Insulin Resistance, Apoptosis, and Colorectal Adenoma Risk

Temitope O. Keku,[1] Pauline Kay Lund,[2] Joseph Galanko,[1] James G. Simmons,[2] John T. Woosley,[3] and Robert S. Sandler[1]

[1]Department of Medicine and Center for Gastrointestinal Biology and Disease and Departments of [2]Cell and Molecular Physiology and [3]Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina

Abstract

Compelling evidence from epidemiologic studies indicates that elevated circulating insulin-like growth factor (IGF)-I, insulin resistance, and associated complications, such as elevated fasting plasma insulin, glucose and free fatty acids, glucose intolerance, increased body mass index, and visceral adiposity, are linked with increased risk of colorectal cancer. However, the role of insulin and markers of glucose control in the development of adenomas, precursors to colorectal cancer, has not been fully explored. We evaluated the relationship between plasma insulin, glucose, IGF-I, IGF-II, IGF-binding protein-3 (IGFBP-3), apoptosis, and colorectal adenomas in a case-control study. Participants were drawn from consenting patients undergoing colonoscopy at the University of North Carolina hospitals (Chapel Hill, NC). Participants were classified as cases or controls based on whether they had one or more colorectal adenomatous polyps. Fasting plasma insulin, IGF-I, IGF-II, and IGFBP-3 levels were assessed by ELISA. Glucose was measured by glucose hexokinase assay. Apoptosis was assessed by morphology on H&E-stained sections. Dietary and lifestyle information were obtained by telephone interview. Logistic regression was used to examine the association between adenoma status and insulin-IGF markers. Adenoma cases (n = 239) and adenoma-free controls (n = 517) provided rectal biopsies and/or blood samples and interview data. Consistent with prior findings, cases were more likely to be males, older, have higher waist-to-hip ratio, lower calcium intake, lower apoptosis, and less likely to report nonsteroidal anti-inflammatory drug use. Those in the highest quartile of insulin (adjusted odds ratio, 2.2; 95% confidence interval, 1.1-4.2) and glucose (adjusted odds ratio, 1.8; 95% confidence interval, 0.9-3.6) were more likely to have an adenoma compared with the lowest quartile. Similarly, subjects in the highest two quartiles of insulin were more likely to be in the lowest two quartiles of apoptosis. Overall, there were no significant differences between mean circulating levels of glucose, IGF-I, IGF-II, and IGFBP-3 among cases and controls and no association between these variables and apoptosis. The results provide novel evidence that elevated insulin and glucose are associated with increased adenoma risk and decreased apoptosis in normal rectal mucosa. These findings suggest that insulin may act early in the adenoma-carcinoma sequence to promote the development of colorectal adenoma by decreasing apoptosis in the normal mucosa. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2076–81)

Introduction

Colorectal adenomas are early intermediates in the pathologic transformation of normal colonic epithelial cells to colorectal cancer. Defining mediators that favor cell growth or limit apoptosis in the normal colonic mucosa is important in the prevention of colorectal cancer. Insulin and insulin-like growth factors (IGF) have been observed to have growth-promoting effects and antiapoptotic actions *in vitro* (1) and in animal models (2-5).

Structurally related insulin receptors and IGF-I receptors mediate the actions of insulin, IGF-I, and IGF-II. Insulin has high affinity for the insulin receptor, which mediates its metabolic actions. At high concentrations, insulin can bind to IGF-I receptors or the hybrid insulin-IGF receptor to activate signal transduction cascades leading to proliferation and apoptosis (2, 5-8). Insulin can also act directly to promote IGF-I biosynthesis, enhancing IGF-I bioavailability and inhibiting the production of IGF-binding protein (IGFBP)-1, IGFBP-2, and IGFBP-3 (6, 9-13).

IGF-I and IGF-II play an important role in normal growth and development (14). IGF-I and IGF-II have autocrine, paracrine, and endocrine actions on cell proliferation and apoptosis (15-17). The IGF-I receptor mediates all of the known proliferative actions of IGF-I and IGF-II. A family of at least seven IGFBPs modulate the actions of IGF (15). Of these, IGFBP-3 is the predominant IGFBP in circulation. IGFBP-3 limits the bioavailability of circulating IGFs for interactions with the IGF-I receptor (15, 18, 19). IGFBP-3 also has independent growth-inhibitory effects through the induction of apoptosis (2, 5, 20). IGFBP-3 has been reported to mediate p53-dependent apoptosis of colon cancer cell lines following γ-radiation-induced DNA damage (21) and to reduce the growth of experimental colon tumors (22).

Considerable evidence indicates that perturbations in insulin, IGFs, or IGFBP-3 increase the risk of colon cancer (18, 23-30) as well as cancers of other organs (3). A disruption of the interrelationship between insulin secretion and insulin action results in hyperinsulinemia, hyperglycemia, and type 2 diabetes. Factors, such as elevated levels of fasting plasma insulin, IGF-I, glucose, and free fatty acids, increased body mass index (BMI), and visceral adiposity, all hallmarks of insulin resistance, are associated with increased risk of colorectal cancer (23, 24, 31, 32). Hyperinsulinemia is associated with increased risk of colorectal cancer in human and animal studies (24, 28). Interestingly, patients with type 2 diabetes are at increased risk of colorectal cancer (33). A recent article reported that exogenous insulin increased the risk of colorectal cancer (34). In addition, studies in animal models (35, 36) and humans suggest that caloric restriction is associated with reduced insulin and IGF-I levels and is also linked to decreased risk of colorectal cancer (37). Studies on acromegaly, a condition characterized by elevated levels of growth hormone and oversecretion of IGF-I, support a link between elevated IGF-I levels, increased proliferation of

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Requests for reprints: Temitope O. Keku, Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, CB 7555, Chapel Hill, NC 27599-7555. Phone: 919-966-5828; Fax: 919-966-7468. E-mail: tokeku@med.unc.edu

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normal mucosa (37), and increased risk of colorectal adenoma and cancer (38-42).

Relatively few studies have evaluated the relationship between insulin or the IGF-axis and the risk of colorectal adenomas. In a case-control study that examined the relationship between serum IGF-I, IGF-II, IGFBP-2, IGFBP-3, and colorectal adenomas, Renehan et al. (43) reported significant associations between increased IGF-II, decreased IGFBP-2, and reduced risk of adenomas. Similarly, Jenkins et al. (44) observed an association between development of new adenomas and elevated serum IGF-I. Two other studies also reported associations between elevated plasma IGF-I levels, decreased IGFBP-3 levels, and increased risk of late-stage adenomas (45, 46). Although Teramukai et al. (47) did not observe a significant relationship between increased IGF-I and colorectal adenomas, they reported strong positive associations between fasting plasma glucose, BMI, and advanced colorectal adenomas (47). However, not all studies have reported positive findings. A recent study found an inverse association between elevated fasting serum insulin levels and risk of colorectal adenomas (48). Although most studies have observed inverse associations between IGFBP-3 levels and colorectal adenoma or cancer risk (26, 45), Kaaks et al. (49) observed a significant increase risk of colorectal cancer among women in the highest quintile of IGFBP-3. Overall, most studies support a role of increased levels of IGFs or insulin and decreased IGFBP-3 as risk factors for colorectal adenoma or cancer. However, at present, few if any studies have addressed mechanisms.

In a recent epidemiologic study, we observed that decreased levels of physiologic apoptosis in the normal colonic mucosa was significantly associated with increased adenoma risk (50). Based on these findings, we postulated that factors that mediate growth or limit apoptosis in the colon will contribute to adenoma development. Defining such factors is important to the development of improved preventive and surveillance strategies. In this case-control study, we examined the relationship between plasma insulin, glucose, IGF-I, IGF-II, or IGFBP-3, colorectal adenomas, and levels of apoptosis within normal colonic mucosa. Importantly, we assessed whether these factors mediate the relationship between apoptosis and colorectal adenomas.

Materials and Methods

Study Population. Study participants in the Diet and Health Study III were drawn from outpatients who underwent colonoscopy for screening and a variety of indications between August 1998 and March 2000 at the University of North Carolina hospitals (Chapel Hill, NC). Eligible subjects were enrolled if they gave informed consent, agreed to participate in a telephone interview, agreed to give rectal biopsies during the procedure, or have blood drawn. Exclusion criteria included incomplete examination (cecum not reached), age <30 years, inability to give informed consent, polyposis (>100 polyps), previous colon resection or cancer, colitis, and previous colon adenoma. The study pathologist (J.T.W.) personally assessed all histology of colon polyps in the study and classified polyps using standard pathologic criteria. Cases were defined as individuals who had one or more adenomatous polyps. Control subjects had no adenomatous polyps. The study was approved by the institutional review board at the University of North Carolina School of Medicine.

Data Collection. Each consenting and eligible participant was asked about the time they last ate (to confirm an overnight fast) and the type of colonoscopy preparation used. The research assistant also measured their waist, hips, height, and weight. During the study period, 2,456 colonoscopies were done in the Gastrointestinal Unit of which 1,530 did not meet eligibility criteria. A total of 926 subjects were eligible to participate, 57 (6.2%) refused to participate, 66 (7.1%) were not asked because the research assistant was not available, and 803 (93.4%) consented to participate in the study.

Enrolled subjects were interviewed over the telephone about their diet and lifestyle by a trained interviewer usually at a time convenient for the subject but no later than 12 weeks of colonoscopy. The lifestyle questionnaire was used to collect data about demographics, family history, education, medical history, physical activity, and other environmental factors. Dietary information was collected using the Block food frequency questionnaire (51) that queried ~106 foods and usual portion size (small, medium, or large) consumed. Lifestyle and dietary interview data were available for 756 subjects, of whom 504 had adequate biopsies for measuring apoptosis and 457 had blood specimens for insulin-IGF measures.

Biological Specimens And Laboratory Assays. In preparation for colonoscopy, subjects used either a balanced electrolyte polyethylene glycol lavage or a phosphate-containing purge. Biopsy specimens were obtained from consenting subjects during colonoscopy. At the beginning of the endoscopic procedure, six mucosal pinch biopsies were obtained 8 to 10 cm from the anal verge using standard (8 mm wing) disposable, fenetrated colonoscopy forceps (Wilson-Cook, Winston-Salem, NC). Biopsies were taken one at a time from normal-appearing mucosa with care taken to avoid raised lesions or larger blood vessels. The same site was sampled in all subjects. Blood samples were obtained at the time of colonoscopy. Plasma was prepared from the blood samples and stored in aliquots at −80°C until assayed. Care was taken to avoid repeated freezing and thawing of samples. Only samples from patients with excellent colonoscopy preparation and confirmed overnight fast (based on verbal response about last food intake and supportive evidence of a clean colon) were analyzed. Plasma insulin, IGF-I, IGF-II, and IGFBP-3 levels were measured by ELISA using reagents from Diagnostic Systems Laboratory (Webster, TX). IGF-I and IGF-II were measured after acid-ethanol extraction to remove IGFBPs. Fasting plasma glucose was measured by the glucose hexokinase assay (Sigma, St. Louis MO). Laboratory personnel were blinded to the case or control status of samples. Intraassay coefficients of variation for insulin and IGF-I were 2.6% and 6.5%, respectively; IGF-II and IGFBP-3 were 1.5% and 4.7% respectively. The intraassay and interassay coefficients of variation for measurements of glucose were <5%. The interassay coefficient of variation was <12% for all the insulin-IGF measures.

Assays of Apoptosis. Colonic biopsies were fixed in 10% buffered formalin and processed by routine histology. Apoptosis was assessed by morphologic identification of apoptotic cells in H&E-stained sections in normal colonic mucosa from adenoma cases and nonadenoma subjects. The terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (Apoptag kit, Intergen Co., Purchase, NY) assay (52) was used to confirm the apoptosis results obtained by morphologic analysis on a subset of samples. The manufacturer's protocol was optimized for specific detection of apoptosis in intestinal epithelium by using one third of the recommended amount of terminal deoxynucleotidyl transferase enzyme in the reaction buffer to minimize nonspecific background staining (53). The crypt selection criteria were described previously (54). Longitudinal crypt sections, 8 to 12 per biopsy, were selected and scored if the base, middle, and top of the crypts were in the plane of the section and the crypt lumen was visible. Apoptosis was observed in isolated single cells not associated with an inflammatory response on H&E-stained sections. Apoptotic cells were recognized by cell shrinkage, chromatin condensation, and formation of apoptotic bodies. Cells were not scored

as apoptotic if the nucleus did not meet these criteria. An experienced technician, blinded to the case-control status of the subjects, scored the tissue sections. The same reader scored selected slides a second time. The intrareader variability for morphologic determination of apoptosis was <1%. Apoptosis was expressed as the average number of apoptotic bodies per crypt counting at least 16 (and as many as 24) crypts from two biopsies per subject. In a previous study, we assessed apoptosis in normal colonic mucosa using two independent measures (50), and we have confirmed agreement between these two measures of apoptosis (morphology and terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling) on a subset of samples in the current study.

Statistical Analysis. Comparison of continuous and categorical variables between adenoma cases and nonadenoma controls were made using t tests and χ^2 tests, respectively. The relationships between plasma insulin, glucose, IGF-I, IGF-II, IGFBP3, and colorectal adenomas were assessed in the study population by comparing mean values between case and control subjects using t tests. The distribution of insulin, glucose, IGF-I, IGF-II, and IGFBP-3 measures among control subjects was used to generate quartile values. The lowest quartile of each measure (insulin, glucose, IGF-I, IGF-II, and IGFBP-3) was considered as the reference. We used values from control subjects to generate quartiles. Apoptosis was expressed as the average number of apoptotic bodies per crypt as described above. Using the median as cut point, we divided apoptosis measures into lower half (below the median) and upper half (above the median).

Logistic regression models were used to examine the association between adenoma status and insulin, glucose, IGF-I, IGF-II, IGFBP3, or apoptosis while controlling for sex, age, and nonsteroidal anti-inflammatory drugs (NSAID). We calculated Ps comparing quartile 4 versus quartile 1. In addition, we also did the trend test to test for linear increase over quartile 1 to quartile 4 (P_{trend}). Potential confounders were age, gender, family history, smoking status (current, former, and never), monthly NSAID use in the past 5 years, and total daily calcium intake (dietary plus supplemental). The relationship between insulin, glucose, IGF-I, IGF-II, and IGFBP-3 and lifestyle/dietary factors, such as BMI, waist-to-hip ratio (WHR) dietary fat, NSAIDs, and calcium were assessed by the Pearson correlation coefficient.

Results

The characteristics of the study participants who provided specimens for insulin-IGF measures, glucose measures, apoptosis, and dietary/lifestyle data are presented in Table 1. There were 239 adenoma cases and 517 adenoma-free controls who had blood and/or biopsy specimens and lifestyle/dietary data. Adenoma cases and nonadenoma controls differed significantly on age, sex, WHR, diabetes history, NSAID use, total daily calcium, and apoptosis. Cases were significantly older (59.7 ± 0.7 years) than controls (54.1 ± 0.5 years). Cases were more likely than controls to have higher WHR (P = 0.004). Consistent with previous observations (50), cases had significantly lower apoptosis (P = 0.0001) and report lower NSAIDs use than controls. Family history of colorectal cancer and total calcium intake were lower in cases than controls. Race, BMI, dietary fat intake, alcohol, and smoking status showed no significant association with case-control status.

Among cases and control subjects who provided blood specimens for insulin-IGF measures (cases, n = 136; controls, n = 321), we examined the relationship between mean fasting plasma insulin, glucose, IGF-I, IGF-II, IGFBP-3, and adenomas (Table 1) Fasting plasma insulin, glucose, IGF-I, IGF-II, and IGFBP-3 levels did not differ significantly between cases and controls overall, although the mean insulin level was somewhat higher in cases. Among men, the mean IGF-I levels were significantly lower in were cases than controls [mean ± SE IGF-I (ng/mL): cases, 126.6 ± 5.7; controls, 145.8 ± 6.3; P = 0.02]. The IGF-I/IGFBP3 ratio was also lower in male cases than male controls (cases, 0.042 ± 0.002; controls, 0.051 ± 0.004; P = 0.05). Among women, there were no significant differences in mean fasting plasma insulin, glucose IGF-I, IGF-II, and IGFBP-3 levels between cases and controls.

Table 1. Selected descriptive characteristics of adenoma cases and adenoma-free controls

Variable	Cases (n = 239)	Controls (n = 517)	P
Mean (SE) age	59.7 (0.7)	54.1 (0.5)	0.001
White (%)	77	80	0.49
Male (%)	54	36	0.001
Family history (%)	19	26	0.06
Mean (SE) BMI	28.3 (0.4)	27.6 (0.3)	0.15
Mean (SE) WHR	0.97 (0.01)	0.95 (0.01)	0.004
Diabetes (% yes)	19	12	0.01
Mean (SE) dietary fat (g/d)	61.6 (1.9)	58.3 (1.3)	0.16
Mean (SE) alcohol (g/d)	5.9 (0.7)	5.1 (0.5)	0.44
Smoking			
Current (%)	16	19	0.06
Former (%)	39	30	
Never (%)	45	50	
Mean (SE) monthly NSAID use in the past 5 y	9.0 (1.4)	13.2 (1.3)	0.02
Mean (SE) total daily calcium (mg)	887 (36)	972 (30)	0.07
Apoptosis (no. labeled cells; SE)	2.47 (0.04)	2.95 (0.04)	0.001
Insulin (SE), microunits/mL	11.4 (1.2)	9.8 (0.7)	0.25
Glucose	115.7 (4.0)	111.6 (2.8)	0.42
IGF-I (SE), ng/mL	121.4 (4.8)	130.7 (3.9)	0.13
IGF-II (SE), ng/mL	527.7 (14.0)	548.6 (10.2)	0.25
IGFBP-3 (SE), ng/mL	3,177 (80)	3,255 (51)	0.41

Comparisons of high versus low levels (quartiles) of insulin, glucose, IGF-I, IGF-II, IGFBP-3, and their potential association with adenoma status while controlling for age, sex, and NSAIDs revealed significant positive associations. Each measure (insulin, glucose, IGF-I, IGF-II, and IGFBP-3) was categorized into quartiles, with the lowest quartile considered as the reference. We did not adjust for BMI and WHR because they were positively correlated with insulin and glucose and felt to be in the casual pathway to adenomas. The results are presented in Table 2. In the study population as a whole, those in the highest quartile of plasma insulin were significantly more likely to have an adenoma [adjusted odds ratio (OR), 2.2; 95% confidence interval (95% CI), 1.1-4.2; P_{trend} = 0.02]. When broken down by gender, the data revealed that women in the highest quartile of insulin were at increased risk for adenoma compared with the lowest quartile (OR, 3.1; 95% CI, 1.1-9.0; P_{trend} = 0.02). However, men did not show a significant association (P = 0.24) between insulin and adenoma risk (Table 2). Similarly, the risk of colorectal adenoma comparing those in the upper quartile of glucose with the lowest quartile was increased (P_{trend} = 0.04). Both men and women showed a trend for a relationship between elevated plasma glucose and adenoma risk (men, P_{trend} = 0.07; women, P_{trend} = 0.09). Compared with the lowest quartile, high levels of IGF-I, IGF-II, and IGFBP-3 were not associated with adenoma risk.

As reported previously (50), apoptosis in the normal colonic mucosa measured by morphology showed a highly significant association with adenoma status (P = 0.0001). Subjects in the highest quartile of apoptosis were less likely to have an adenoma compared with those in the lowest quartile (adjusted OR, 0.1; 95% CI, 0.06-0.2; P_{trend} = 0.0001). In both men and women, high apoptosis was inversely associated with adenomas (P = 0.0001 for men and women separately). To assess the

Table 2. Adjusted ORs and 95% CIs for the relationship between insulin-IGF axis and adenoma

Factor	Overall			Men			Women		
	Q4 case/control	Q1 case/control	OR* (95%CI)	Q4 case/control	Q1 case/control	OR† (95%CI)	Q4 case/control	Q1 case/control	OR† (95%CI)
Insulin	38/73	18/78	2.2 (1.1-4.2)	26/31	13/29	1.6 (0.7-3.8)	14/46	5/50	3.1 (1.1-9.0)
Glucose	44/76	19/73	1.8 (0.9-3.6)	31/36	14/34	1.9 (0.8-4.2)	16/42	6/44	2.5 (0.9-7.1)
IGFBP-3	25/76	39/75	0.9 (0.4-1.7)	18/28	25/40	1.7 (0.7-4.0)	9/50	16/39	0.5 (0.2-1.3)
IGF-I	28/75	29/75	1.0 (0.5-2.0)	18/38	14/19	0.8 (0.3-2.0)	11/38	17/59	1.3 (0.5-3.2)
IGF-II	21/77	27/71	1.0 (0.5-2.1)	15/23	23/38	1.5 (0.6-3.7)	8/54	7/39	0.9 (0.3-2.8)

NOTE: OR comparing Q4 versus Q1.
*Adjusted for sex, age, and NSAIDs.
†Adjusted for age and NSAIDs.

relationship between insulin and apoptosis, we examined the association between plasma insulin levels and the odds of being in the lowest two quartiles of apoptosis (Table 3). Subjects in the highest two quartiles for plasma insulin were significantly more likely to be in the lowest two quartiles for apoptosis. There were no significant associations between plasma glucose, IGF-1, IGF-II, IGFBP-3, and apoptosis. Circulating levels of insulin, glucose, IGF-I, IGF-II, and IGFBP-3 were not associated with adenoma size among case subjects (data not shown).

We also examined the relationship between known risk factors, such as BMI and WHR, which were related to both colorectal adenomas and insulin resistance. We observed modest but highly significant positive correlations between insulin and BMI (Spearman correlation coefficient $r = 0.26$; $P < 0.0001$), insulin and WHR ($r = 0.14$; $P = 0.005$), glucose and WHR ($r = 0.15$; $P = 0.003$), and glucose and BMI ($r = 0.14$, $P = 0.002$).

Discussion

Evidence from epidemiologic studies support a link between markers of insulin resistance, hyperglycemia, increased plasma IGF levels, reduced IGFBP-3, and increased risk of colorectal cancer (18, 23, 24, 27-29). The role of the insulin, IGFs, and IGFBP-3 in the development of colorectal adenomas has not been extensively explored. We assessed the relationship between insulin, glucose, IGF-I, IGF-II, IGFBP-3, and colorectal adenomas in a case-control study among participants undergoing routine colonoscopy at one university hospital. In addition, we examined whether the relationship between adenomas and apoptosis was modified by insulin, glucose, IGF-I, IGF-II, and IGFBP-3. We found that a high fasting insulin level particularly in women was associated with increased risk of adenomas, although there was a trend for an association between elevated fasting plasma glucose and adenoma risk. We also observed that elevated circulating insulin levels were associated with low apoptosis in the normal colonic mucosa. No significant associations were observed between plasma IGF-I, IGF-II, or IGFBP-3 and adenoma or apoptosis.

The observed association between elevated plasma insulin concentrations and adenoma risk are consistent with most studies linking hyperinsulinemia and insulin resistance with increased risk of colorectal cancer (18, 27, 28, 32, 37, 49, 55) Reports from the few previous studies that examined the relationship between plasma insulin or other markers of glycemic control and colorectal adenomas are mixed. For example, Misciagna et al. (48) found an inverse association between elevated fasting insulin levels and colorectal adenoma risk in nondiabetic subjects. Saydah et al. (56) observed no association between elevated insulin and colorectal cancer. However, these studies reported positive associations between plasma glucose, BMI, and colorectal adenomas (47, 48) and cancer (56). The reasons for the discrepancy in results are not clear.

Elevated plasma glucose and diabetes are associated with increased risk of colorectal cancer (24, 47, 57, 58). We observed a trend for an association between elevated plasma glucose and increased risk for adenomas. Our findings support reports from other studies on colorectal adenoma/cancer and glucose control (47, 59). One study found no association between fasting serum glucose and adenoma risk but reported an increased risk of colorectal adenoma with high levels of fructosamine, a marker of glycated proteins in serum and blood glucose. The risk for adenomas was increased 2.3-fold in those with high fructosamine levels compared with subjects with low fructosamine (48). Some studies failed to confirm the positive relationship between glucose and colorectal adenomas (60, 61). The study by Platz et al. observed no increased risk of colorectal adenoma or cancer among women with elevated glycosylated hemoglobin (HbA1c) levels, a clinical marker of long term glucose control.

Table 3. ORs and 95% CIs for the association between insulin, glucose, IGF-I, IGF-II, IGFBP-3, and apoptosis

Factor	Apoptosis, *n* (lower half/upper half)	OR*,† (95% CI)	*P*
Insulin			
Q1	36/33	1.0 (Reference)	—
Q2	51/32	1.5 (0.8-2.9)	0.22
Q3	55/23	2.4 (1.2-4.7)	0.01
Q4	50/23	2.2 (1.1-4.4)	0.05
P_{trend}			0.01
Glucose			
Q1	39/29	1.0 (Reference)	—
Q2	44/31	1.1 (0.6-2.2)	0.73
Q3	51/22	1.8 (0.9-3.6)	0.10
Q4	58/29	1.5 (0.8-3.0)	0.22
P_{trend}			0.14
IGF-I			
Q1	56/35	1.0 (Reference)	—
Q2	53/28	1.0 (0.5-1.9)	0.96
Q3	48/23	1.1 (0.5-2.1)	0.82
Q4	34/25	0.7 (0.3-1.4)	0.30
P_{trend}			0.57
IGF-II			
Q1	44/32	1.0 (Reference)	—
Q2	52/26	1.5 (0.8-3.0)	0.21
Q3	54/29	1.4 (0.7-2.6)	0.35
Q4	42/24	1.3 (0.7-2.7)	0.43
P_{trend}			0.27
IGFBP-3			
Q1	49/31	1.0 (Reference)	—
Q2	45/20	1.5 (0.7-3.0)	0.27
Q3	52/31	1.0 (0.5-2.0)	0.89
Q4	46/29	1.0 (0.5-1.9)	0.89
P_{trend}			0.84

*Odds of being in the lower two quartiles of apoptosis.
†Adjusted for age, sex, and NSAIDs usage.

Spontaneous apoptosis may remove genetically damaged colonic crypt cells and prevent further proliferation or expansions (62). Consistent with this concept, previous reports by us (50) and others (63-65) indicate that individuals with low apoptosis in the normal colonic mucosa are more predisposed to develop colorectal adenoma. Our observations of lower rates of apoptosis in normal mucosa distant from adenomatous tissue suggest a field effect in which a generalized decrease in apoptosis throughout the colon is predictive of increased adenoma risk. This finding raises the possibility that low of apoptosis is an early event in the carcinogenesis process. Indeed, our observations that elevated levels of circulating insulin are associated with low apoptosis in normal colorectal mucosa suggest a possible mechanism of insulin action and support reports of other studies. In addition to promoting glucose metabolism, insulin has also been shown to promote DNA synthesis, stimulate cell division, and inhibit apoptosis *in vitro* (66) and *in vivo* (4). Elevated insulin may inhibit apoptosis by interacting with IGF-I receptor, enhancing nuclear factor-κB activation (67) or decreasing peroxisome proliferator-activated receptor-γ activation (68).

Obesity and central adiposity are associated with increased risk of colon cancer. We observed modest positive correlations between insulin, glucose, BMI, and WHR. These observations agree with epidemiologic evidence that obesity and especially central adiposity are risk factors for colorectal adenomas and cancer (23, 69) Elevated BMI and increased intra-abdominal fat stores are associated with elevated fasting insulin levels and insulin resistance. Hyperinsulinemia generally leads to lower levels of IGFBP-1 (70, 71). This physiologic relationship is reflected in our data as shown by the positive association between insulin, BMI, and WHR and negative correlations between IGFBP3, insulin, and WHR. Although increased WHR was related to increased adenoma risk in our study, the risk was attenuated after adjustment for insulin. The data suggest that the risk from obesity is mediated at least in part by insulin.

Although several studies suggest an association between members of the IGF-axis, colorectal adenomas (47), and cancer (49, 72, 73), we found no association between IGF-I, IGF-II, IGFBP-3, and adenoma risk overall, but among women we observed a nonsignificant trend for an inverse association between high levels of plasma IGFBP-3 and adenoma risk. Possible explanations for the discrepancy in findings across IGF studies include variation in methods of detecting circulating IGFs between studies, methods of specimen collection (serum or plasma-EDTA/heparin), and specimen handling. For example, IGF-I levels have been found to be 10% higher in serum than plasma, whereas no differences in IGF-I levels were observed in plasma collected from EDTA and heparin tubes (74). IGFBP-3 levels in serum and plasma from heparin tubes were significantly different from EDTA plasma (74). These observations suggest a need for standardization for IGF assays to make comparisons across studies. In future studies, we will assess IGF-I in serum.

The major strengths of this study include the study design to simultaneously measure anthropometrics, apoptosis, fasting insulin, glucose, IGFs, and adenomas in a population of average-risk subjects undergoing colonoscopy. Patients undertook an overnight fast, which likely reduces the variability in fasting insulin and other measures of glycemic control. Another advantage is the availability of potentially confounding dietary and lifestyle information. Colonoscopy-based case-control studies have some limitations. The study population may not be representative of the general population because they are all going for colonoscopy. There is a potential family history bias. Controls may be at higher risk than the general population, and because of a family history of colorectal cancer, controls may be getting colonoscopies before polyps appear, thereby making them more like cases and attenuating associations. Another potential limitation of our study is the availability of plasma samples for one-time measures of insulin, glucose, and IGF levels. Single measurements of circulating insulin, glucose, or IGFs may not accurately reflect levels over time. This may lead to misclassification and bias our results toward the null. However, evidence suggests that IGF-I and IGFBP-3 are stable over time (75, 76). In this study, we evaluated only IGFBP-3 because it is the major IGFBP; however, future analysis of other IGFBPs in similar study population would be interesting.

In summary, we confirmed that low apoptosis was a significant predictor of adenoma in normal rectal mucosa. We found that elevated insulin was significantly associated with increased adenoma risk and low apoptosis in the normal mucosa. Our observations suggest a possible mechanism by which insulin increases adenoma risk possibly through down-regulation of apoptosis. Our findings also indicate that insulin but not IGFs may be more relevant to the development of colorectal adenoma. Patients with elevated insulin may benefit from frequent screening for polyps/colon cancer.

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