A Prospective Study of Plasma Insulin-like Growth Factor-1 and Binding Protein-3 and Risk of Colorectal Neoplasia in Women[1]

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Abstract

Insulin-like growth factor-1 (IGF-1) is an important mitogen, and IGF binding protein-3 (IGFBP-3) has opposing effects. Acromegalics, who have abnormally elevated levels of IGF-1, are at increased risk of colorectal tumors. Recent studies have found that IGF-1 levels correlate with risk of prostate cancer and colorectal cancer in men, premenopausal breast cancer in women, and lung cancer in men and women. We examined whether prediagnostic plasma levels of IGF-1 and IGFBP-3 influence risk of colorectal cancer and adenoma in women. From 1989 to 1990, a total of 32,826 women from the Nurses' Health Study provided blood specimens that were archived in liquid nitrogen. During 6 years of follow-up from 1989 to 1994, we documented 79 new cases of colorectal cancer, 90 cases of intermediate/late-stage adenoma (≥1 cm or tubulovillous/villous histology), and 107 cases of early-stage adenoma (<1 cm and tubular histology). After matching controls (2:1 for cancers and 1:1 for adenomas) to cases by age, month of blood draw, fasting status, and indication for endoscopy (for adenoma controls), plasma IGF-1 and IGFBP-3 levels were measured. Controlling for IGFBP-3 level, relative to women in the low tertile of IGF-1, those in the high tertile were at elevated risk of intermediate/late-stage colorectal neoplasia adenoma [multivariate relative risk (RR), 2.78; 95% confidence interval (CI), 0.76–9.76] and cancer (RR, 2.18; 95% CI, 0.94–5.08). Controlling for IGF-1 level, relative to women in the low tertile of IGFBP-3, women in the high tertile of IGFBP-3 were at lower risk of intermediate/late-stage colorectal adenoma (RR, 0.28; 95% CI, 0.09–0.85) and cancer (RR, 0.28; 95% CI, 0.10–0.83). Neither IGF-1 nor IGFBP-3 had any appreciable relation with early-stage adenoma. These analyses indicate that high levels of circulating IGF-1 and particularly low levels of IGFBP-3 are associated independently with an elevated risk of large or tubulovillous/villous colorectal adenoma and cancer.

Introduction

IGF-1[3] is an important mitogen required for progression through the cell cycle (1). More than 90% of circulating IGFs are complexed with IGFBP-3. Most IGFs and IGFBP-3 found in the circulation are produced in the liver and are up-regulated by growth hormone (2). The actions of IGFBPs can oppose those of IGF-1, in part by binding IGF-1 (3), but also by direct inhibitory effects on target cells (4). Tissue IGF bioactivity is determined not only by circulating IGF-1 and IGFBP levels but also by local production of IGFs, IGFBPs, and IGFBP proteases (2) that enhance IGF-1 availability by cleaving IGFBPs. Despite this complexity, determinants of tissue IGF bioactivity appear to be regulated in parallel with circulating IGF-1 level (5, 6); thus, circulating IGF-1 level may represent tissue IGF bioactivity.

Normal colorectal epithelia and cancer cells express IGF-1 receptors, which stimulate mitogenesis when activated by IGF-1 *in vitro* (7, 8). As mitogens, IGFs may be important in colorectal carcinogenesis, possibly by increasing the risk of cellular transformation by enhancing cell turnover. Moreover, in colon cancer cell lines, IGF-1 increases production of vascular endothelial growth factor, an angiogenic factor that supports cancer growth (9), and overexpression of IGF-1 receptors is also important for the survival and maintenance of transformed cells (10). Indeed, acromegaly, characterized by chronically elevated growth hormone levels that cause IGF-1 hypersecretion, is associated with increased epithelial cell proliferation in the sigmoid colon (11) and elevated risk of tubulovillous adenomas and colorectal cancer (12).

In recent studies, high but normal plasma IGF-1 and low IGFBP-3 levels were independently associated with a greater risk of prostate cancer (13), premenopausal breast cancer (14), and lung cancer (15). A recent analysis in the Physicians' Health Study found high IGF-1 levels and, in particular, low IGFBP-3 levels, to be related to a high risk of colorectal cancer in men (16). Here, we examine the relationship between plasma IGF-1 and IGFBP-3 levels and risk of colorectal adenoma and cancer in a case-control study of women nested within the prospective NHS.

[3] The abbreviations used are: IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; RR, relative risk; CI, confidence interval; NHS, Nurses' Health Study; BMI, body mass index.

Materials and Methods

Study Population. Cases of colorectal cancer and adenoma and controls were drawn from among participants in the NHS, an on-going prospective cohort study of 121,700 United States female registered nurses. Details of the design and follow-up of this cohort have been described previously (17). Briefly, in 1976, 121,700 United States female registered nurses, who were 30–55 years of age and married, completed a mailed questionnaire providing information on risk factors for cancer and cardiovascular disease to form the NHS. Biennially, information on updated exposure, medical procedures, and disease history was collected by mail. Food frequency questionnaires were administered in 1980, 1984, 1986, and 1990. From 1989 to 1990, 32,826 of the NHS participants provided a chilled blood specimen via overnight courier. After receipt by our laboratory, the heparinized blood was centrifuged; separated into plasma, erythrocytes, and buffy coat aliquots; and stored in liquid nitrogen freezers. Among cancer-free women who had provided a blood sample, the follow-up response rate to 1994 was 98%. We used the National Death Index to assess vital status among nonrespondents.

When women reported a diagnosis of colorectal cancer or adenoma on the questionnaires, these were confirmed through histopathological reports reviewed by a study investigator. On the basis of the endoscopy and pathology reports, we recorded adenoma number, site, size, and histology (tubular, tubulovillous, and villous). For cancers, we recorded site and Duke's stage at diagnosis.

Eligible colorectal cancer cases were women who supplied a blood sample between 1989 and 1990, were diagnosed with adenocarcinoma of the colon or rectum between the date of return of the blood sample and June 1, 1994, and had no prior (noncutaneous) cancer diagnosis. Controls were selected from the 32,826 women who provided a blood sample. Controls were required to not have had a cancer diagnosis at the time the matched case was diagnosed. We chose at random two controls matched to each case on year of birth, fasting status (for 68% of blood samples, the participant had fasted for $\geq$8 h), and month of blood draw. For the analyses, 79 cases and 158 matched controls were included. Seventy-eight % of the cases were diagnosed at least 1 year after the blood sample was provided. Thirty-one of the 158 had had an endoscopy between 1980 and 1989.

To be eligible for selection as a case or control for the colorectal adenoma analyses, women must have supplied a blood sample between 1989 and 1990, must have undergone sigmoidoscopy or colonoscopy after the date of return of the blood sample (1989–1994), and not have had a cancer or adenoma diagnosis, excluding nonmelanoma skin cancer, prior to the date of endoscopy. Because the majority of women who underwent endoscopy had a sigmoidoscopy, we included as cases only women with adenomas of the distal colorectum. Controls were matched to cases on year of birth, month of blood draw, fasting status, time period of endoscopy (within 2 years), and routine screening, gastrointestinal symptoms, or family history of colorectal cancer as indication(s) for endoscopy. A total of 207 matched pairs were included in the analysis, including 90 large ($\geq$1 cm) or tubulovillous/villous adenomas (69 were large; 21 were $<$1 cm and tubulovillous/villous) and 107 small, tubular adenomas (10 adenomas had missing data on size or histological subtype). Seventy-six % of the adenoma cases were diagnosed at least 1 year after the blood sample was provided.

Plasma IGF-1 and IGFBP-3 Assays. Plasma IGF-1 and IGFBP-3 were assayed using ELISAs with reagents from Diagnostic Systems Laboratory Inc. (Webster, TX). The IGF-1 values obtained by the ELISA were highly correlated (Pearson $r = 0.97$) with values obtained by radioimmunoassay after acid chromatography. All assays were carried out blinded to case-control status, and quality control samples were included within assay runs. Matched pairs were analyzed in the same run. The mean intrapair coefficients of variation were 7.2 and 8.6% for IGF-1 and IGFBP-3, respectively, for the adenoma analyses, and 2.5 and 3.9% for the cancer analyses. We have demonstrated previously that our collection methods did not adversely affect sample integrity (13).

Assessment of Other Factors. Mean values for demographic, dietary, and other covariates were computed from the 1980 through 1990 questionnaires, including BMI (weight in kg/square of height in m), physical activity (MET-hours/week), aspirin use (days/month), cigarette pack-years smoked, alcohol intake (g/day), red meat intake (servings/day), and dietary intake of vitamin D, calcium, phosphorus, folate, and methionine (all nutrients were adjusted for total energy intake by residual analysis; Ref. 18). We also assessed current (1990) use of postmenopausal hormones.

Statistical Analysis. We computed odds ratios to estimate the RR and corresponding 95% CI using conditional logistic regression (SAS version 6.12; SAS Institute, Cary, NC). Because IGF-1 and IGFBP-3 levels are positively correlated but have opposing effects biologically, it was necessary to adjust simultaneously for these factors to observe their independent effects. Tests for trend using two-sided *P*s were calculated by entering the tertile-specific median value for IGF-1 and IGFBP-3 as continuous variables in logistic regression models.

We examined the relation of IGF on three empirical stages of colorectal carcinogenesis: (*a*) early-stage small, tubular adenomas that may represent adenoma formation or "initiation", (*b*) intermediate/late-stage adenomas $\geq$1 cm in diameter or those that had a villous component (tubulovillous, villous), representing adenoma progression (these include *in situ* cancers); and (*c*) adenocarcinomas.

We computed independent effects of IGF-1 and IGFBP-3 in models with each of the following established or potential risk factors averaged from 1980 to 1990 for colorectal cancer (19): intake of fat, carbohydrate, protein, folate, methionine, red meat, and alcohol, physical activity, BMI, pack-years smoked, aspirin use, and current (1990) postmenopausal hormone use. These covariates were included only if there was indication of confounding, *i.e.*, if the RRs for IGF-1 or IGFBP-3 were appreciably different when these were added to models.

Results

Table 1 shows the levels of IGF-1 and IGFBP-3, their interrelationships, and their correlations with age, among controls, who represent the underlying population. The higher mean plasma IGF-1 for the adenoma controls reflects their lower median age compared with the cancer controls. For the controls for small tubular adenoma cases, the mean plasma IGF-1 level was 170.6 ng/ml, and the mean plasma IGFBP-3 level was 3822 ng/ml; for controls for large or villous adenoma cases, these respective levels were 162.5 and 3755 ng/ml. As expected, IGF-1 and IGFBP-3 were strongly correlated, and a moderate inverse correlation existed between age and total IGF-1, and age and IGF-1 adjusted for IGFBP-3 level (Table 1).

Table 2 shows results for total colorectal cancer, intermediate late-stage adenoma, and early-stage adenoma in relation

Table 1 Characteristics of IGF-1 and IGFBP-3 in the underlying (control) population

	Cancer controls	Adenoma controls
n	158	207
Median age (yr)	62	59
IGF (ng/ml)		
Mean	149.6	166.7
Median	140.0	158.5
IGFBP-3 (ng/ml)		
Mean	3724	3837
Median	3705	3809
Pearson correlation (*r*)		
IGF-1 and IGFBP-3	0.53	0.50
IGF-1 and age	−0.23	−0.27
IGF-1 and age[a]	−0.34	−0.29
IGFBP-3 and age	−0.10	−0.04

[a] IGF-1 is adjusted for IGFBP-3 level by residual analysis.

to IGF-1 and IGFBP-3 levels. The patterns were similar for intermediate/late-stage adenoma and cancer but different for small, tubular adenomas (Table 2). High levels of IGF-1 were associated with increased risk of both cancer and intermediate/late stage adenoma, although conventional statistical significance was not achieved for either. Even stronger (inverse) associations were observed for these endpoints with IGFBP-3 levels. IGF-1 and IGFBP-3 were strong negative confounders of each other, and associations for both were considerably stronger for cancer and intermediate/late-stage adenoma when IGF-1 and IGFBP-3 were adjusted for simultaneously.

The relations between IGF-1 and IGFBP-3 and intermediate/late-stage colorectal adenoma and cancer were evident for both women <60 and ≥60 years of age. BMI and alcohol intake were both risk factors for colorectal cancers (cases: mean alcohol, 8.1 g; mean BMI, 26.0 kg/m^2; controls: mean alcohol, 6.7 g; mean BMI, 24.7 kg/m^2). For total adenomas, alcohol intake and BMI were slightly but nonsignificantly higher in cases, but alcohol was significantly related to higher risk of large or villous adenomas. The results were slightly weaker but still statistically significant for IGFBP-3 when alcohol and BMI were not included. The associations were similar whether we used average alcohol intake and BMI from 1980 to 1990 or the 1990 status only. Results were not materially altered when we controlled individually in separate models for intake of fat, carbohydrate, protein, folate, methionine, and red meat, physical activity, pack-years smoked, aspirin use, and current postmenopausal hormone use. The relationships observed in the overall analyses were broadly similar for results limited to colon cancer (n = 55) and for metastatic colorectal cancer (n = 37).

No appreciable relationship was observed for either IGF-1 or IGFBP-3 and the early-stage small, tubular adenomas (Table 2). With IGF-1 and IGFBP-3 modeled simultaneously, the RR for total distal colorectal adenoma was 1.01 (95% CI, 0.53–1.93; P, trend, 0.96) and 0.87 (95% CI, 0.48–1.60; P, trend, 0.65), respectively, comparing the high to the low tertiles for each.

Table 2 RR and 95% CI of colorectal cancer, intermediate/late-stage colorectal adenoma, and early-stage colorectal adenoma according to tertiles of IGF-1 and IGFBP-3 in the NHS, 1989–1994

	Tertile 1	Tertile 2	Tertile 3	*P*, trend
Colorectal cancer				
IGF-1				
No. of Cases	21	27	31	
No. of controls	52	53	53	
RR[a]	1.0	0.80	1.21	
RR[b]	1.0	0.91	2.18	
95% CI		0.42–1.99	0.94–5.08	0.10
IGFBP-3				
No. of cases	29	32	18	
No. of controls	53	53	52	
RR[a]	1.0	1.15	0.53	
RR[b]	1.0	0.97	0.28	
95% CI		0.48–1.96	0.10–0.83	0.05
Intermediate/Late-stage adenoma[c]				
IGF-1				
No. of cases	32	38	20	
No. of controls	37	28	25	
RR[a]	1.0	1.14	0.82	
RR[b]	1.0	2.10	2.78	
95% CI		0.92–4.83	0.76–9.76	0.24
IGFBP-3				
No. of cases	30	39	21	
No. of controls	29	28	33	
RR[a]	1.0	1.13	0.47	
RR[b]	1.0	1.04	0.28	
95% CI		0.46–2.32	0.09–0.85	0.04
Early-stage adenoma[c]				
IGF-1				
No. of cases	35	31	41	
No. of controls	28	38	41	
RR[a]	1.0	0.64	0.99	
RR[b]	1.0	0.54	0.65	
95% CI		0.24–1.24	0.27–1.58	0.52
IGFBP-3				
No. of cases	37	35	35	
No. of controls	38	36	33	
RR[a]	1.0	0.98	1.02	
RR[b]	1.0	1.28	1.41	
95% CI		0.58–2.83	0.59–3.36	0.68

[a] Adjusted for alcohol intake and BMI. Cutpoints for IGF-1 and IGFBP-3 tertiles for cancer and adenoma matched pairs were determined separately. For cancers, mediums and ranges for increasing tertiles of IGF-1 (ng/ml) are 108 (56–132), and 159 (133–182), and 223 (183–401); and for IGFBP-3 (ng/ml), these are 3140 (939–3465), 3809 (3466–4212), and 4543 (4213–5753). For adenomas, for IGF-1 these are 96 (44–116), 139 (117–165), and 199 (166–387); and for IGFBP-3, these are 2834 (1628–3230), 3694, (3231–4063), and 4602 (4064–6790).

[b] Adjusted for alcohol intake and BMI; IGF-1 and IGFBP-3 are mutually adjusted for each other.

[c] Intermediate/late-stage adenomas include those ≥1 cm in diameter or that have a tubulovillous/villous histology, and early-stage adenomas are those <1 cm in diameter and with a tubular histology.

Discussion

Several lines of evidence point to the IGF axis as important in colorectal carcinogenesis. IGF-1 has important mitogenic and antiapoptotic properties, whereas IGFBP-3 binds IGF-1, attenuating the influence of any circulating IGF-1 (1), and has direct apoptotic actions independent of binding IGF-1, possibly mediated through IGFBP-3 membrane-associated receptors (4). In colon cancer cell lines, IGF-1 increases production of vascular endothelial growth factor, an angiogenic factor that supports cancer growth (9). Colorectal carcinogenesis involves an accumulation of specific molecular alterations (20). Because the IGF axis is a determinant of the cellular turnover rate, chronically high IGF bioactivity may increase cellular turnover, thereby increasing the rate that these alterations accumulate. Both normal gut epithelial cells and transformed cells are IGF responsive; thus, IGF can influence the carcinogenic cascade at various stages. IGF-1 is also important for the survival and maintenance of transformed cells (10).

We found in women that a single measure of circulating IGF-1 and IGFBP-3 levels predicted risk of colorectal cancers and adenomas that were large or had a villous component. In contrast, no consistent relation between IGF-1 and IGFBP-3 and small tubular adenomas was observed, indicating that the IGF axis may be more important in late (adenoma growth and transformation) stages, or that only a subset of adenomas are primed to respond to IGF. Large or villous adenomas are clinically relevant, whereas smaller, tubular adenomas are of questionable relevance.

Growth hormone increases production of both IGF-1 and IGFBP-3, accounting in part for the relatively high correlation between plasma IGF-1 and IGFBP-3. Because of this correlation, it is important to control for these simultaneously to observe their independent effects. Our finding that, controlling for level of IGF-1, IGFBP-3 is a strong independent protective factor may reflect binding of IGF-1, making IGF-1 not available, or direct apoptotic effects by IGFBP-3, or both (4). The 3–4-fold risk differential for colorectal cancer, also observed in men in a recent analysis (16), suggests a more important role for this binding protein for colorectal cancer than had been indicated for prostate (13) or breast cancer (14).

We considered the potential impact of chance or methodological biases in our study. Chance alone was unlikely to account for our findings because, individually, for large adenomas, tubulovillous/villous adenomas, and colorectal cancer, positive trends were observed for IGF-1, and highly significant inverse trends with IGFBP-3 were apparent. Although blood specimens were collected prior to the diagnosis, an undiagnosed cancer could theoretically have increased circulating IGF-1 while lowering IGFBP-3. This bias is unlikely because IGF-1 levels were not differentially elevated and IGFBP-3 levels were not depressed for the colorectal cases diagnosed earlier during follow-up rather than later. Moreover, these biases would be very unlikely for the large or tubulovillous/villous adenomas, preinvasive lesions.

Other measures, such as adolescent IGF-1 and IGFBP-3, mean circulating levels assessed over adolescence and adulthood, mean growth hormone level, or direct measures of tissue IGF bioactivity, might better capture the aspects of IGF physiology relevant for colorectal risk. We did not measure growth hormone level because it fluctuates widely over time, and hepatic IGF-1 production and release (and hence circulating IGF-1 level) integrates growth hormone level. To the extent that a single measure of circulating IGF-1 and IGFBP-3 is a proxy for the biologically relevant exposure and that measurement errors are random between cases and controls, our study design would tend to underestimate the magnitude of any true association. Over a short-term basis, the circulating IGF-1 level is quite stable; in one study, $r = 0.97$ between two measures taken an average of 5.8 days apart in 10 subjects, and $r = 0.94$ between two measures taken an average 42 days apart in 24 subjects (21).

Childhood and adolescent levels of IGF-1 influence linear growth and correlates with height, and tallness is a risk factor for colorectal cancer (22–25). In the current study, height in 1976 was poorly correlated with adult IGF-1 level ($r = 0.02$ for 367 cancer and adenoma controls combined; $P = 0.75$), consistent with previous studies indicating that adolescent, but not adult, IGF-1 level correlates with height (26). Integrated mean growth hormone level would be of interest because growth hormone stimulates local production of IGF-1 in addition to increasing circulating IGF-1 level.

Hyperinsulinemia has been proposed to explain why the Western lifestyle is related to colon cancer risk (27). Some of the cancer-enhancing effect of insulin may be mediated through the IGF axis. Although supraphysiologic levels of insulin are required to activate the IGF receptor and stimulate cell division, insulin lowers circulating levels of IGFBP-1, a serum protein produced primarily by the liver (28, 29). IGFBP-1 binds the IGFs with high affinity and inhibits IGF action *in vitro* (30–32). Conditions associated with decreased insulin levels, including fasting, exercise, and poorly controlled juvenile-onset diabetes mellitus, are associated with elevated IGFBP-1 (33–38). Serum IGFBP-1 fluctuates throughout the day, in parallel with insulin levels. Plausibly, IGFBP-1 may be an important mediator of the mitogenic effects of insulin.

If confirmed, our ability to demonstrate an association between IGF parameters with large, villous adenomas and localized colorectal cancers has potential clinical utility, because detecting and removing these lesions is the basis of secondary prevention of colorectal cancer incidence and mortality. However, a considerably larger study would be required to evaluate clinically utility. Of note, the relative risk of colorectal neoplasia related to "high risk" IGF profile is of the range of 2–4-fold. Although this relative risk is considerably weaker than those for hereditary syndromes (*e.g.*, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer), the "high risk" profile as defined for IGF (*e.g.*, top tertile) is far more common than the hereditary syndromes and thus influences a substantial number of cases.

Our results also suggest that lifestyle or pharmacologic approaches to decrease IGF-1 bioactivity may warrant investigation as risk reduction strategies targeted at individuals with increased IGF-1 levels relative to IGFBP-3. Unfortunately, relatively little is known about determinants of normal variation of IGF-1 and IGFBP-3 levels (39). In cases of severe energy or protein restriction, IGF-1 levels are lowered (40–42), and in various rodent tumors, IGF-1 appears to mediate the benefits of caloric restriction (43, 44). However, the influence of feasible reductions in energy or protein intake on IGF-1 is unclear. A recent study reported a moderate correlation between IGF-1 levels and alcohol consumption, but this association was observed only for men, and IGFBPs were not considered (21). Pharmaceutical suppression of the growth hormone-IGF-1 axis by somatostatin analogues (45) or growth hormone-releasing hormone antagonists (46) may provide a more potent strategy, but issues of cost and side effects become important. Whether anti-IGF therapy has efficacy to treat colorectal cancer is unclear; our results apply directly to the prediagnostic stage. Finally, our results as well as other recent reports (4, 13, 15, 16, 47) raise concern that the chronic administration of growth hormone or IGF-1 over long periods may increase the risk of epithelial cancers. Such therapy has been proposed to delay some of the effects of aging (48), but it would be prudent to evaluate any objective benefits of such intervention in the context of the potential risks associated with it.

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