4
Glycated hemoglobin (HbA1c) (27)	221	5.7
Type 2 diabetes (26)	268	2.0

<sup>a</sup>SNP = single nucleotide polymorphism.

research ethics committee or institutional review board. The effect estimates for associations of each individual glycemic trait related SNP with colorectal cancer from the GECCO, CORECT, and CCFR meta-analysis are presented in [Supplementary Table 1](#) (available online). For sensitivity analyses, summary-level data for the associations for glycemic trait related variants with colorectal cancer were also obtained from a FinnGen consortium GWAS of 2435 colorectal cancer cases and 147 282 non-cancer cases (31).

### Statistical Power

Post hoc statistical power was calculated using an online tool at <https://shiny.cnsgenomics.com/mRnd/>. We had sufficient statistical power (>80%) to detect relatively small causal effect estimates with minimum expected odds ratios (ORs) per 1 SD ranging from 1.09 to 1.24 for glycemic traits in relation to colorectal cancer risk ([Supplementary Table 3](#), available online).

### Statistical Analysis

Two-sample random-effects inverse variance weighted methods were implemented. Odds ratios were scaled to a 1-SD increase in log of fasting insulin (mean approximately 57 pmol/mol; SD approximately 42 pmol/mol), 2-hour glucose (mean approximately 5 mmol/L; SD approximately 0.6 mmol/L), fasting glucose (mean approximately 6 mmol/L; SD approximately 1.6 mmol/L), and HbA1c (mean approximately 36 mmol/mol; SD approximately 6.7 mmol/mol) concentrations; and a 1-unit increase in log odds of type 2 diabetes. False discovery rate correction was computed (q-value; statistical significance level <.05) for the primary analyses—sexes combined inverse variance weighted models for colorectal cancer—using the Benjamini-Hochberg method (32). Heterogeneity by sex and anatomical subsite (colon, proximal colon, distal colon, and rectum) was assessed by calculating  $\chi^2$  statistics. Cochran's Q statistics quantified heterogeneity across individual SNPs. Sensitivity analyses were conducted to assess and correct for the presence of horizontal pleiotropy (ie, genetic variants influencing colorectal cancer via an alternate biological pathway, independent of the glycemic exposure of interest). To evaluate the extent to which directional pleiotropy (nonbalanced horizontal pleiotropy in the MR risk estimates) may have affected the causal estimates, we used MR-Egger regression (33). We also computed odds ratios using the complementary weighted median method that can provide valid MR estimates under the presence of pleiotropy when up to 50% of the included instruments are invalid (34). The presence of pleiotropy was also assessed using the MR pleiotropy residual sum and outlier test (MR-PRESSO), in which outlying SNPs are excluded from the instruments and the effect estimates are reassessed (35).

The GWAS used for the fasting insulin genetic instrument adjusted for BMI, however, conditioning on BMI (a heritable covariable) may introduce bias if BMI is a collider in the pathway between the genetic instrument of fasting insulin and/or the genetic instrument to colorectal cancer relationships. Therefore, we conducted a sensitivity analysis excluding variants related to BMI at the  $P$  less than  $5 \times 10^{-8}$  ( $n = 9$ ) level (identified by searching <http://www.phenoscanner.medschl.cam.ac.uk/>; date checked May 2021). For type 2 diabetes, the genetic instrument included GWAS estimates unadjusted for BMI, but to assess the possible influence of collider bias on our MR estimates, we conducted a sensitivity analysis using BMI-adjusted GWAS summary estimates in the genetic instrument. Finally, in a sensitivity analysis, separate MR analyses were also conducted using data from the FinnGen consortium, and estimates were combined with those from our main analyses (GECCO, CORECT, and CCFR) using fixed-effects meta-analysis.

All statistical tests were 2-sided. Thresholds for nominal significance (for the secondary and sensitivity analyses) were set at  $P$  less than .05. All statistical analyses were performed using the *MendelianRandomization* R package (36).

## Results

### Effect of Fasting Insulin and Colorectal Cancer

Higher fasting insulin levels increased colorectal cancer risk (OR per 1-SD, 1.65, 95% confidence interval [CI] = 1.15 to 2.36,  $q$ -value = 0.035). Evidence of effect heterogeneity by SNP was found (Cochran's  $Q$   $P = 1.6 \times 10^{-7}$ ), but little evidence of directional pleiotropy was detected (MR-Egger intercept  $P = .78$ ). Positive effect estimates were also found in the weighted median, MR-Egger, and MR-PRESSO models ([Table 2](#)). There was little evidence of heterogeneity by sex in the inverse variance weighted models ( $P_{\text{heterogeneity}} = .9$ ), although evidence of pleiotropy was detected for women in the weighted median and MR-Egger models. Similar effect estimates were also found for all colorectal cancer subsites ( $P_{\text{heterogeneity}}$  for colon vs rectal cancer = .98;  $P_{\text{heterogeneity}}$  for proximal colon vs distal colon cancer = .98) ([Table 2](#)). In the sensitivity analysis that excluded genetic variants associated with BMI ( $n = 9$  SNPs removed), similar strength positive effect estimates were found ([Supplementary Table 4](#), available online). Scatter plots (with colored lines representing the slopes of the different regression analyses) for the fasting insulin, plus other glycemic traits, and colorectal cancer association are presented in [Supplementary Figure 1](#) (available online). A similar association without evidence of heterogeneity ( $I^2 = 0\%$ ) was found for fasting insulin with colorectal cancer when estimates using data from GECCO, CORECT, and CCFR and FinnGen were pooled (OR per 1-SD = 1.68, 95% CI = 1.12 to 2.23) ([Supplementary Table 5](#), available online).

### Effects of 2-Hour Glucose, Fasting Glucose, and HbA1c on Colorectal Cancer

We found no evidence of any effects of 2-hour glucose (OR per 1-SD increase = 1.02, 95% CI = 0.86 to 1.21,  $q$ -value = 0.81) or fasting glucose (OR per 1-SD increase = 1.04, 95% CI = 0.88 to 1.23,  $q$ -value = 0.81) on colorectal cancer in the inverse variance weighted models. Similar null effect estimates were found for men and women ( $P_{\text{heterogeneity}} > .2$ ), across anatomical subsites ( $P_{\text{heterogeneity}}$  for colon vs rectal cancer >.2;  $P_{\text{heterogeneity}}$  for

Table 2. MR estimates for glyemic traits and risk of colorectal cancer

Glyemic trait	IVW random effects		$P_{\text{heterogeneity}}^a$	Weighted median OR (95% CI)	MR-Egger OR (95% CI)	MR-Egger intercept $P^b$	MR-PRESSO	
	OR (95% CI)	OR (95% CI)					OR (95% CI)	SNPs excluded
<b>Fasting insulin<sup>c</sup></b>								
<b>Colorectal cancer</b>								
All	1.65 (1.15 to 2.36)	1.48 (1.01 to 2.16)	$1.60 \times 10^{-7}$	1.39 (0.43 to 4.57)	.78	1.52 (1.11 to 2.10)	rs11727676	
Men	1.72 (1.05 to 2.80)	1.72 (1.05 to 2.80)	$5.70 \times 10^{-7}$	2.32 (0.47 to 11.02)	.7	1.55 (1.00 to 2.39)	rs11727676	
Women	1.65 (1.14 to 2.39)	1.05 (0.66 to 1.70)	.05	0.68 (0.21 to 2.16)	.11	No outliers		
<b>Colon cancer</b>								
All	1.73 (1.16 to 2.59)	1.60 (1.04 to 2.46)	$8.70 \times 10^{-6}$	1.63 (0.44 to 6.05)	.93	1.57 (1.12 to 2.23)	rs11727676	
Men	1.57 (0.96 to 2.59)	1.22 (0.69 to 2.16)	.003	1.80 (0.36 to 9.03)	.86	1.39 (0.91 to 2.14)	rs11727676	
Women	1.92 (1.21 to 3.06)	1.55 (0.88 to 2.75)	.01	1.35 (0.30 to 6.17)	.63	No outliers		
<b>Proximal colon cancer</b>								
All	1.77 (1.14 to 2.75)	2.05 (1.22 to 3.46)	.005	1.55 (0.37 to 6.49)	.85	1.62 (1.08 to 2.41)	rs11727676	
Men	1.43 (0.81 to 2.56)	1.60 (0.78 to 3.29)	.07	1.34 (0.20 to 9.03)	.94	No outliers		
Women	2.23 (1.34 to 3.67)	2.23 (1.15 to 4.35)	.16	1.77 (0.34 to 9.21)	.77	No outliers		
<b>Distal colon cancer</b>								
All	1.79 (1.08 to 2.94)	2.03 (1.16 to 3.56)	$1.50 \times 10^{-4}$	2.10 (0.41 to 10.49)	.84	1.62 (1.02 to 2.53)	rs11727676	
Men	1.79 (1.02 to 3.10)	1.70 (0.83 to 3.42)	.07	2.66 (0.44 to 16.28)	.65	No outliers		
Women	1.79 (0.95 to 3.39)	1.77 (0.79 to 3.90)	.01	1.46 (0.18 to 11.59)	.84	No outliers		
<b>Rectal cancer</b>								
All	1.72 (1.14 to 2.56)	1.79 (1.07 to 3.00)	.04	1.19 (0.32 to 4.39)	.57	No outliers		
Men	2.08 (1.14 to 3.78)	2.16 (1.09 to 4.31)	.003	2.66 (0.38 to 18.17)	.79	2.34 (1.34 to 4.06)	rs73013411	
Women	1.39 (0.81 to 2.39)	1.86 (0.87 to 3.94)	.3	0.46 (0.08 to 2.56)	.18	No outliers		
<b>2-hour glucose<sup>c</sup></b>								
<b>Colorectal cancer</b>								
All	1.02 (0.86 to 1.21)	1.05 (0.92 to 1.20)	$6.40 \times 10^{-7}$	0.82 (0.52 to 1.28)	.3	1.12 (0.99 to 1.27)	rs1260326, rs117643180	
Men	0.97 (0.81 to 1.17)	1.06 (0.90 to 1.25)	$9.90 \times 10^{-4}$	0.75 (0.45 to 1.23)	.26	1.02 (0.87 to 1.20)	rs1260326	
Women	1.06 (0.90 to 1.26)	1.07 (0.90 to 1.26)	.01	0.90 (0.57 to 1.45)	.47	No outliers		
<b>Colon cancer</b>								
All	1.00 (0.84 to 1.17)	1.02 (0.88 to 1.19)	$3 \times 10^{-4}$	0.79 (0.50 to 1.23)	.28	1.03 (0.89 to 1.20)	rs1260326	
Men	0.98 (0.84 to 1.15)	1.02 (0.84 to 1.25)	.14	0.78 (0.50 to 1.22)	.28	No outliers		
Women	1.02 (0.83 to 1.26)	0.99 (0.81 to 1.21)	.003	0.81 (0.45 to 1.46)	.41	No outliers		
<b>Proximal colon cancer</b>								
All	0.98 (0.85 to 1.12)	0.90 (0.75 to 1.06)	.26	0.77 (0.54 to 1.11)	.17	No outliers		
Men	0.95 (0.79 to 1.14)	0.94 (0.73 to 1.21)	.69	0.90 (0.55 to 1.48)	.84	No outliers		
Women	1.01 (0.84 to 1.22)	1.00 (0.79 to 1.27)	.22	0.70 (0.43 to 1.16)	.13	No outliers		
<b>Distal colon cancer</b>								
All	1.04 (0.84 to 1.30)	1.03 (0.85 to 1.25)	$5.00 \times 10^{-4}$	0.87 (0.47 to 1.58)	.52	1.11 (0.93 to 1.31)	rs1260326	
Men	1.04 (0.84 to 1.30)	0.96 (0.75 to 1.23)	.09	0.79 (0.43 to 1.46)	.34	No outliers		
Women	1.05 (0.77 to 1.42)	0.97 (0.73 to 1.28)	$9.00 \times 10^{-4}$	0.98 (0.40 to 2.36)	.86	1.12 (0.84 to 1.48)	rs1260326	
<b>Rectal cancer</b>								
All	1.05 (0.89 to 1.26)	1.06 (0.89 to 1.27)	.02	0.84 (0.52 to 1.34)	.29	No outliers		

(continued)

Table 2. (continued)

Glycemic trait	IVW random effects		P <sub>heterogeneity</sub> <sup>a</sup>	Weighted median		MR-Egger		MR-Egger intercept p <sup>b</sup>	MR-PRESSO	
	OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)	OR (95% CI)	SNPs excluded			
Men	1.05 (0.84 to 1.32)	1.03 (0.81 to 1.30)	.03	0.95 (0.50 to 1.82)	1.12 (0.91 to 1.35)	rs1260326	rs1260326	.74	1.12 (0.91 to 1.35)	rs1260326
	1.05 (0.86 to 1.30)	0.93 (0.71 to 1.21)	.3	0.74 (0.43 to 1.27)	No outliers					
Women	1.04 (0.88 to 1.23)	1.05 (0.89 to 1.25)	4.9 × 10 <sup>-10</sup>	1.01 (0.75 to 1.36)	0.97 (0.84 to 1.12)	rs1260326, rs174583	rs1260326, rs174583	.84	0.97 (0.84 to 1.12)	rs1260326, rs174583
	0.90 (0.71 to 1.14)	0.96 (0.72 to 1.30)	4.8 × 10 <sup>-4</sup>	1.03 (0.68 to 1.57)	0.90 (0.76 to 1.07)					
Men	1.11 (0.92 to 1.34)	1.01 (0.80 to 1.28)	.003	1.02 (0.73 to 1.42)	1.07 (0.90 to 1.27)	rs174583	rs174583	.54	1.07 (0.90 to 1.27)	rs174583
	0.96 (0.79 to 1.16)	0.90 (0.73 to 1.09)	1.40 × 10 <sup>-7</sup>	0.95 (0.68 to 1.34)	0.93 (0.79 to 1.11)					
Women	0.90 (0.71 to 1.14)	0.97 (0.72 to 1.30)	4.80 × 10 <sup>-4</sup>	1.03 (0.68 to 1.57)	0.90 (0.73 to 1.11)	rs174583	rs174583	.47	0.90 (0.73 to 1.11)	rs174583
	1.22 (0.83 to 1.26)	0.87 (0.66 to 1.14)	.02	0.89 (0.61 to 1.30)	0.99 (0.81 to 1.21)					
Men	0.89 (0.73 to 1.06)	0.82 (0.64 to 1.06)	.04	0.85 (0.61 to 1.19)	0.87 (0.73 to 1.04)	rs174583	rs174583	.78	0.87 (0.73 to 1.04)	rs174583
	0.89 (0.68 to 1.16)	0.92 (0.63 to 1.36)	.1	1.03 (0.64 to 1.65)	No outliers					
Women	0.88 (0.70 to 1.11)	0.71 (0.51 to 1.00)	.45	0.72 (0.48 to 1.07)	No outliers	rs174583	rs174583	.22	No outliers	rs174583
	1.05 (0.83 to 1.34)	0.99 (0.75 to 1.30)	6 × 10 <sup>-7</sup>	0.97 (0.63 to 1.49)	0.98 (0.80 to 1.20)					
Men	0.94 (0.71 to 1.25)	0.99 (0.68 to 1.43)	.01	0.98 (0.59 to 1.60)	0.90 (0.70 to 1.16)	rs174583	rs174583	.86	0.90 (0.70 to 1.16)	rs174583
	1.19 (0.88 to 1.60)	0.97 (0.66 to 1.42)	.003	0.94 (0.55 to 1.62)	1.13 (0.85 to 1.51)					
Men	1.17 (0.96 to 1.43)	1.12 (0.85 to 1.48)	.01	1.03 (0.73 to 1.46)	1.14 (0.94 to 1.38)	rs174583	rs174583	.38	1.14 (0.94 to 1.38)	rs174583
	1.11 (0.86 to 1.42)	1.17 (0.83 to 1.65)	.06	1.05 (0.67 to 1.63)	No outliers					
Women	1.23 (0.96 to 1.60)	1.00 (0.67 to 1.49)	.54	0.98 (0.63 to 1.54)	No outliers	rs174583	rs174583	.21	No outliers	rs174583
	1.09 (1.00 to 1.19)	1.06 (0.95 to 1.17)	2.80 × 10 <sup>-21</sup>	0.93 (0.78 to 1.11)	1.06 (0.99 to 1.14)					
Men	1.09 (0.98 to 1.21)	1.06 (0.92 to 1.23)	4.80 × 10 <sup>-9</sup>	0.95 (0.77 to 1.18)	1.07 (0.97 to 1.17)	rs174549, rs76895963, rs61927768, rs10784889, rs11065979	rs174549, rs76895963, rs61927768, rs10784889, rs11065979	.16	1.07 (0.97 to 1.17)	rs174549, rs61927768, rs10784889, rs11065979
	1.09 (0.99 to 1.21)	1.03 (0.90 to 1.20)	1.60 × 10 <sup>-6</sup>	0.91 (0.74 to 1.11)	1.07 (0.97 to 1.17)					
Men	1.06 (0.95 to 1.17)	1.05 (0.92 to 1.20)	8.00 × 10 <sup>-17</sup>	0.94 (0.77 to 1.15)	1.03 (0.95 to 1.13)	rs11065979	rs11065979	.2	1.03 (0.95 to 1.13)	rs11065979
	1.08 (0.95 to 1.23)	1.03 (0.86 to 1.23)	5.60 × 10 <sup>-9</sup>	0.95 (0.72 to 1.24)	1.08 (0.95 to 1.22)					
Women	1.05 (0.94 to 1.17)	1.01 (0.84 to 1.20)	9.80 × 10 <sup>-4</sup>	0.94 (0.75 to 1.18)	1.03 (0.93 to 1.15)	rs11065979	rs11065979	.28	1.03 (0.93 to 1.15)	rs11065979
	1.06 (0.95 to 1.17)	1.00 (0.85 to 1.17)	1.20 × 10 <sup>-9</sup>	0.87 (0.69 to 1.10)	1.06 (0.95 to 1.17)					
Men	1.06 (0.95 to 1.19)	1.00 (0.85 to 1.17)	1.20 × 10 <sup>-9</sup>	0.87 (0.69 to 1.10)	1.06 (0.95 to 1.17)	rs10784889, rs11065979	rs10784889, rs11065979	.06	1.06 (0.95 to 1.17)	rs10784889, rs11065979
	1.10 (0.94 to 1.28)	1.06 (0.85 to 1.33)	2.40 × 10 <sup>-4</sup>	0.94 (0.67 to 1.26)	1.08 (0.94 to 1.26)					
Women	1.03 (0.90 to 1.18)	1.04 (0.84 to 1.30)	.01	0.83 (0.63 to 1.09)	No outliers	rs10784889, rs11065979	rs10784889, rs11065979	.19	1.08 (0.94 to 1.26)	rs10784889, rs11065979
	1.03 (0.90 to 1.18)	1.04 (0.84 to 1.30)	.01	0.83 (0.63 to 1.09)	No outliers					

(continued)

Table 2. (continued)

Glycemic trait	IVW random effects		$P_{\text{heterogeneity}}^a$	Weighted median		MR-Egger intercept		MR-PRESSO	
	OR (95% CI)			OR (95% CI)		OR (95% CI)		OR (95% CI)	SNPs excluded
All	1.08 (0.96 to 1.22)		$1.90 \times 10^{-10}$	0.96 (0.82 to 1.14)		1.07 (0.83 to 1.36)	.9	1.07 (0.96 to 1.19)	rs7766070, rs174549, rs61927768, rs11065979
Men	1.07 (0.92 to 1.26)		$3.80 \times 10^{-7}$	1.02 (0.81 to 1.29)		0.99 (0.72 to 1.40)	.61	1.06 (0.90 to 1.24)	rs3104369
Women	1.09 (0.94 to 1.26)		.01	1.06 (0.84 to 1.32)		1.13 (0.83 to 1.53)	.79	1.08 (0.94 to 1.25)	rs11065979
Rectal cancer									
All	1.19 (1.06 to 1.33)		$1.60 \times 10^{-6}$	1.07 (0.95 to 1.30)		1.03 (0.82 to 1.30)	.16	1.14 (1.03 to 1.27)	rs9273363, rs61927768, rs11065979
Men	1.21 (1.05 to 1.40)		$5.90 \times 10^{-4}$	1.37 (1.11 to 1.70)		1.26 (0.94 to 1.69)	.77	No outliers	
Women	1.16 (0.99 to 1.35)		.01	0.95 (0.75 to 1.22)		0.78 (0.57 to 1.06)	.004	1.14 (0.98 to 1.32)	rs3130453
Type 2 diabetes <sup>d</sup>									
Colorectal cancer									
All	1.04 (1.01 to 1.07)		$1.90 \times 10^{-16}$	1.00 (0.96 to 1.04)		0.97 (0.90 to 1.04)	.04	1.04 (1.01 to 1.07)	rs1260326, rs9379084, rs7756992, rs76895963
Men	1.02 (0.98 to 1.06)		$1.30 \times 10^{-6}$	1.00 (0.94 to 1.05)		0.96 (0.88 to 1.05)	.15	1.02 (0.99 to 1.06)	rs76895963, rs2736177
Women	1.06 (1.02 to 1.09)		$4.00 \times 10^{-6}$	0.99 (0.94 to 1.05)		0.98 (0.90 to 1.07)	.06	1.07 (1.03 to 1.11)	rs7756992
Colon cancer									
All	1.03 (1.00 to 1.07)		$3.30 \times 10^{-10}$	0.98 (0.94 to 1.03)		0.97 (0.90 to 1.05)	.08	1.04 (1.01 to 1.08)	rs7756992, rs1561927
Men	1.01 (0.97 to 1.06)		.002	0.98 (0.91 to 1.05)		0.93 (0.85 to 1.03)	.08	1.02 (0.98 to 1.07)	rs76895963
Women	1.05 (1.01 to 1.09)		$1.20 \times 10^{-4}$	1.01 (0.94 to 1.07)		1.01 (0.91 to 1.12)	.34	1.06 (1.02 to 1.11)	rs7756992
Proximal colon cancer									
All	1.03 (0.99 to 1.07)		$4.20 \times 10^{-5}$	0.97 (0.92 to 1.03)		0.96 (0.88 to 1.05)	.1	1.03 (0.99 to 1.06)	rs6518681
Men	1.01 (0.96 to 1.06)		.47	1.02 (0.94 to 1.11)		0.93 (0.83 to 1.04)	.12	No outliers	
Women	1.05 (1.00 to 1.11)		.002	1.00 (0.91 to 1.08)		1.00 (0.89 to 1.13)	.31	No outliers	
Distal colon cancer									
All	1.04 (1.00 to 1.08)		$1.25 \times 10^{-7}$	1.04 (0.98 to 1.11)		0.98 (0.89 to 1.08)	.19	1.05 (1.01 to 1.09)	rs7756992, rs2736177, rs10811647
Men	1.02 (0.97 to 1.08)		.002	0.95 (0.88 to 1.04)		0.95 (0.84 to 1.07)	.21	No outliers	
Women	1.06 (1.01 to 1.13)		$1.70 \times 10^{-4}$	1.02 (0.92 to 1.12)		1.03 (0.90 to 1.17)	.56	No outliers	
Rectal cancer									
All	1.04 (1.00 to 1.08)		$2.90 \times 10^{-7}$	1.00 (0.93 to 1.07)		0.97 (0.88 to 1.07)	.11	1.04 (1.00 to 1.08)	rs149717632
Men	1.03 (0.97 to 1.08)		.001	1.03 (0.94 to 1.13)		0.98 (0.87 to 1.11)	.41	1.02 (0.97 to 1.08)	rs149717632
Women	1.06 (1.00 to 1.12)		.004	0.99 (0.90 to 1.08)		0.96 (0.84 to 1.09)	.1	No outliers	

<sup>a</sup>Cochran's Q statistics (2-sided) quantified heterogeneity across individual SNPs. CI = confidence interval; IVW = inverse-variance-weighted; MR = Mendelian randomization; OR = odds ratio; PRESSO = pleiotropy residual sum and outlier test; SNP = single nucleotide polymorphism.

<sup>b</sup>MR-Egger intercept test (2-sided P value).

<sup>c</sup>Odds ratios scaled to 1-SD increase in log of genetically predicted 2-hour glucose, fasting glucose, glycated hemoglobin, and fasting insulin levels.

<sup>d</sup>Odds ratios scaled to 1-unit increase in log odds of genetic liability to type 2 diabetes.

proximal colon vs distal colon cancer  $>.3$ ), and for the weighted median, MR-Egger, and MR-PRESSO models (Table 2).

In the inverse variance weighted model, a positive effect was found for HbA1c concentration with colorectal cancer risk (OR per 1-SD increase = 1.09, 95% CI = 1.00 to 1.19;  $q$ -value = 0.08), with similar effects in men and women ( $P_{\text{heterogeneity}} = 1$ ) (Table 2). However, evidence of effect heterogeneity (Cochran's  $Q P = 2.8 \times 10^{-21}$ ) and directional pleiotropy was detected (MR-Egger intercept  $P = .04$ ), with no evidence of causal effects found in the weighted median, MR-Egger, and MR-PRESSO models. Little evidence of heterogeneity was observed across anatomical subsites ( $P_{\text{heterogeneity}}$  for colon vs rectal cancer = 0.14;  $P_{\text{heterogeneity}}$  for proximal colon vs distal colon cancer = .83). A positive effect of HbA1c on rectal cancer was found (OR per 1-SD increase = 1.19, 95% CI = 1.06 to 1.33), but this effect was attenuated towards the null in the weighted median and MR-Egger models. For men, however, a positive effect was found for HbA1c concentration and rectal cancer (OR per 1-SD = 1.21, 95% CI = 1.05 to 1.40), with evidence of effect heterogeneity (Cochran's  $Q P = 5.9 \times 10^{-4}$ ) but little evidence of directional pleiotropy (MR-Egger intercept  $P = .77$ ). Similar effect estimates were observed for rectal cancer in men in the weighted median, MR-Egger, and MR-PRESSO models (Table 2).

### Effects of Type 2 Diabetes and Colorectal Cancer

In the inverse variance weighted model, a weak positive effect was found between genetic liability to type 2 diabetes and colorectal cancer (OR per 1-unit increase in log odds = 1.04, 95% CI = 1.01 to 1.07,  $q$ -value = 0.05), with similar magnitude of effects by sex ( $P_{\text{heterogeneity}} = .14$ ) and anatomical subsites ( $P_{\text{heterogeneity}}$  for colon vs rectal cancer = .71;  $P_{\text{heterogeneity}}$  for proximal colon cancer vs distal colon cancer = .73) (Table 2). However, no evidence of causal effects was detected in the weighted median (OR = 1.00, 95% CI = 0.96 to 1.04) or MR-Egger models (OR = 0.97, 95% CI = 0.90 to 1.04), with evidence of effect heterogeneity (Cochran's  $Q P = 1.9 \times 10^{-16}$ ) and directional pleiotropy detected (MR-Egger intercept  $P = .04$ ). A similar pattern of results to the inverse variance weighted model was found when the MR-PRESSO test detected outlier SNPs were excluded from the models (Table 2) and when type 2 diabetes GWAS summary estimates adjusted for BMI were used in the genetic instrument (Supplementary Table 6, available online).

### Discussion

We conducted the largest and most comprehensive study to date on the effects of multiple glycemic traits with colorectal cancer risk. We found that higher circulating fasting insulin levels increased colorectal cancer risk, with minimal evidence of heterogeneity by sex or anatomical subsite found. There was no evidence of effects of 2-hour glucose and fasting glucose on colorectal cancer risk. Genetic liability to type 2 diabetes and higher HbA1c concentration also appeared to increase colorectal cancer risk, but horizontal pleiotropy may have influenced these findings. Higher HbA1c concentrations increased rectal cancer risk in men.

Many experimental and observational epidemiological studies have examined the insulin and colorectal cancer relationship. Experimental studies have demonstrated that insulin, through binding to its cognate receptor or the insulin-like growth factor receptor, activates the phosphoinositide 3-

kinase-protein kinase B -mammalian target of rapamycin (PI3K-AKT-mTOR) and Ras-mitogen-activated protein kinase (RAS to MAPK) pathways, which in turn can lead to downstream cellular proliferation and protein synthesis in tumor cells (37,38). Rat models have demonstrated that insulin can induce proliferation of colorectal epithelial cells and the development of aberrant crypt foci, the primary neoplastic lesions in colorectal development (39). In colonic tumor cells, the expression of the insulin receptor protein is elevated, particularly isoform A, which exerts mitogenic effects (40,41).

This experimental evidence is supported by results from epidemiological studies that have examined the association between prediagnostic C-peptide concentrations and colorectal cancer risk (17). Two US-based prospective studies from the early 2000s reported positive associations between circulating C-peptide levels and colorectal cancer risk (16–18). More recently, a meta-analysis of 8 prospective studies reported a pooled odds ratio of 1.39 (95% CI = 1.04 to 1.87) for the comparison of the highest vs lowest C-peptide-level groups (16). Prior prospective studies that assessed the association between circulating fasting insulin levels and colorectal cancer have yielded inconsistent results, with positive associations found in some studies that were attenuated after statistical adjustment for other colorectal cancer risk factors (19–21), and null results found in 2 studies that did not measure insulin levels in fasting blood samples (22,23). The use of nonfasting biospecimens, differences in laboratory assays, and the vulnerability of observational epidemiological studies to confounding or reverse causality limit causal inference of the fasting insulin and colorectal cancer association. In our MR analyses, we found a positive effect of fasting insulin on colorectal cancer, with consistent effect estimates in men and women, according to anatomical subsite, and for all the sensitivity analyses that assessed horizontal pleiotropy. This result, taken together with experimental data showing mitogenic and antiapoptotic effects of insulin (37,38), provides supportive evidence of a positive causal relationship between fasting insulin concentrations and colorectal cancer.

We found inconclusive evidence of causal effects of glucose on colorectal cancer. For 2-hour glucose and fasting glucose, our findings suggesting no evidence of an association are consistent with some (42,43) but not other (12,14,44) prior prospective observational studies. For HbA1c concentrations, we found a positive effect with colorectal cancer, but our sensitivity analyses indicated that alternate biological pathways (ie, horizontal pleiotropy) may have influenced this result. However, for rectal cancer, particularly for men, a positive effect was found that was robust to all the sensitivity analyses we used to assess the influence of horizontal pleiotropy. It is unclear why a robust positive causal effect was found for rectal cancer and for men only. Growing evidence indicates that the clinical features, genetic architecture, and risk factor profiles may differ for tumors across different anatomical locations in the colorectum (45–47). There are also emerging data that risk factors differ between men and women (45,47). However, we also cannot rule out the possibility that the HbA1c effect found for rectal cancer in men only is a chance finding. Additional well-powered studies are needed to examine the sex-specific relationship between different markers of metabolic dysregulation, including hyperglycemia, and risk of colorectal cancer at different anatomical regions.



Type 2 diabetes has been consistently associated with higher risk of developing colorectal cancer in prospective cohort studies, with a large umbrella review reporting a pooled relative risk of 1.27 (95% CI = 1.21 to 1.34) for the diabetes vs nondiabetes comparison (5,6). The results from this study, and those from 2 smaller MR studies (7,8), are generally unresponsive of a causal relationship between genetic liability to type 2 diabetes and colorectal cancer. Bias from reverse causality or residual confounding in the observational studies is a possible explanation for the divergent findings with the MR estimates. However, comparing results from these different study designs is challenging because we examined the genetic liability to type 2 diabetes, rather than the disease itself. In contrast, observational studies have included participants with or without an actual type 2 diabetes diagnosis. Collectively, our MR results suggest that elevated levels of insulin—a characteristic of prediabetes and uncontrolled diabetes—rather than glucose may be driving the positive association found between type 2 diabetes and colorectal cancer risk reported in observational studies. In support of this hypothesis, a recent Nurses' Health Study and Health Professionals Follow-up Study analysis found that the positive association between type 2 diabetes and colorectal cancer diminished over time as circulating insulin levels lowered (48). Additional studies are required to further examine which specific aspects of the pathophysiology of type 2 diabetes may promote colorectal cancer development.

Our study has several notable strengths. This was the largest MR study to date to estimate the causal effects of glycemic traits on colorectal cancer risk. We conducted multiple sensitivity analyses to examine the possible influence of pleiotropy in biasing our results. Crucially, the positive effects found for fasting insulin and colorectal cancer were generally robust according to these various sensitivity analyses. Several limitations of our study should be noted. First, our use of summary-level data precluded analyses according to subgroups of other colorectal cancer risk factors (eg, BMI, physical inactivity) and examination of possible nonlinear effects. In addition, the GWAS used to identify the fasting insulin genetic instruments was adjusted for BMI, which may have introduced collider bias into our MR estimates. However, we found similar results when we excluded variants associated with BMI from the fasting insulin genetic instrument. Further, similar MR estimates were found for the type 2 diabetes and colorectal cancer association using BMI unadjusted and adjusted GWAS estimates for type 2 diabetes, suggesting that collider bias had minimal influence on this relationship. In addition, results from a recent empirical study suggest that the use of covariate-adjusted GWAS summary estimates should not markedly influence downstream MR effect estimates (49). Finally, we acknowledge that the null effect estimates we observed in some of our analyses may have been a consequence of inadequate statistical power. However, our post hoc power calculation found that we had sufficient power (>80%) to detect relatively small causal effect estimates (minimum expected ORs per 1 SD ranging from 1.09 to 1.16 for 2-hour glucose, fasting glucose, HbA1c, and type 2 diabetes with colorectal cancer) (50).

In conclusion, our results support a causal effect of higher fasting insulin, but not glucose traits and genetic liability to type 2 diabetes, on colorectal cancer risk. These results suggest that high circulating insulin levels, rather than high glucose levels, may be the main driver of the positive associations found between type 2 diabetes and colorectal cancer in observational studies. The findings suggest that pharmacological or lifestyle

interventions that lower circulating insulin levels may be beneficial in preventing colorectal tumorigenesis.

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