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A Prospective Evaluation of C-peptide Levels and Colorectal Adenoma Incidence

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Abstract

Background: Obesity is a recognised positive risk factor for colorectal adenoma and colorectal cancer. Obesity is associated with insulin resistance and compensatory hyperinsulinaemia, and circulating insulin and C-peptide, a biomarker of insulin levels, have been positively associated with colorectal cancer risk. However, whether a similar relationship exists for colorectal adenomas, an established colorectal cancer precursor, is unclear.

Methods: In a nested case-control study of 273 colorectal adenoma cases and 355 matched controls from the screening arm of the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, serum C-peptide levels were measured by a chemiluminescent immunometric assay. Multivariable unconditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) for colorectal adenoma within quartiles of C-peptide. Further, to explore the temporal stability of C-peptide, repeat samples from the incident adenoma cases (n = 50) and controls (n = 30), over a 5 year period were assayed and the intra-class correlations (ICC) estimated.

Results: In a multivariable model that included established colorectal adenoma risk factors, C-peptide levels were not associated with colorectal adenoma (Q4 vs. Q1, OR 0.83, 95% CI: 0.52–

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Analysed the data: NM. Wrote the paper: NM, MJG. Commented on the manuscript and interpretation of the findings: AJC, WYH, VRH, FS, and RH.

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1.31; P -trend 0.32); similar null associations were observed by gender, by adenoma subsite and for advanced adenomas. Among control participants, the ICC value over a 5-year period was 0.66.

Conclusion: Our results suggest that higher C-peptide levels were not associated with colorectal adenoma incidence in this study population. Other biological pathways associated with obesity may be more relevant to the early stages of colorectal tumorigenesis.

Keywords

colorectal; adenoma; cancer; insulin; C-peptide, hyperinsulinaemia; resistance

Introduction

Obesity has been established as a convincing risk factor for colorectal cancer [1,2], although the biological mechanisms which mediate this relationship remain uncertain.

Hyperinsulinaemia and insulin resistance are commonly present in obese individuals and have been hypothesised to play a role in colorectal cancer aetiology [3,4]. Insulin has been shown to stimulate mitogenesis [5,6] and reduce apoptosis [7], leading to enhanced cellular proliferation. Experimental studies have also shown that insulin receptor expression is increased in colonic tumour cells compared to normal cells [8].

Epidemiological studies have generally reported a positive association between circulating insulin, or C-peptide, a marker of insulin secretion, and colorectal cancer risk [9–11], though not all studies have shown the association to be independent of related factors such as body mass index (BMI) [12]. Further, data on the association of circulating insulin levels and colorectal adenoma, an established precursor of colorectal cancer, are limited. Investigating the association of insulin production with colorectal adenoma risk may help establish a role for insulin resistance in the early stages of colorectal cancer development. The relationship between insulin levels or C-peptide levels and colorectal adenoma has rarely been investigated prospectively. Case-control studies have usually reported positive associations between circulating levels of C-peptide or insulin and colorectal adenomas risk [13–15], but the two previous prospective investigations have reported contrasting findings, with one reporting a positive relationship [16] and the other a null result [17].

To further knowledge on the potential role of insulin on the early stages of colorectal cancer development we investigated the associations between circulating C-peptide levels and sigmoidoscopy-screened, colonoscopy-confirmed incident colorectal adenoma.

Methods

Study design

This case-control study was nested within the screening arm of the Prostate, Lung, Colorectal and Ovarian Cancer Screening (PLCO) trial [18]. The PLCO trial is a large, randomized trial designed to test the efficacy of cancer screening and to investigate the etiology and early markers of cancer [19–21]. Between 1993 and 2001, 154,942 men and women aged 55–74 years, with no prior history of prostate, lung, colorectal, or ovarian cancer were enrolled from 10 U.S. states and randomly assigned to the screening or control

(non-screened) arm. At study entry (T_0), participants in the screening arm ($n = 77,465$) were offered flexible sigmoidoscopy examinations of the distal colorectum (60cm); of which, 83% were compliant ($n = 64,658$), and 89% ($n = 57,559$) of those were considered successful (insertion to at least 50cm, with greater than 90% of the mucosa visible or a suspect lesion identified). Women refused the sigmoidoscopy procedure more often than men and this increased with age; no such age related increase in non-adherence was found for men [22]. Screening arm participants were then offered a follow-up sigmoidoscopy either 3 (T_3) or 5 (T_5) years after baseline; 41,858 participants (64.7%) were screened at either of these time points. Participants who had neoplastic lesions detected were referred for colonoscopic examinations. All study participants completed a self-administered questionnaire querying demographic characteristics, medical history, family history of cancer, use of tobacco, selected drugs and hormones, and height and weight as well as a food frequency questionnaire at study entry (T_0). Available medical and pathologic reports were obtained and coded by trained medical record abstractors to follow-up on cancer and mortality outcomes up to 13 years. The institutional review boards of the U.S. National Cancer Institute and the 10 screening centers approved the study and all participants provided informed consent.

Study sample

Included within the current nested case-control study were PLCO trial screening arm participants who were assigned between September 1993 and September 1999 and had undergone a successful sigmoidoscopy at T_0 and who met the study inclusion criteria: (i) Completion of baseline risk factor/dietary questionnaire; (ii) Provided a blood sample for use in etiological investigations; (iii) No self-reported history of Crohn's disease, ulcerative colitis, familial polyposis, Gardner's syndrome, colorectal polyps or cancer (excluding basal cell skin cancer). As previously described elsewhere [23], incident colorectal adenoma cases were identified from all participants who had a negative sigmoidoscopy at T_0 , but were then found to have a left-sided colorectal adenoma at the T_3 or T_5 repeat sigmoidoscopies. Controls were individuals who had negative sigmoidoscopies at both the T_0 and the T_3 or T_5 screens and therefore deemed to be polyp-free in the distal colon and rectum. Cases were frequency matched to controls by age at study entry (55–59, 60–64, 65–69, or 70–74 years), sex, fiscal year at study entry, race/ethnicity (White, Black, Hispanic, Asian, or Pacific Islander), screening center, study protocol (T_3 or T_5 rescreen), and season of blood draw (May–September or October–April). C-peptide levels were measured in 701 participants (305 cases and 396 controls). Individuals with missing baseline BMI information and/or prevalent or unknown diabetes status were excluded from all analyses. Analyses therefore included 273 cases and 355 controls. A random sample of 30 control participants with blood samples from three time points (T_0 ; 1 year after study entry - T_1 ; and T_5) were selected to test the reproducibility of C-peptide measurements over a 5-year period. Similarly, to test whether C-peptide levels change during the development of colorectal adenoma, a random sample of 50 case participants had repeat blood samples at the T_3 or T_5 screen assayed for C-peptide levels.

Laboratory assays

Non-fasting serum samples were assayed in batches of 40 with cases and matched control sets arranged within the same batch. Specimens from participants obtained at multiple time points were assayed together in the same batch and for quality control purposes, blinded repeat samples ($n = 90$) were embedded within and between batches to test for assay reproducibility. C-peptide levels were quantified using a solid phase chemiluminescent immunometric assay using the Immulite analyzer (Siemens Medical Solutions Diagnostics) in the laboratory of Dr Frank Stanczyk at the University of Southern California, Los Angeles, USA. The intrabatch and interbatch coefficients of variation were 6.9% and 8.9%, respectively.

Statistical methods

The baseline characteristics of cases and controls were compared using the Wilcoxon two-sample test for continuous variables and the Pearson's χ^2 test for categorical variables. Serum C-peptide levels were logarithmically transformed due to not being normally distributed in the control population. Amongst the 50 cases and 30 controls with multiple C-peptide measurements, differences in medians at the various multiple time-points were assessed by calculating the Friedman's statistic. Also, in this subset of participants, intraindividual and interindividual variances in C-peptide levels were assessed using random effects models and the intraclass correlations (ICC) were assessed from these values.

Unconditional logistic regression models were used to compute odds ratios (OR) and 95% confidence intervals (95% CI) for the associations between serum C-peptide levels and incident colorectal adenoma. For the C-peptide analysis, participants were divided into quartiles based on the distribution of C-peptide levels among the control group. Additionally, C-peptide levels were also assessed continuously by entering the logarithmically transformed variable into the models. Tests of trend across C-peptide categories were calculated by entering the quartile variable into the model as a single continuous variable. The univariate models were controlled for the matching factors only. The multivariable models were adjusted for an *a priori* determined set of colorectal neoplasia risk factors, including: cigarette smoking status (never, former, or current), BMI at baseline (<25 , $25\text{--}30$, or ≥ 30 kg/m²; C-peptide analysis only), regular use of non-steroidal anti-inflammatory drugs (NSAIDs; no or yes), use of menopausal hormone therapy (never or ever; women only), family history of colorectal cancer (no or yes), and educational attainment (no college education, or some college education). Additional adjustment for alcohol consumption, physical activity, frequency of NSAID use, hypertensive status, and dietary intakes of fibre, calcium, folate, saturated fat, and red meat resulted in virtually unchanged risk estimates; so as a consequence, these factors were not included in the final multivariable model.

Subgroup analyses for C-peptide tertiles (based on the distribution among the control group) and colorectal adenoma according to sex, BMI (<25 , $25\text{--}30$, or ≥ 30 kg/m²), regular NSAID use (no or yes), and use of hormone therapy (never or ever; women only) were also undertaken. The association of serum C-peptide levels and adenoma location (descending colon vs. rectum) and advanced histology (defined as adenomas ≥ 1 cm, high-grade dysplasia

or villous components) was also assessed. Statistical tests used in the analysis were all two-sided and a P -value of <0.05 was considered statistically significant. Analyses were conducted using Stata version 11.0.

Results

Compared to the control group participants, colorectal adenoma cases were more likely to have a higher daily consumption of alcoholic drinks; however, for all other baseline characteristics considered the case and control participants were relatively similar (Table 1). The median C-peptide level in case and control participants was 2.3 ng/mL and 2.4 ng/mL, respectively (P -value 0.25).

The distributions of known colorectal neoplasia risk factors across quartiles of serum C-peptide levels amongst the control participants are shown in Table 2. Compared to control participants in the lowest quartile of serum C-peptide levels, those in the fourth quartile had higher baseline BMI and CRP levels, and were more likely to be current smokers, regular users of NSAIDs, and be hypertensive.

The reproducibility of C-peptide measurements over multiple time-points among the 50 adenoma case and 30 control participants was assessed (Table 3). For control participants, median levels of C-peptide at time-points T₁ and T₅ were significantly higher than those measured at baseline (T₀) (P -value 0.0001). For the adenoma cases, there was no difference in C-peptide levels at T₀ and at T₃ or T₅ (P -value 0.20). The ICC values for the case and control groups were 0.73 and 0.66, respectively.

Table 4 provides ORs and 95% CIs for the associations between serum C-peptide levels and colorectal adenoma. In the multivariable model, C-peptide levels were not associated with incident colorectal adenoma in the categorical analyses (Q4 vs. Q1, OR 0.83, 95% CI: 0.52–1.31; P -trend 0.32) or when C-peptide was investigated on the continuous scale (OR per 1-unit increment in log-C-peptide concentration 0.84, 95% CI: 0.64–1.09). This result did not materially differ when the outcome was restricted to advanced adenomas only, and when split by sub-site (colon vs. rectum: P -heterogeneity 0.61) (Supplementary Table 1). Similar findings were observed for men (tertile 3 vs. tertile 1, OR 0.79, 95% CI: 0.47–1.34; P -trend 0.38) and women (tertile 3 vs. tertile 1, OR 0.85, 95% CI: 0.41–1.77; P -trend 0.59) (P -interaction 0.32) (Supplementary Table 2). In the subgroup analyses, similar strength non-significant associations were observed across categories of BMI (P -interaction 0.85), NSAID use (P -interaction 0.50), smoking status (P -interaction 0.16), and ever use of hormone therapy (P -interaction 0.77) (Supplementary Table 2).

Discussion

In this prospective investigation of colorectal adenoma, serum C-peptide levels, a marker of insulin secretion, were not associated with incident colorectal adenoma. If replicated, these findings suggest that insulin may not be associated with the early stages of colorectal tumorigenesis.

The relationship between circulating C-peptide levels and colorectal cancer risk has been relatively well studied, with a positive relationship usually observed [9–11]. For colorectal adenomas, however, the relationship with C-peptide levels is less certain. The majority of previous studies investigating the circulating C-peptide-colorectal adenoma relationship have been case-control studies, which have usually reported positive relationships [13–15]. These studies are vulnerable to reverse causality as serum samples were collected from cases after adenoma diagnosis. Three prospective studies have investigated the relationship between C-peptide levels and colorectal adenomas. Firstly, a study of colorectal adenoma recurrence found that C-peptide levels did not predict risk [24]. The other two prospective studies analysed the relationship between circulating C-peptide levels and incident colorectal adenoma, with contrasting results reported. Firstly, in a nested case-control analysis in the Nurses' Health Study (NHS), higher circulating C-peptide levels were positively associated with distal colon and rectal adenomas (Q4 vs. Q1, OR 1.63, 95% CI: 1.01–2.66; *P*-trend 0.01) [25]. More recently, the CLUE II cohort reported a null association between C-peptide levels and incident colorectal adenoma (Q4 vs. Q1, OR 1.04, 95% CI: 0.50–2.17; *P*-trend 0.90) [17]. The reason for the discrepancy in the findings from the current study and CLUE II analysis to that reported by the NHS is unclear. The NHS only included women whereas the current study and CLUE II analysis included both males and females. It is possible that the association between C-peptide and colorectal adenoma may vary by gender, though there was no apparent heterogeneity by gender in the findings presented in the current analysis. The NHS analysis matched cases and controls by fasting status, while the current study lacked information on fasting/time since last meal, thus it is possible that C-peptide levels were misclassified and could have biased the results towards the null. However, the CLUE II study observed similar results when analyses were limited to fasting participants only. Overall, the C-peptide quartile cut-points, sample size and analytic methods were relatively similar between all three studies. It is clear that further research in this area is required to clarify the association of markers of insulin resistance and colorectal adenoma development, preferably in large-scale prospective cohorts with fasting blood taken at multiple time-points.

Methodological limitations of the current analysis should be taken into consideration when evaluating the observed associations, specifically imprecision in the measurement of C-peptide levels. Single serum samples from baseline were used to assess the observed relationships, meaning that intra-individual variation in circulating C-peptide levels were unaccounted for, which could have caused attenuation of the observed risk estimates. We also lacked data on time since last meal at the time of blood draw; optimally, fasting serum samples would better reflect long-term insulin exposures, rather than post-prandial elevations; however, when we additionally adjusted for time of day of blood draw, as a proxy for fasting status, similar results were observed. Despite these limitations, the ICC values for C-peptide among both the case and control participants were in the moderate to high range (0.66–0.73), and higher than what was previously found in the Health Professionals Follow-up Study [9], suggesting relative stability of C-peptide levels over a 5-year period.

The observed null association for circulating C-peptide and colorectal adenomas may have been confounded by unmeasured factors, such as medication use. Higher circulating C-peptide levels are associated with greater cardiovascular disease and metabolic syndrome

risks. As a consequence, to lower cholesterol levels and protect against cardiovascular diseases, individuals with higher C-peptide levels may be more likely to use medications such as NSAIDs or statins, which have been associated with lower colorectal adenoma and cancer risks [26–30]. Indeed, in our analysis, control participants with higher circulating C-peptide levels reported higher NSAID use, and were more likely to be hypertensive at baseline. Although baseline NSAID use was controlled for in the multivariable models, usage patterns during the follow-up period were unknown; this was also the case for all other covariates in the multivariable models for which we only had baseline measurements. Further, information on the use of other medications, such as statins, was not collected at any time point.

Strengths of the current analysis include its prospective design which meant that serum samples and risk factor information were collected prior to colorectal adenoma diagnosis. All case and control participants underwent a flexible sigmoidoscopy at baseline, so as a consequence all participants were known to be free of neoplasia in the left-side of the colorectum. A limitation was that control participants may have had proximal adenomas which were undetected as these lie outside the range of the 60cm flexible sigmoidoscopy; however, none of the cases included in this analysis would have had proximal adenomas which would have been identified by the follow-up colonoscopy. A further limitation was that the relatively small number of cases did not allow a more thorough examination of the C-peptide relationship by adenoma subtypes. Finally, the possibility exists that adenomas in cases may have been missed at study entry and that undetected lesions may have affected C-peptide levels leading to the potential of reverse causation bias [31]; however, C-peptide levels were shown to be very stable for case participants throughout the study (ICC value of 0.73) and thus undetected adenomas are unlikely to have led to significant alterations in C-peptide concentrations.

In summary, in this prospective investigation, circulating C-peptide levels were not associated with colorectal adenomas. Further data are required to understand the role of insulin on colorectal tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Higher insulin levels have been associated with greater colorectal cancer risk.
- Whether insulin is similarly related to colorectal adenomas (colorectal cancer precursors) is unclear.
- We prospectively examined the relationship between C-peptide levels (a marker of insulin) and colorectal adenoma.
- No relationship between C-peptide and colorectal adenoma was observed.
- Our results suggest that non-insulin related molecular pathways may be more relevant to early stages of colorectal tumorigenesis.

Table 1.

Baseline characteristics of incident colorectal adenoma cases and matched controls in the PLCO trial.

Characteristic	Cases (<i>n</i> = 273)	Controls (<i>n</i> = 355)	<i>P</i> -value [*]
Age, y	62.8 (5.1)	62.6 (5.0)	Matched
Female, %	34.1	35.2	Matched
Caucasian, %	87.2	90.7	Matched
Family history of colorectal cancer, %	12.1	7.9	0.08
Some college education and above, %	60.4	55.8	0.24
BMI at baseline, kg/m ²	27.2 (4.4)	27.0 (4.4)	0.35
Hours spent of vigorous physical activity per week, %			0.45
0	15.0	11.0	
1–2	44.0	44.8	
3	38.8	41.1	
Cigarette smoking status			0.74
Never, %	44.3	46.8	
Former, %	42.5	39.4	
Current, %	13.2	13.8	
Ever use of female menopausal hormone therapy, %	53.8	59.2	0.40
Regular use of NSAIDs, %	49.5	60.0	0.12
Daily intakes			
Alcohol, g	15.7 (26.1)	12.1 (27.9)	0.04
Folate, µg	596.8 (351)	615.6 (350)	0.37
Red meat, g	87.1 (73.5)	80.7 (66.5)	0.86
Saturated fat, g	23.8 (12.6)	24.7 (14.9)	0.62
Fiber, g	24.7 (11.6)	25.1 (10.3)	0.26
Calcium, mg	1,199 (634)	1,256 (664)	0.31
Serum C-peptide, ng/mL [‡]	2.3 (1.6–3.6)	2.4 (1.7–3.7)	0.25
Serum CRP, mg/L [‡]	1.6 (0.8–3.6)	1.7 (0.8–4.0)	0.55

Values are means (SD) unless stated otherwise.

[‡]Values are medians (IQR).^{*}Calculated using Wilcoxon two-sample tests for continuous variables and χ^2 tests for categorical variables.

Table 2.

Distribution of selected colorectal adenoma risk factors by quartile of serum C-peptide among control group participants (n = 355).

Characteristic	Quartile of C-peptide (ng/mL)				P-value
	<1.8	1.8–<2.5	2.5–<3.8	3.8	
Age, y	62.7 (4.6)	61.6 (4.9)	63.0 (5.5)	62.8 (5.0)	0.59
Female, %	39.2	38.0	34.1	29.1	0.11
Caucasian, %	87.3	92.4	90.9	93.0	0.26
Family history of colorectal cancer, %	7.8	12.7	1.1	10.5	0.25
Some college education and above, %	53.0	57.0	52.3	61.6	0.30
BMI at baseline, kg/m ²	25.3 (3.7)	26.8 (3.9)	28.3 (4.4)	28.0 (4.8)	<0.001
4–5 hours of vigorous physical activity per week, %	41.2	48.1	43.2	35.6	0.90
Current smoker, %	13.7	16.5	8.0	17.4	0.90
Ever use of female menopausal hormone therapy, %	57.5	63.3	56.7	60.0	0.35
Regular use of NSAIDs, %	52.5	56.7	60.0	76.0	0.007
Alcohol intake, g/day	9.8 (14.5)	13.1 (44.6)	15.0 (26.3)	11.1 (20.4)	0.62
Folate intake, µg/day	642.2 (303)	592.1 (339)	622.3 (383)	600.0 (378)	0.54
Red meat intake, g/day	65.8 (45.1)	82.2 (87.2)	86.9 (71.0)	90.1 (57.8)	0.01
Saturated fat intake, g/day	22.2 (10.6)	25.6 (20.6)	25.0 (13.0)	26.4 (14.5)	0.08
Fiber intake, g/day	26.0 (11.0)	25.0 (11.1)	24.1 (9.7)	25.2 (9.3)	0.48
Calcium intake, mg/day	1,192 (627)	1,230 (749)	1,284 (656)	1,324 (631)	0.15
CRP, mg/L [‡]	0.9 (0.5–1.8)	1.6 (0.8–3.6)	2.7 (1.2–5.4)	2.3 (1.4–5.4)	<0.001
Hypertension, %	25.5	27.9	25.0	31.4	0.36

Values are means (SD) unless otherwise stated.

[‡]Values are medians (IQR).

Table 3.

Median levels and interquartile ranges for C-peptide levels in incident colorectal adenoma cases and controls sampled at multiple time points.

Screen					
Controls (<i>n</i> = 30)	T ₀		T ₁	T ₅	P-value *
	Intraclass correlation coefficient (95% CI)				
C-peptide, ng/mL	2.6 (1.4–3.8)	4.6 (2.6–6.4)	3.3 (1.9–5.2)	0.0001	0.66 (0.38–0.83)
Adenoma cases (<i>n</i> = 50)	T ₀		T ₃ or T ₅ [‡]		P-value *
	Intraclass correlation coefficient (95% CI)				
C-peptide, ng/mL	2.1 (1.6–3.7)	2.7 (1.8–4.5)	0.20	0.73 (0.52–0.85)	

* Difference in median values assessed by calculating the Friedman's χ^2 statistic.

[‡] Whenever the colorectal adenoma was diagnosed.

Table 4.

ORs and 95% CI for colorectal adenoma according to quartiles of serum C-peptide concentration in the PLCO Cancer Screening Trial.

	Quartile of C-peptide (ng/mL)				P-trend [*]	OR per unit change in log C-peptide
	<1.8	1.8–<2.5	2.5–<3.8	3.8		
Median, ng/mL	1.3	2.0	3.1	5.5		
Interquartile range, ng/mL	1.0–1.6	1.9–2.2	2.8–3.4	4.8–6.9		
Cases/controls, <i>n</i>	85/102	65/79	59/88	64/86		
Univariate OR (95% CI) [‡]	1.00	0.99 (0.64–1.53)	0.80 (0.52–1.25)	0.89 (0.58–1.38)	0.44	0.87 (0.68–1.11)
Multivariable OR (95% CI) [‡]	1.00	0.93 (0.59–1.45)	0.76 (0.47–1.20)	0.83 (0.52–1.31)	0.32	0.84 (0.64–1.09)

^{*}Statistical tests for trend were calculated using ordinal variables entered into the models as single continuous variables.

[‡]Univariate model adjusted for matching factors only.

[‡]Multivariable model adjusted for matching factors, plus cigarette smoking status (never, former, or current), BMI at baseline (<25, 25–<30, or ≥30 kg/m²),

use of non-steroidal anti-inflammatory drugs (NSAIDs; no or yes), use of menopausal hormone therapy (never or ever; women only), family history of colorectal cancer (no or yes), and educational attainment (no college education, or some college education).