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Western style diets and lifestyles are associated with increasing rates of obesity, diabetes and insulin resistance. Higher circulating insulin levels may modulate cell proliferation and apoptosis either directly or indirectly by increasing the bioactivity of IGF-I and decreasing the bioactivity of some of its binding proteins. The objective of this study was to determine the association of increasing levels of serum C-peptide, a biomarker of pancreatic insulin secretion, and IGF binding proteins (IGFBP) -1 and -2 with color-

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ectal cancer risk in a case–control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC), a large cohort involving 10 Western European countries. A total of 1,078 colorectal cancer cases were matched (age, date of blood donation, fasting status, gender, study center) to an equal number of control subjects. Relative cancer risks were estimated using conditional logistic regression models. Serum C-peptide concentration was positively associated with an increased colorectal cancer risk for the highest *versus* the lowest quintile (OR = 1.56, 95% CI = 1.16–2.09, $p_{trend} < 0.01$), which was slightly attenuated after adjustment for BMI and physical activity (OR = 1.37, 95% CI = 1.00–1.88, $p_{trend} = 0.10$). When stratified by anatomical site, the cancer risk was stronger in the colon (OR = 1.67, 95% CI = 1.14–2.46, $p_{trend} < 0.01$) than in the rectum (OR = 1.42, 95% CI = 0.90–2.25, $p_{trend} = 0.35$). The cancer risk estimates were not heterogeneous by gender or fasting status. No clear colorectal cancer risk associations were observed for IGFBP-1 or -2. This large prospective study confirms that hyperinsulinemia, as determined by C-peptide levels, is associated with an increased colorectal cancer risk.

Key words: colorectal cancer; IGF; insulin; C-peptide; EPIC

Dietary and lifestyle factors play a large role in colorectal cancer (CRC) etiology and may account for a majority of CRC cases.[1,2] In particular, high intakes of fats, red/processed meats and low intakes of dietary fiber, fruits and vegetables accompanied with physical inactivity and overweight are thought to be associated with an increased risk of colon and rectal cancers.[3–6] Such a regimen has major metabolic consequences, including the development of insulin resistance and hyperinsulinemia.[7] Insulin is key to the regulation of energy metabolism and may directly influence CRC risk *via* mitogenic/antiapoptotic effects through the insulin receptor[8,9] or by increasing energy provision to colon and rectal cancer cells with growth-promoting consequences.[10] Indirectly, insulin may also increase the bioactivity of Insulin-like Growth Factor I (IGF-I) by reducing the levels of 2 IGF-binding proteins (IGFBP), IGFBP-1 and -2,[6,11] although total IGF-I concentrations in blood are primarily mediated by growth hormone levels and less so by insulin.[12] In previous studies, elevated blood concentration of total IGF-I has been associated with increased CRC risk.[13] Thus, chronically elevated fasting and postprandial insulin levels may be closely associated with increased colon and rectal cancer risk.[14,15] Indeed, recent reviews suggest that prevention of weight gain and a greater level of physical activity, both of which inhibit loss of insulin sensitivity and tend to lower blood insulin levels, may help to reduce the risk of colon cancer, although evidence for an association with rectal cancer is currently insufficient.[5,16]

Evidence for the association of blood insulin levels with CRC risk comes from both experimental[17] and epidemiologic studies, showing a higher CRC risk in type-II diabetics[18] and in individuals presenting risk factors thought to be related to chronic hyperinsulinemia.[19] However, only a few prospective studies have directly assessed baseline levels of circulating insulin in association with CRC risk. In a small cohort of elderly subjects, Schoen *et al.*[20] show an almost 2-fold increased CRC risk with higher nonfasting insulin levels 2 hr after a glucose challenge, while in a Swedish population, baseline insulin levels have been associated with a nonstatistically significant positive risk of colon and rectal cancers.[21] Some other prospective studies have utilized C-peptide, which is considered as a valid marker of pancreatic insulin secretion because it has a longer half life than insulin itself. A study based on a cohort of New York women showed that higher C-peptide level was significantly associated with increased CRC risk, which was even stronger when analyses were limited to the colon.[22] Results from other North American cohorts have also shown a positive association between C-peptide and CRC risk, which was statistically significant in men (effects similar in the colon and rectum)[23] but not in women (effect appeared stronger in the colon, but was not statistically significant).[24] Similarly, a Norwegian study has shown a nonstatistically significant positive cancer risk association in the colon, and no association in the rectum.[25]

Although the results of these prospective studies tend to support the hyperinsulinemia-CRC hypothesis, they were all based on relatively small numbers of cancer cases drawn from select North American and Scandinavian populations. To date, the hyperinsulinemia-CRC hypothesis has not been tested in larger cohorts and it is unclear whether similar effects to the above can be observed in diverse population groups with different dietary and lifestyle patterns. Furthermore, although higher blood levels of both IGFBP-1 and -2 have been associated with lower colon and rectal cancer risk in some studies,[22,24] the same has not been observed elsewhere.[21,26] Thus, the aim of this case–control study, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, was to determine the CRC risk association of circulating baseline C-peptide, IGFBP-1 and -2. The large number of CRC cases within the EPIC cohort allows a clearer differentiation between the colon and rectum. The CRC risk associations of blood IGF-I and IGFBP-3 concentrations are presented elsewhere (Kaaks R, et al, unpublished).

Material and methods

EPIC study population and collection of blood samples

The rationale and methods of EPIC, a large prospective study with over 520,000 subjects enrolled, have been previously discussed.[27] Briefly, EPIC consists of 23 centers in 10 Western European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom). Between 1992 and 1998, country-specific dietary questionnaires, standardized lifestyle and personal history questionnaires, anthropometric data and blood samples were collected from all study subjects.

In most recruitment centers, blood samples ($\geq$30 ml) were drawn from all participants and stored at 5–10°C protected from light and transported to local laboratories for processing and aliquoting.[27] In the EPIC-Oxford centre (UK), blood samples were collected from a network of general practitioners and transported to a central laboratory by post. In centers in Denmark and Sweden, blood was aliquoted within 1 and 2 hr, respectively, after drawing. In all countries, except Denmark and Sweden, blood was separated into 0.5 ml fractions (serum, plasma, red cells and buffy coat for DNA extraction). Each fraction was placed into straws, which were heat sealed and stored (−196°C) under liquid nitrogen. In Denmark, blood fraction aliquots of 1.0 ml were stored locally in Nunc tubes at −150°C under nitrogen vapor. In Sweden, samples were stored in −80°C freezers.

This study was approved by the Ethical Review Board of the International Agency for Research on Cancer (IARC; Lyon, France) and those of all EPIC centers. All EPIC participants have provided written consent for the use of their blood samples and all data.

Follow-up for cancer incidence and vital status

Follow-up for vital status is collected by record linkage with regional and national mortality registries in all countries except Germany and Greece, where data are continuously collected through active follow-up. Currently, vital status is known for 98.4% of all EPIC subjects. Cancer incidence is determined through record linkage with regional cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom; complete up to December 2001) or *via* a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries and active follow-up through study subjects and their next-of-kin (France, Germany, Greece; complete up to December 2002).

Nested case–control design and subject selection

Case ascertainment and selection. For the present study, colon cancers were defined as tumors in the cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon (C18.0–C18.7 as per the 10th Revision of

TABLE I – DESCRIPTION OF CASES AND MATCHED CONTROLS, BY COLON AND RECTAL ANATOMICAL SITE GROUPINGS

Variable	Colon			Rectum		
	Cases	Matched controls	*p* value difference[1]	Cases	Matched controls	*p* value difference[1]
Number of subjects	674[2]	674	–	404[3]	404	–
Percentage of Male Subjects	52.8	52.8	–	58.2	58.2	–
Percentage of Non-Fasting Subjects	73.6	73.6	–	81.2	81.2	–
Mean age (years) (minimum–maximum value)						
At recruitment	59.2 (36.7–76.9)	59.2 (36.7–76.5)	0.43	58.3 (39.9–75.0)	58.3 (39.8–75.3)	0.93
At blood donation	59.2 (36.7–76.9)	59.3 (36.7–76.5)	0.66	58.3 (39.9–75.0)	58.3 (39.8–75.3)	0.98
At diagnosis	62.3 (37.0–81.0)	–	–	61.6 (40.0–78.0)	–	–
Mean years of follow-up (minimum–maximum value)	3.5 (0.1–8.6)	–	–	3.7 (0.1–8.8)	–	–
Mean BMI (minimum–maximum value)	27.3 (17.6–52.5)	26.8 (16.9–42.3)	0.04	26.9 (18.2–41.1)	26.5 (17.6–44.5)	0.14
Smoking status (%)[4]			0.36			0.96
Never	39.8	44.2		35.6	35.6	
Former	35.8	34.0		33.4	34.2	
Smoker	23.9	21.1		30.5	29.5	
Physical activity (%)[5]			0.17			0.09
Inactive	16.5	12.6		15.8	14.1	
Moderately inactive	29.5	27.3		29.0	25.5	
Moderately active	43.8	48.7		43.3	42.8	
Active	9.3	10.7		11.4	15.4	
Geometric mean/median of peptide concentrations (5–95th percentile)[6]						
Fasting subjects (ng/mL)						
C-peptide	2.9/3.1 (1.7–5.6)	2.8/2.9 (1.7–4.8)	0.27	2.8/3.1 (1.6–5.2)	2.9/3.2 (1.6–6.0)	0.58
IGFBP-1	14.0/19.7 (3.9–60.3)	14.2/19.9 (3.4–64.5)	0.85	16.3/22.0 (2.4–64.6)	15.4/17.8 (4.3–62.9)	0.54
IGFBP-2	285.1/284.0 (93.6–717.8)	281.6/298.6 (93.4–659.9)	0.80	292.6/305.0 (61.3–1082.3)	288.2/301.2 (73.1–720.6)	0.89
Nonfasting subjects (ng/mL)						
C-peptide	4.7/4.8 (2.1–11.1)	4.2/4.4 (1.7–10.2)	<0.01	4.2/4.5 (1.7–12.3)	4.0/4.4 (1.5–11.3)	0.27
IGFBP-1	7.9/6.6 (1.63–37.3)	8.6/7.6 (1.9–46.9)	0.13	7.8/6.9 (1.6–39.2)	8.4/7.3 (1.7–40.2)	0.27
IGFBP-2	376.4/399.2 (111.1–1072.9)	372.4/411.1 (100.3–971.3)	0.69	361.2/391.2 (95.6–1061.0)	371.7/395.6 (112.1–993.4)	0.60

[1]*p* values for differences in means between cases and controls determined by paired *t*-tests, except for smoking status and physical activity where *p* values determined by Chi-square tests.– [2]Indicates number of cases and matched controls for C-peptide analyses. For IGFBP-1 *n* cases/matched controls = 668/668, for IGFBP-2 *n* cases/matched controls = 656/656.– [3]Indicates number of cases and controls for C-peptide analyses. For IGFBP-1 *n* cases/matched controls = 399/399, for IGFBP-2 *n* cases/matched controls = 397/397.– [4]Smoking status of the remaining subjects is missing.– [5]Physical activity status of the remaining subjects is missing.– [6]Mean/median and 5–95th percentile values refer to the non-logarithmically transformed distribution.

TABLE II – PEARSON'S PARTIAL CORRELATION COEFFICIENTS FOR MAIN STUDY VARIABLES STRATIFIED BY FASTING STATUS, IN THE COLON AND RECTUM ANATOMICAL SITES COMBINED, ADJUSTED BY AGE, GENDER, LABORATORY ANALYSIS BATCH AND CASE–CONTROL STATUS

Fasting subjects	Nonfasting Subjects					
	C-peptide	IGFBP-1	IGFBP-2	BMI	Waist circumference	Physical activity
C-peptide	–	−0.47*	−0.32*	0.35*	0.36*	−0.09*
IGFBP-1	−0.48*	–	0.48*	−0.39*	−0.48*	0.04
IGFBP-2	−0.45*	0.56*	–	−0.48*	−0.40	0.08*
BMI	0.39*	−0.37*	−0.33*	–	0.87*	−0.06*
Waist circumference	0.46*	−0.42*	−0.36*	0.83*	–	−0.10*
Physical activity	−0.04	0.02	0.02	−0.02	0.01	–

Values are Pearson partial correlations coefficients. Values on the left-hand triangular section pertain to fasting subjects and values on the right-hand triangular section refer to nonfasting subjects; *$p < 0.05$.

the International Statistical Classification of Diseases, Injury and Causes of Death), as well as tumors that were overlapping or unspecified (C18.8 and C18.9). Cancers of the rectum were defined as tumors occurring at the recto-sigmoid junction (C19) or rectum (C20). Anal canal tumors were excluded. CRC is defined as a combination of the colon and rectal cancer cases.

The EPIC database contained 1,587 incident CRC cases. Of these, the following were excluded: 444 women cases (use of exogenous hormones at blood collection), 56 cases (missing information on fasting status [nonfasting: ≤6 hr after eating; fasting: >6 hr after eating]), 9 cases (C-peptide measurements could not be obtained). Thus, the present study includes a total of 1,078 incident CRC cases (number colon = 674; number rectal = 404) identified after recruitment into EPIC. The distribution of cases (colon/rectum) by country was Denmark = 193/167, France = 14/1, Greece = 14/13, Germany = 77/50, Italy = 102/41, Netherlands = 65/38, Spain = 78/41 and United Kingdom = 131/53. Cases were not selected from Norway (blood samples only recently collected; few CRCs diagnosed after blood donation) and Sweden (an independent study of blood insulin levels and CRC risk is ongoing). Due to some incomplete analyses, the number of cases for analyses of IGFBP-1 (number colon = 668; number rectal = 399) and IGFBP-2 (number colon = 656; number rectal = 397) were less.

Control selection. Controls were selected from all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the cases and were matched by age (±6 months at recruitment), gender, study centre (to account of centre specific differences in questionnaire design, blood collection procedures, *etc.*), follow-up time since blood collection (to account for follow-up time), time of the day at blood collection (to account for any potential changes that may have occurred in the blood samples over time during storage), and fasting status (<3, 3–6, >6 hr; to account for differences in analyte values by fasting status). Women were further matched by menopausal status (premenopausal, postmenopausal, perimenopausal/unknown) and phase of menstrual cycle at blood collection (to account for potential differences in blood analyte levels by these factors).

Laboratory assays. For all laboratory analyses, serum samples were retrieved from frozen storage, thawed and analyzed immediately, without any consecutive freeze–thaw cycles. C-peptide (radioimmunoassay; Diagnostic System Laboratories, Webster, TX), IGFBP-1 (immunoradiometric assay; Diagnostic System Laboratories) and IGFBP-2 (radioimmunoassay; Diagnostic System Laboratories) were assayed in serum samples of all subjects. Case/control match-sets were always analyzed in the same batch. Each batch contained 3 additional sera randomly inserted as quality control measures. All assays were performed at IARC by laboratory personnel blinded to the subjects' case–control status. The mean intra-batch and inter-batch coefficients of variation were 4.6 and 7.5%, respectively, for C-peptide (at a concentration of 5 ng/ml), 5.1 and 19.5% for IGFBP-1 (concentration:40 ng/ml) and 5.4 and 29% for IGFBP-2 (concentration:500 ng/ml). Some measurements could not be obtained for IGFBP-1 (colon [case/control]: 6/1; rectum: 5/1) and IGFBP-2 (colon: 13/12; rectum: 3/6).

Statistical methods. All peptide measurements were logarithmically transformed to approximate normality. Differences between cases and controls in mean levels for each peptide and anthropometric variables were tested by paired *t*-tests of the log-transformed value in each case–control set. Differences between cases and controls for categorical variables (smoking status, physical activity) were assessed by chi-square tests. Partial Pearson correlations (adjustments: age, gender, analysis batch, case–control status) were calculated for the matrix of log-transformed analytes, as well as for correlations with BMI (kg/m^2, continuous variable) and physical activity (index of combined recreational/household, continuous variable calculated as metabolic equivalent units per day). To examine the degree of between-center variations in average serum peptide levels, analysis of variance models were run with age at blood donation, study center, gender, analysis batch, fasting status and case–control status as explanatory variables, with and without further adjustment for BMI and physical activity.

Odds ratios (OR) and 95% confidence intervals (95% CI) for risk of colon and rectal cancer anatomical site groupings and the colon and rectum combined (*i.e.* CRC), in relation to log-transformed serum peptide concentrations were calculated by conditional logistic regression (SAS statistical software, version 9, SAS Institute, Cary, NC), stratified by case–control set. For each peptide, category cut-points were based on log-transformed variable distributions in all the controls combined, stratified by fasting status. The effects of potential confounders, other than matching criteria, were examined by including additional regression terms into the logistic regression models. Potential confounders included smoking status (never, former, current, missing), alcohol consumption, waist circumference, and intake of total energy, dietary fiber, fruits, vegetables and meats. It was decided that only those variables that resulted in a greater than 10% change in the estimate would be included in the final model. However, none of these variables altered the risk estimates and so ''crude'' models were run as univariate analyses conditioned on the matching factors. BMI (kg/m^2) and physical activity (metabolic equivalents; METS) are 2 other potential confounding variables that are of special interest since, both are thought to affect cancer risk by modulating insulin resistance and hyperinsulinemia, although their effects may vary in the colon *versus* the rectum.[5,16] To allow comparisons to previous publications showing results with and without adjustments for these factors, multivariate models (referred to as ''adjusted'') with adjustments for BMI and physical activity were also run. For all models, linear trend tests were determined using continuous variables. All statistical tests were 2-tailed and $p < 0.05$ was considered statistically significant.

Since the degree of abdominal adiposity may reflect the extent of insulin resistance, a 3 by 3 interaction model between each serum peptide and waist circumference (cm) was run using the ''crude'' analysis models described previously. For each model, the lowest category of the peptide being analyzed and waist circumference was set as the reference category. Similar analyses were also made for BMI and physical activity, in order to further explore potential interactions with these variables. For all variables, models were run separately for CRC, colon and rectum, and

TABLE III – ASSOCIATION OF SERUM C-PEPTIDE WITH CANCER RISK IN THE COLON, RECTUM AND COLON AND RECTUM COMBINED, BY FASTING STATUS

Quintiles of serum C-peptide concentration	Fasting and nonfasting subjects combined[1]			Fasting subjects only[1]			Non-fasting subjects only[1]		
	N cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)	*N* cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)	*N* cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)
Colon[2]									
1 (reference)	104/121	1.00	1.00	27/32	1.00	1.00	77/89	1.00	1.00
2	139/150	1.08 (0.76–1.55)	1.05 (0.73–1.50)	40/41	1.15 (0.58–2.26)	1.20 (0.59–2.45)	99/109	1.06 (0.70–1.61)	1.04 (0.68–1.59)
3	120/136	1.07 (0.75–1.55)	1.06 (0.72–1.54)	32/38	0.99 (0.48–2.04)	0.98 (0.47–2.06)	88/98	1.10 (0.72–1.69)	1.10 (0.71–1.70)
4	143/136	1.29 (0.89–1.86)	1.17 (0.80–1.71)	38/36	1.27 (0.62–2.62)	1.22 (0.56–2.67)	105/100	1.30 (0.85–1.98)	1.19 (0.77–1.85)
5	168/131	1.67 (1.14–2.46)	1.48 (0.98–2.25)	41/31	1.66 (0.80–3.45)	1.58 (0.70–3.56)	127/100	1.66 (1.06–2.65)	1.49 (0.91–2.42)
p trend[3]		<0.01	<0.01		0.18	0.49		0.01	0.01
Rectum[2]									
1 (reference)	78/93	1.00	1.00	15/18	1.00	1.00	63/75	1.00	1.00
2	71/68	1.26 (0.80–1.99)	1.25 (0.79–1.99)	12/11	1.37 (0.46–4.10)	1.25 (0.41–3.83)	59/57	1.25 (0.75–2.06)	1.25 (0.75–2.09)
3	94/80	1.43 (0.93–1.20)	1.29 (0.82–2.04)	20/13	1.99 (0.60–5.72)	1.62 (0.49–5.39)	74/67	1.33 (0.83–2.15)	1.26 (0.76–2.08)
4	68/81	1.02 (0.65–1.20)	0.94 (0.59–1.51)	14/16	0.92 (0.30–2.80)	0.68 (0.20–2.31)	54/65	1.03 (0.63–1.68)	0.97 (0.58–1.63)
5	93/82	1.42 (0.90–2.25)	1.25 (0.76–2.03)	15/18	1.11 (0.38–3.25)	0.78 (0.23–2.58)	78/64	1.53 (0.92–2.55)	1.37 (0.80–2.36)
p trend[3]		0.35	0.78		0.86	0.16		0.25	0.49
Colorectum[2]									
1 (reference)	182/214	1.00	1.00	42/50	1.00	1.00	140/164	1.00	1.00
2	210/218	1.14 (0.87–1.51)	1.11 (0.84–1.47)	52/52	1.20 (0.68–2.12)	1.20 (0.67–2.16)	158/166	1.13 (0.82–1.55)	1.09 (0.79–1.51)
3	214/216	1.20 (0.91–1.59)	1.13 (0.85–1.51)	52/51	1.24 (0.69–1.21)	1.11 (0.61–2.03)	162/165	1.20 (0.87–1.55)	1.14 (0.82–1.58)
4	211/217	1.19 (0.90–1.58)	1.09 (0.81–1.46)	52/52	1.22 (0.67–2.21)	1.09 (0.57–2.05)	159/165	1.19 (0.86–1.63)	1.10 (0.79–1.53)
5	261/213	1.56 (1.16–2.09)	1.37 (1.00–1.88)	56/49	1.41 (0.78–2.55)	1.22 (0.64–2.35)	205/164	1.61 (1.15–2.26)	1.44 (1.00–2.06)
p trend[3]		<0.01	0.03		0.31			0.01	0.02

[1]Values for ''Crude'' are OR (95%CI) derived from univariate models conditioned on the matching factors, Values for ''Adjusted'' are OR (95% CI) derived from univariate models conditioned on the matching factors and further adjusted for body mass index and physical activity.– [2]Category cut-points were the same for all anatomical site groupings but differed by fasting status. For fasting subjects: Q1, <2.2; Q2, ≥2.2–2.8; Q3, ≥2.8–3.2; Q4, ≥3.2–3.8; Q5, ≥3.8 ng/mL. For nonfasting subjects: Q1, <2.7; Q2, ≥2.7–3.8; Q3, ≥3.8–5.0; Q4, ≥5.0–6.8; Q5, ≥6.8 ng/mL.– [3]*p* for linear trends in risk calculated by likelihood ratio tests with increasing peptide concentrations using the log-transformed continuous variables.

TABLE IV – INTERACTION OF SERUM C-PEPTIDE AND WAIST CIRCUMFERENCE WITH CANCER RISK IN THE COLON, RECTUM AND COLON AND RECTUM COMBINED

Tertiles of serum C-peptide levels	Tertiles of waist circumference (cm)[1]			*p* value for interaction
	<84.5	≥84.5–<95.0	≥95.0	
Colorectum[2]				0.62
1	1.00	1.19 (0.81–1.74)	1.01 (0.63–1.61)	
2	1.24 (0.88–1.76)	1.30 (0.91–1.86)	1.61 (1.10–2.33)	
3	1.09 (0.71–1.68)	1.44 (0.99–2.09)	1.68 (1.19–2.35)	
Colon[2]				0.49
1	1.00	1.07 (0.66–1.75)	0.93 (0.52–1.63)	
2	1.29 (0.83–2.01)	1.19 (0.75–1.89)	1.86 (1.15–3.01)	
3	1.25 (0.74–2.13)	1.49 (0.92–2.41)	2.00 (1.30–3.10)	
Rectum[2]				0.98
1	1.00	1.31 (0.71–2.42)	1.20 (0.52–2.73)	
2	1.18 (0.66–2.10)	1.50 (0.85–2.67)	1.33 (0.73–2.44)	
3	0.84 (0.40–1.77)	1.34 (0.74–2.43)	1.29 (0.74–2.24)	

[1]Values are OR (95% CI) derived from univariate models conditioned on the matching factors, with the lowest category of waist circumference and serum C-peptide level set as the reference.– [2]Category cut-points were the same for colorectum, colon and rectum but differed by fasting status. For fasting subjects: T1, <2.6; T2, ≥2.6–3.4; T3, ≥3.4 ng/mL. For nonfasting subjects: T1, <3.4; T2, ≥3.4–5.5; T3, ≥5.5 ng/mL.

p values for interaction were calculated using the likelihood ratio test.

Additionally, for each peptide, potential heterogeneity by gender, fasting status and the time to diagnosis of cancer of less than 2 years or more than 2 years was tested separately using chi-square tests. For the assessment of time to diagnosis of cancer, each control set was assigned the value for years of follow-up of its matched case. No overall significant interactions were observed for any of these variables. Nonetheless, models stratified by gender, fasting status and the time to diagnosis of cancer of less than and equal to 2 years or more than 2 years were also run using the ''crude'' models described earlier.

Results

The mean age at recruitment was equal in cases and controls (colon: 59.2 [range = 36.7–76.9]; rectum: 58.3 [range = 39.8–75.3]) (Table I). Colon cancer cases had 3.5 (range = 0.1–8.6) mean years of follow up while that of rectal cancer cases was slightly longer (3.7, range = 0.1–8.8). Colon, but not rectal, cancer cases had a significantly higher ($p = 0.04$) BMI than their matched controls (Table I). Fasting subjects had significantly lower serum C-peptide/IGFBP-2 and significantly higher serum IGFBP-1 than nonfasting subjects in the colon and rectal cancer anatomical site groupings ($p < 0.01$ for all comparisons).

No significant between-center heterogeneity was observed in serum concentrations of C-peptide ($p_{\text{heterogeneity}} = 0.98$), IGFBP-1 ($p_{\text{heterogeneity}} = 0.32$) and IGFBP-2 ($p_{\text{heterogeneity}} = 0.68$), and results for CRC risk by centre did not differ considerably. The percent of variation in analyte values explained by differences in study centers was <1% for C-peptide and IGFBP-1 and <1.3% for IGFBP-2, after adjustments for age, gender, case–control status, fasting status, analysis batch, BMI and physical activity.

Table II shows Pearson's partial correlation coefficients by fasting status between all 3 peptides, BMI, waist circumference and physical activity. In general, in both fasting and nonfasting subjects C-peptide was negatively correlated with both IGFBP-1 and IGFBP-2 and BMI and waist circumference were positively correlated with C-peptide but negatively correlated with IGFBP-1 and -2 (Table II).

In conditional logistic regression models, higher baseline serum C-peptide level was associated with increased cancer risk in the colon (OR highest *versus* lowest quintile = 1.67, 95% CI = 1.14–2.46, $p_{\text{trend}} < 0.01$) and less so in the rectum (OR highest *versus* lowest quintile = 1.42, 95% CI = 0.90–2.25, $p_{\text{trend}} = 0.35$) (Table III). When the colon and rectal anatomical site groupings were combined, the OR of the highest *versus* lowest quintile was 1.56 (95% CI = 1.16–2.09, $p_{\text{trend}} < 0.01$). In each anatomical site grouping, further adjustment for BMI and physical activity slightly attenuated the risk estimates but did not alter the pattern of results (Table III).

The cancer risk association of serum C-peptide levels showed no heterogeneity of effect by fasting status in any of the anatomical site groupings ($p_{\text{heterogeneity}}$: colon = 0.81; rectum = 0.40; CRC = 0.62). Results of subgroup analyses by fasting status did not show any statistically significant cancer risk associations for C-peptide (Table III). Results in left and right colon subsites were not heterogeneous for the C-peptide cancer risk associations by fasting status. Omission of 291 case-sets where either a case or control subject had responded in the affirmative to a lifestyle questionnaire item regarding a previous medical diagnosis of diabetes did not show any changes in the OR estimates of C-peptide, although the statistical significance of the trend was slightly reduced, likely due to smaller number of subjects (data not shown).

Table IV shows the results for 3 by 3 interaction models run for serum C-peptide and waist circumference by anatomical site groupings. Although the *p* values for interaction were not statistically significant (Colon = 0.49; Rectum = 0.98; CRC = 0.62), higher C-peptide was more strongly associated with CRC risk in subjects with larger waist circumference. This association appears to have been driven more by the colon than the rectum. For IGFBP-1 and IGFBP-2, no significant interactions or remarkable differences in results were noted with waist circumference (results not shown).

Higher serum IGFBP-1 levels were negatively associated with risk of colon (OR highest *versus* lowest quintile = 0.72, 95% CI = 0.49–1.05, $p_{\text{trend}} = 0.16$) and rectal cancers (OR highest *versus* lowest quintile = 0.88, 95% CI = 0.55–1.39, $p_{\text{trend}} = 0.42$), although none of the results were statistically significant (Table V). Further adjustment by BMI and physical activity did not change the pattern of results (Table V). Subgroup analyses by fasting status, showed no heterogeneity of effect ($p_{\text{heterogeneity}}$: colon = 0.65; rectum = 0.33; CRC = 0.36) and did not change the pattern of results.

Higher serum IGFBP-2 was positively associated with colon cancer risk (OR highest *versus* the lowest quintile = 1.17, 95% CI = 0.80–1.69, $p_{\text{trend}} = 0.98$), and negatively associated with rectal cancer risk (OR highest *versus* lowest quintile = 0.75, 95% CI = 0.47–1.18, $p_{\text{trend}} = 0.36$), although the associations were not statistically significant in either anatomical site grouping (Table V). Similar to the other peptides, there was no difference of effect of IGFBP-2 by fasting status ($p_{\text{heterogeneity}}$: colon = 0.97; rectum = 0.74; CRC = 0.77).

For all 3 peptides, results from interaction models for BMI (likelihood ratio $p_{\text{interaction}}$ for CRC risk: C-peptide = 0.53;

TABLE V – ASSOCIATION OF SERUM IGFPB-1 AND IGFBP-2 AND CANCER RISK IN THE COLON, RECTUM AND COLON AND RECTUM COMBINED, FOR FASTING AND NON-FASTING SUBJECTS TOGETHER

Quintiles of serum peptide concentration	Colon[1]			Rectum[1]			Colorectum[1]		
	N cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)	*N* cases/controls	OR (95% CI)	Adjusted OR (95% CI)	*N* cases/controls	OR (95% CI)	Adjusted OR (95% CI)
IGFBP-1[2]									
1 (reference)	139/128	1.00	1.00	85/86	1.00	1.00	224/214	1.00	1.00
2	149/138	0.99 (0.71–1.38)	1.06 (0.75–1.49)	84/74	1.14 (0.74–1.75)	1.18 (0.76–1.84)	233/212	1.05 (0.80–1.36)	1.11 (0.85–1.45)
3	125/139	0.82 (0.58–1.16)	0.90 (0.62–1.29)	80/76	1.03 (0.67–1.60)	1.10 (0.70–1.73)	205/215	0.89 (0.68–1.17)	0.96 (0.73–1.28)
4	145/129	1.01 (0.71–1.45)	1.15 (0.79–1.69)	80/84	0.95 (0.61–1.46)	1.01 (0.63–1.62)	225/213	0.99 (0.75–1.30)	1.11 (0.83–1.49)
5	110/134	0.72 (0.49–1.05)	0.82 (0.55–1.24)	70/79	0.88 (0.55–1.39)	1.01 (0.60–1.68)	180/213	0.78 (0.58–1.04)	0.89 (0.65–1.22)
p trend[3]		0.16	0.56		0.42			0.11	0.53
IGFBP-2[2]									
1 (reference)	125/138	1.00	1.00	89/73	1.00	1.00	214/211	1.00	1.00
2	145/126	1.28 (0.91–1.81)	1.38 (0.97–1.96)	77/84	0.76 (0.50–1.16)	0.78 (0.50–1.21)	222/210	1.04 (0.80–1.36)	1.11 (0.84–1.45)
3	138/129	1.18 (0.84–1.68)	1.28 (0.89–1.83)	77/82	0.76 (0.49–1.20)	0.81 (0.50–1.29)	215/211	1.00 (0.76–1.31)	1.07 (0.81–1.42)
4	114/136	0.92 (0.64–1.32)	1.09 (0.75–1.61)	76/74	0.84 (0.54–1.32)	0.93 (0.57–1.51)	190/210	0.89 (0.67–1.17)	1.02 (0.75–1.37)
5	134/127	1.17 (0.80–1.69)	1.48 (0.97–2.24)	78/84	0.75 (0.47–1.18)	0.83 (0.50–1.39)	212/211	0.98 (0.74–1.31)	1.18 (0.86–1.63)
p trend[3]		0.98	0.09		0.36	0.83		0.55	0.17

[1]Values for ''Crude'' are OR (95% CI) derived from univariate models conditioned on the matching factors. Values for ''Adjusted'' are OR(95% CI) derived from univariate models conditioned on the matching factors and further adjusted for body mass index and physical activity.–[2]Category cut-points were the same for colorectum, colon and rectum but differed by fasting status. For fasting subjects: IGFBP-1 = Q1, <7.9; Q2, ≥7.9–<16.6; Q3, ≥16.6–<24.6; Q4, ≥24.6–<39.7; Q5, ≥39.7 ng/mL. IGFBP-2 = Q1, <160.0; Q2, ≥160.0–<253.0; Q3, ≥253.0–<354.0; Q4, ≥354.0–<500.0; Q5, ≥500.0 ng/mL. For nonfasting subjects: IGFBP-1 = Q1, <3.1; Q2, ≥3.1–<5.5; Q3, ≥5.5–<10.5; Q4, ≥10.5–>21.2; Q5, ≥21.2 ng/mL. IGFBP-2 = Q1, <219.0; Q2, ≥219.0–<344.0; Q3, ≥344.0–<475.0; Q4, ≥475.0–<684.0; Q5, ≥684.0 ng/mL.–[3]*p* for linear trends in risk calculated by likelihood ratio tests with increasing peptide concentrations using the log-transformed continuous variables.

IGFBP-1 = 0.59; IGFBP-2 = 0.13) were very similar to results for waist circumference (results for BMI not shown). Similarly, interaction models for physical activity did not show any remarkable differences for C-peptide (likelihood ratio $p_{interaction}$ for CRC risk = 0.87; results not shown), but did demonstrate a trend towards a reduced CRC risk with higher physical activity and higher serum IGFBP-1 (likelihood ratio $p_{interaction}$ for CRC risk = 0.52; OR for the category representing IGFBP-1 ≥3.3 ng/ml [fasting] or 2.5 ng/ml [nonfasting] and physical activity ≥100.7 METS = 0.68, 95% CI = 0.47–0.98) and IGFBP-2 levels (likelihood ratio $p_{interaction}$ for CRC risk = 0.26; OR for the category representing IGFBP-2 ≥5.9 ng/ml [fasting] or 6.3 ng/ml [nonfasting] and physical activity ≥100.7 METS = 0.67, 95%CI = 0.46–0.97).

For all 3 peptides, tests for heterogeneity showed no differences in relative risk estimates in subjects with less than or equal to 2 years of follow-up *versus* those with more than 2 years in any of the anatomical site groupings (C-peptide $p_{heterogeneity}$: colon = 0.63; rectum = 0.96; CRC = 0.68). Exclusion of cases diagnosed within the first 2 years (n CRC cases excluded = 292), so as to reduce any potential influence of an undiagnosed tumor on blood peptide levels, slightly altered the risk estimates obtained but did not change the pattern of results observed in the colon and rectal anatomical site groupings combined (C-peptide: OR highest *versus* lowest quintile = 1.47, 95% CI = 1.04–2.07, p_{trend} = 0.04; IGFBP-1: OR = 0.76, 95% CI = 0.54–1.07, p_{trend} = 0.12; IGFBP-2: OR = 0.95, 95% CI = 0.68–1.32, p_{trend} = 0.53) or separate (data not shown).

Analyses of the cancer risk estimates of serum C-peptide levels by gender showed no heterogeneity of effect in any of the anatomical site groupings ($p_{heterogeneity}$: colon = 0.63; rectum = 0.90; CRC = 0.69). For C-peptide, the subgroup analyses stratified by gender showed similar cancer risk estimates in the colon (men: OR highest *versus* lowest quintile = 1.67, 95% CI = 0.98–2.85, p_{trend} = 0.04; women: OR = 1.63, 95% CI = 0.90–2.96, p_{trend} = 0.08) and rectum anatomical site groupings (men: OR highest *versus* lowest quintile = 1.40, 95% CI = 0.78–2.54, p_{trend} = 0.63; women: OR = 1.28, 95% CI = 0.61–2.69, p_{trend} = 0.37). Subgroup analyses by gender showed no heterogeneity of effect in any of the anatomical site groupings for IGFBP-1 ($p_{heterogeneity}$: colon = 0.65; rectum = 0.60; CRC = 0.49) or IGFBP-2 ($p_{heterogeneity}$: colon = 0.94; rectum = 0.40; CRC = 0.56). Gender stratification did not change the pattern of results for any of these peptides.

Discussion

This large, multicenter European prospective study has shown that elevated serum levels of C-peptide are associated with an increased colon cancer risk. The association between C-peptide concentration and rectal cancer was less clear, but a statistically significant association was maintained for the highest *versus* the lowest quintile when the colon and rectal anatomical site groupings were combined. Although the magnitude of the C-peptide–colon cancer association did not differ by fasting status, it was statistically significant only in nonfasting subjects, which may be indicative of a potentially clearer effect of postprandial hyperinsulinemia or be due to the larger number of nonfasting subjects. These observed associations were not heterogeneous by gender. In addition to serum C-peptide, this study also considered serum concentrations of IGFBP-1 and -2. These 2 binding proteins are known to be modulated by insulin levels and can regulate the bioactivity of IGF-I. Although IGF-I itself is associated with increased CRC risk,[13] its levels are principally mediated by growth hormone and, in well nourished populations, are only weakly affected by insulin. In the present study, no clear cancer risk associations were observed for the serum concentrations of either IGFBP-1 or IGFBP-2, despite the observed negative correlations of both with serum C-peptide concentrations.

Obesity, type-2 diabetes, and various risk factors for increased insulin resistance—which are all related to hyperinsulinemia—have been previously associated in prospective studies with higher risk of colon cancer,[19,28–31] rectal cancer[32] or colon and rectal cancers combined.[19,32–34] Higher blood insulin level is thought to influence CRC risk by enhancing cell proliferation and reducing apoptosis[8,9] and by increasing the biosynthesis of IGF-I and reducing that of IGFBP-1 and -2.[11] Many recent studies considering the CRC-insulin hypothesis have utilized C-peptide as a blood marker for pancreatic insulin secretion. It is thought to be a better marker than insulin itself because it has a much longer half life than insulin and its levels do not fluctuate as much as those of insulin, especially in nonfasting subjects. Thus, it is more indicative of average circulating insulin levels, which is of particular importance in situations, such as the present study, where blood samples have not systematically been collected under fasting conditions.[35] Nonetheless, the marker does have its drawbacks, since it is still only a relatively short-term indicator of insulin production, and can be affected by food intake.[36]

To date, the prospective studies testing the CRC-insulin hypothesis that have utilized blood C-peptide levels as a biomarker of insulin secretion[22–25] have been of relatively small sample size, ranging from 102[22] to 378[25] cases. In addition, with 1 exception,[25] these studies have mostly focused on North American populations, which may differ from other populations in terms of food intake patterns, lifestyle, physical activity levels and prevalence of obesity. Although all of these studies show a generally positive association between blood C-peptide concentrations and risk of colon and rectal cancers, the results have been statistically significant in only 2 studies, one in men[23] and the other in women.[22] A significant association of elevated blood C-peptide has also been shown with risk of colorectal adenomas.[26]

Interestingly, in the study based on the cohort of New York women[22] and elsewhere,[24] the C-peptide and cancer risk association was strengthened when the analyses were limited to the colon, while in the Physicians' Health Study[23] the risk estimates were similar between the colon and rectum. One of the key strengths of the present study is its large number of cases and hence its ability to clearly differentiate between colon and rectal anatomical sites. Here, although the risk estimates for colon and rectal cancers were not statistically heterogeneous from each other, the results tended to be stronger for the colon *versus* the rectum. Taken together, these results may suggest that the colon is more susceptible than the rectum to any growth-promoting effects of hyperinsulinemia—although, it must also be noted that the number of colon cancer cases in this study was higher than the number of rectal cancer cases. Interestingly, both excess body weight and physical activity have been suggested to be more strongly related to the risk of colon than rectal cancer, with excess weight thought to be promotive and exercise purported to be protective.[5,16] Indeed, both of these variables are thought to affect cancer risk by modulating insulin resistance and hyperinsulinemia. However, in the present study, as in other studies,[22–24] the positive association between C-peptide levels and risk of colon and rectal cancers was only slightly attenuated after adjustment for BMI and physical activity. To further explore the role of hyperinsulinemia, an interaction was tested between C-peptide and waist circumference, a variable that is thought to reflect the level of insulin resistance. Although the interaction term was not statistically significant, the interaction analysis did show an increasing CRC risk with increasing serum C-peptide concentrations and higher waist circumference (particularly in the colon), strengthening the suggestion that hyperinsulinemia is part of the underlying etiologic mechanism of these cancers.

No clear effects of IGFBP-1 and -2 were observed in the present study, similar to some previous studies.[21,26] However, others have observed that higher levels of these IGFBPs are associated with a decrease in CRC risk, particularly in the colon.[22,24] These previous observations are in line with the understanding that higher levels of IGFBP-1 and -2 may decrease the bioactivity of IGF-I—which could lead to a reduction in CRC risk—and that higher insulin levels may inhibit the biosynthesis of IGFBP-1 and -2 resulting in greater IGF-I bioactivity, which could lead to an increase in CRC risk. But the IGF/IGFBP system is very complex and not only are all the potential inter-relationships of these compounds not fully elucidated, it is also not precisely known how IGF-I and its binding proteins may interact within cancer cells.[37]

In the present study, blood donations were not systematically collected under fasting conditions, and only a subset of subjects were fasting at the time of blood donation. The fasting status is important because it allows comparison of chronically elevated blood insulin levels (*i.e.* higher fasting serum C-peptide levels) with postprandially elevated blood insulin levels (*i.e.* higher nonfasting serum C-peptide levels). The etiologic importance of these 2 phases of blood insulin levels on development of colon and rectal cancers is relatively unexplored. Nevertheless, it has been suggested that elevated postprandial blood insulin level may be of greater relevance, since it precedes the development of fasting hyperinsulinemia.[38] This has, in part, been confirmed by the prospective study of Schoen *et al.*,[20] which showed fasting insulin levels were not as strongly associated with colon cancer risk than insulin and glucose levels measured after a glucose challenge. Although the risk estimates observed in the present study showed no statistically significant heterogeneity by fasting status, the positive CRC association appears to be slightly stronger in nonfasting than fasting subjects. However, it must be noted that the number of fasting subjects in the present study is somewhat limited. In addition, the intercorrelations of the 3 peptides did not differ by fasting status, although blood concentrations of all 3 were higher in nonfasting than fasting subjects. These findings suggest that both chronically elevated insulin levels and postprandially elevated insulin levels may be associated with colon and rectal cancer risk to some extent.

In summary, this study has shown that hyperinsulinemia, as determined by blood C-peptide concentrations, is associated with an increased risk of colon cancer as well as colon and rectal cancers combined. The observed association did not differ by fasting status or gender. Further studies are necessary to elucidate any potential roles of IGFBP-1 and IGFBP-2 and to better understand the cancer promotive mechanisms of insulin action. These results may be important from a public health perspective for primary and secondary prevention of colorectal cancer.

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