Serum IGF1, IGF2 and IGFBP3 and risk of advanced colorectal adenoma

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The insulin-like growth factor (IGF) signaling pathway is involved in cell proliferation and differentiation. Elevated serum IGF1 levels have been associated with increased colorectal cancer risk; however, studies of this association with colorectal adenoma are inconclusive. We examined serum IGF1, IGF2 and IGFBP3 levels in relation to risk of advanced colorectal adenoma in a case-control study within the prostate, lung, colorectal and ovarian cancer screening trial. A total of 764 advanced, left-sided colorectal adenoma cases and 775 controls frequency-matched on gender and ethnicity, without evidence of a left-sided polyp on sigmoidoscopy were included in the current study. Serum levels of IGF1, IGF2 and IGFBP3 were measured using an enzyme linked immunosorbent assay in serum samples collected at baseline. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the associations adjusting for age, race, sex, year of blood draw, body mass index, smoking and education. Higher IGF1 levels were associated with increased adenoma risk: ORs = 1.58 (95% CI = 1.16–2.16), 1.42 (95% CI = 1.04–1.93), and 1.80 (95% CI = 1.30–2.47) for the second, third and fourth quartiles, respectively (p_{trend} = 0.002). Elevated IGF2 levels were also associated with increased adenoma risk (OR = 1.43, 95% CI = 1.05–1.96 for the fourth *vs.* first quartile, p_{trend} = 0.02), but the association was no longer significant after adjustment for IGF1 (p_{trend} = 0.28). IGFBP3 levels were not associated with adenoma risk. Our analysis showed a significant positive association between circulating IGF1 levels and risk of advanced colorectal adenoma, suggesting that IGF1 is associated with the pivotal precursor to colorectal cancer.

Key words: colorectal adenoma, insulin-like growth factor
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Insulin-like growth factor (IGF) signaling pathway is involved in cell proliferation and differentiation, having a central role in growth regulation at both cellular and organism levels.[1] IGFs are produced in the liver and several other tissues, *e.g.*, bone and muscle, and are bound to IGF binding proteins (IGFBPs) in the circulation.[2,3] IGFs also regulate apoptosis through the Ras-MAPK/PI3K pathway,[4] and disruption of normal IGF pathway regulation has been proposed to promote the development of several human cancers. Both *in vivo* and *in vitro* experiments have suggested that IGF1 can stimulate cellular proliferation[5–7] and inhibit apoptosis.[8,9] Prospective population-based studies have observed positive relationships between elevated circulating IGF1 levels and risk of prostate, breast and colorectal cancers,[10–13] supporting the hypothesis that IGF1 plays an integral role in the development of specific malignancies. However, it is unclear whether circulating IGF1 levels contribute to risk in the early stages of carcinogenesis.

Colorectal cancer is the third most common cancer in the United States for both men and women.[14] Most colorectal cancers are believed to have originated from a preexisting colorectal adenoma. Elevated serum IGF1 levels have been

consistently associated with moderately increased risk of colorectal cancer as reviewed by Rinaldi *et al.*[12] Emerging evidence suggests that IGF1 levels might also be associated with colorectal adenoma risk. However, to date, most studies on colorectal adenoma have been small[15–21] and statistically underpowered, resulting in uncertainty regarding the role of IGF1 levels in adenoma pathogenesis and, if the association is real, whether IGF1 is primarily involved in tumor initiation or progression. Limited results on the relationship between serum IGF2[16,19] and insulin-like growth factor binding protein 3 (IGFBP3)[15,18,20,21] and colorectal adenoma are inconclusive. Furthermore, whether the effects of IGF1 on colorectal adenoma differ by important clinical characteristics and whether its effects can be modified are largely unknown.

To explore the role of IGF signaling in colorectal carcinogenesis, we conducted a large case-control study in the prostate, lung, colorectal and ovarian cancer screening (PLCO) trial testing whether circulating levels of IGF1, IGF2 and IGFBP3 were associated with risk of prevalent advanced colorectal adenoma.

Methods

The prostate, lung, colorectal and ovarian cancer trial (PLCO)

The PLCO study is a randomized clinical trial, designed to assess the efficacy of screening tests to reduce death from cancers of the prostate, lung, colon and rectum and ovary. As described previously,[22,23] 154,938 cancer-free men and women aged 55–74 were recruited from 10 sites in the USA between 1993 and 2001. Participants were randomly assigned to the control group or the colorectal cancer screening group, where they underwent a 60-cm flexible sigmoidoscopy examination at study entry. Those found to have a suspicious lesion were referred to their personal physician for subsequent diagnostic follow-up. Cases of colorectal adenoma were pathologically verified with medical records. Information on demographics, personal and family medical history, and lifestyle factors (*e.g.*, smoking and dietary intake) were collected by standard questionnaire at baseline. This trial was approved by the institutional review boards of the 10 screening centers and the National Cancer Institute in Bethesda, Maryland, USA, and all participants provided written informed consent.

Study population

A case-control study was conducted among the PLCO participants in screening group, who had consented to participate in etiologic studies of cancer and related diseases, completed a risk factor questionnaire, provided blood samples, and had no previous history of inflammatory bowel disease, colorectal polyps, Gardner's syndrome, familial polyposis, or cancer other than basal or squamous cell skin cancer. Cases included 772 subjects found to have prevalent "advanced" colorectal adenoma ($\geq$1cm in size, containing villous/tubulovillous characteristics, high-grade dysplasia or carcinoma *in situ*) of the distal colon or rectum at study entry (*i.e.*, at the time of the initial screening examination). Controls ($n = 777$) were participants who had successful sigmoidoscopy at baseline, were negative for polyps in the distal colon and rectum, and were frequency-matched to cases on ethnicity and gender. Among the selected subjects, 764 cases and 775 controls had sufficient serum for IGF1, IGF2 and IGFBP3 measurements.

Serum measurement

Serum was extracted from baseline nonfasting blood samples and stored at -70°C until analysis. Serum levels of IGF1, IGF2 and IGFBP3 were measured using an enzyme linked immunosorbent assay (ELISA) with reagents from DSL (Webster, Texas). Reagents were validated with dilution series of recombinant protein samples and interwell variability of ELISA plates. All test samples were randomly distributed in 49 plates with four blinded quality control samples randomly dispersed within each plate. The laboratory was blinded to the disease status of samples.

Duplicate serum aliquots were analyzed for each subject and the average was used for statistical analysis. The coefficients of variation (CV) for the duplicates were less than 10% for all three markers tested here, and inter-plate CV ranges were 5.8–6.2%, 3.4–6.4% and 4.1–6.5% for IGF1, IGF2 and IGFBP3, respectively.

Statistical analysis

Serum IGF1, IGF2 and IGFBP3 levels were classified as quartiles for statistical analysis according to their distributions in controls. Molar concentrations of IGF1, IGF2 and IGFBP3 were calculated as IGF1 (ng ml^{-1})/7.5 kDa, IGF2 (ng ml^{-1})/7.5 kDa and IGFBP3 (ng ml^{-1})/28.7 kDa, respectively. Molar ratios were calculated as the fractions of IGF1/IGFBP3 and IGF2/ IGFBP3, and they were also classified as quartiles according to the distribution in controls.

Logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (95% CI) for the association between serum measurements (IGF1, IGF2 and IGFBP3 levels), as well as molar ratios, and risk of colorectal adenoma. We conducted two sets of analyses adjusting for different covariates: (*i*) basic model: adjusting for age (55–59, 60–64, 65–69 and 70–74 years), gender, ethnicity, year of blood draw and study center; (*ii*) full model: adjusting for age (55–59, 60–64, 65–69, 70–74 years), gender, ethnicity, year of blood draw, study center, BMI ($\leq$25, 25.1–30, $>$30 and missing), smoking (never, former cigarette smoking, current cigarette smoking and cigar/pipe smoking) and education ($\leq$11 years, 12 years or high school, post high school and college graduate). p value for trend was calculated by coding the quartiles of IGF1, IGF2 and IGFBP3 serum levels as ordinal variables in the models.

To explore potential modification of IGF1-adenoma association by other factors, we conducted the above analysis stratifying by age, gender, family history, hormone replacement treatment (among women), smoking status, body mass index categories and time of blood draw [early morning (6 am–9 am), late morning (10 am–noon), and afternoon

Table 1. Baseline characteristics of advanced colorectal adenoma cases and controls in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial

		Controls	Cases	*p*-value*
N		775	764	
Age in years (%)	55–60	406 (52)	301 (39)	<0.0001
	61–65	187 (24)	224 (29)	
	66–70	124 (16)	167 (22)	
	71–75	58 (8)	72 (10)	
Gender (%)	Male	535 (69)	530 (69)	0.88
	Female	240 (31)	234 (31)	
Ethnic group (%)	Caucasian	728 (94)	718 (94)	0.95
	African American	23 (3)	21 (3)	
	Other	24 (3)	25 (3)	
Family history of colorectal cancer first-degree relative (%)	No	705 (91)	669 (88)	0.03
	Yes	70 (9)	95 (12)	
Aspirin/ibuprofen use in the last year (%)	No	307 (39)	317 (42)	0.31
	Yes	468 (61)	446 (58)	
Hormone replacement therapy use in females (%)	Never	78 (33)	80 (34)	0.34
	Former	32 (13)	41 (18)	
	Current	129 (54)	113 (48)	
Smoking (%)	Never	314 (41)	257 (34)	<0.0001
	Former cigarette smoker	365 (47)	361 (47)	
	Current cigarette smoker	53 (7)	109 (14)	
	Cigar/pipe smoker	43 (6)	36 (5)	
Body mass index in kg m^{-2} (%)	≤25	214 (28)	199 (26)	0.17
	25.1–30	358 (46)	345 (45)	
	>30	194 (25)	217 (28)	
	Missing	9 (1)	3 (0.4)	
Education (%)	≤11 years	50 (6)	69 (9)	0.002
	12 yr/high school	176 (23)	192 (25)	
	Post high school	246 (32)	272 (36)	
	College graduate	302 (39)	231 (30)	
IGF1 (mean ± SD) (ng ml^{-1})		196.6 ± 71.7	206.7 ± 75.8	0.13
IGF2 (mean ± SD) (ng ml^{-1})		954.4 ± 213.1	973.1 ± 224.2	0.16
IGFBP3 (mean ± SD) (ng ml^{-1})		4420.4 ± 866.0	4483.3 ± 887.9	0.49

*p value from χ^2 test for categorical variables and T test for continuous variables.

(1 pm–4 pm)]. Interactions were formally tested with log-likelihood ratio test. We also examined differences in the associations by clinical characteristics, such as anatomic site, histology type, size and multiplicity of the adenoma lesions. Homogeneity between subtypes was formally tested with the Wald test.

All analyses were two-sided, and statistical significance was defined as a p-value less than 0.05. SAS 9.1 statistical analysis software was used for the analyses.

Results

A total of 764 colorectal adenoma cases and 775 frequency-matched controls with successful serum measurements were included in the current analyses (Table 1). More than 90% of study subjects were white; less than 10% were African American or other ethnicities. Compared with controls, cases were older, more likely to be: current smokers, having a family history of colorectal cancer and less educated. Although not statistically significant ($p > 0.1$, unadjusted t test), the means of serum IGF1, IGF2 and IGFBP3 levels were higher in cases than controls (Table 1). When classified by quartiles, higher levels of IGF1 were associated with an increased risk of adenoma ($p_{trend} = 0.005$), with the ORs of 1.50 (95%CI = 1.11–2.04), 1.36 (95%CI = 1.00–1.85) and 1.68 (95%CI = 1.23–2.29) for the second, third, and fourth quartile respectively,

Table 2. Risk of advanced colorectal adenoma for IGF-1, IGF-2, and IGFBP-3 levels by quartiles in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial[1]

	Quartiles				
Measure	**1**	**2**	**3**	**4**	***p* trend**
IGF1 (ng ml^{-1})	120.3	167.4	211.6	281.5	
Median (range)	(15.7–144.7)	(144.8–187.7)	(188.4–236.1)	(236.7–484.6)	
N case/control	153/194	202/193	185/194	224/194	
Basic model	1	1.50 (1.11–2.04)	1.36 (1.001–1.85)	1.68 (1.23–2.29)	0.005
Full model	1	1.58 (1.16–2.16)	1.42 (1.04–1.93)	1.80 (1.30–2.47)	0.002
IGF2 (ng ml^{-1})	719.0	887.4	1011.8	1198.7	
Median (range)	(248.4–811.6)	(813.4–950.3)	(950.8–1079.8)	(1085.4–1798.7)	
N case/control	183/194	187/194	170/194	224/193	
Basic model	1	1.11 (0.83–1.49)	1.08 (0.80–1.46)	1.42 (1.06–1.92)	0.02
Full model	1	1.16 (0.86–1.57)	1.12 (0.82–1.53)	1.49 (1.09–2.02)	0.02
IGFBP3 (ng ml^{-1})	3462.4	4172.7	4845.8	5396.6	
Median (range)	(782.7–3917.5)	(3921.2–4406.1)	(4406.3–5003.5)	(5005.6–7463.2)	
N case/control	185/194	176/194	190/194	213/193	
Basic model	1	1.02 (0.76–1.37)	1.14 (0.85–1.54)	1.28 (0.95–1.72)	0.08
Full model	1	1.06 (0.78–1.43)	1.20 (0.88–1.62)	1.32 (0.98–1.79)	0.05
IGF1/IGFBP3 molar ratio	0.115	0.150	0.183	0.226	
Median (range)	(0.054–0.135)	(0.135–0.166)	(0.166–0.200)	(0.200–0.389)	
N case/control	168/193	183/194	188/195	225/193	
Basic model	1	1.13 (0.83–1.54)	1.15 (0.83–1.58)	1.47 (1.06–2.04)	0.02
Full model	1	1.12 (0.82–1.53)	1.17 (0.84–1.62)	1.49 (1.07–2.08)	0.02
IGF2/IGFBP3 molar ratio	0.733	0.801	0.852	0.917	
Median (range)	(0.400–0.770)	(0.770–0.824)	(0.825–0.878)	(0.879–1.565)	
N case/control	170/193	188/194	202/195	204/193	
Basic model	1	1.10 (0.82–1.48)	1.23 (0.92–1.65)	1.32 (0.98–1.78)	0.05
Full model	1	1.15 (0.85–1.55)	1.28 (0.95–1.74)	1.34 (0.99–1.82)	0.04
(IGF1+IGF2)/IGFBP3 molar ratio	0.891	0.974	1.023	1.087	
Median (range)	(0.547–0.939)	(0.940–0.999)	(1.000–1.051)	(1.052–1.792)	
N case/control	162/193	189/194	195/194	218/194	
Basic model	1	1.20 (0.89–1.61)	1.30 (0.96–1.75)	1.47 (1.09–1.98)	0.009
Full model	1	1.22 (0.90–1.66)	1.34 (0.98–1.82)	1.50 (1.11–2.03)	0.007

[1]Basic model is adjusted for age at T_0 screen (four categories), year of blood draw (continuous), gender, ethnicity, and study left; full model is adjusted for age at T_0 screen (four categories), year of blood draw (continuous), gender, ethnicity, study left, BMI (four categories, including missing class), smoking status (four categories), and education (four categories).

compared with to the first quartile in the basic model (Table 2). When the second, third, and fourth quartiles of IGF1 were combined, it was associated with 1.51-fold increased adenoma risk (95% CI = 1.16–1.95) compared with the lowest quartile. Further adjustment for BMI, smoking status, and education in the full model strengthened all the associations observed in the basic model, with ORs of 1.58, 1.42 and 1.80 for the second, third and fourth quartile of IGF1, respectively ($p_{trend} = 0.002$).

For IGF2 levels, only the highest quartile was associated with significantly increased adenoma risk (OR = 1.42, 95%CI = 1.06–1.92 in the basic model and OR = 1.49, 95%CI = 1.09–2.02 in the full model). We observed a monotonically increased risk of adenoma with increasing IGFBP3 level, but no any individual association reached significance and the *p*-value for trend was only borderline significant in the fully adjusted model ($p_{trend} = 0.05$).

Because the molar ratios reflect the bioactive (unbound) fraction of IGF1 and IGF2, we also evaluated the associations between the molar ratios and risk of adenoma (Table 2). A monotonically increasing association was observed between IGF1/IGFBP3 and adenoma risk ($p_{trend} = 0.02$), with the

Table 3. Risk of advanced adenoma for quartiles of IGF1 stratified by select baseline characteristics of the participants in the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial[1]

		Quartiles				
Measure		**1**	**2**	**3**	**4**	***p* interaction**[2]
Age	<60	1	1.39 (0.85–2.27)	1.17 (0.72–1.92)	1.39 (0.84–2.28)	0.16
	≥60	1	**1.75 (1.17–2.61)**	**1.57 (1.04–2.38)**	**2.15 (1.40–3.31)**	
Gender	Male	1	**1.86 (1.22–2.83)**	**1.67 (1.11–2.53)**	**2.12 (1.43–3.16)**	0.60
	Female	1	1.20 (1.74–1.93)	1.15 (0.62–1.76)	1.28 (0.64–2.54)	
FH	+	1	3.30 (1.06–10.2)	1.06 (0.37–3.00)	1.40 (0.43–4.57)	0.42
	–	1	**1.56 (1.12–2.17)**	**1.49 (1.06–2.09)**	**1.92 (1.37–2.70)**	
HRT (♀)[3]	Never	1	1.19 (0.71–3.46)	1.26 (0.43–3.64)	1.45 (0.40–5.27)	0.82
	Ever	1	1.12 (0.63–1.98)	0.81 (0.40–1.63)	1.14 (0.43–3.02)	
Cigarettes	Never	1	**1.81 (1.08–3.02)**	1.40 (0.83–2.36)	**2.07 (1.22–3.49)**	0.72
	Former	1	1.46 (0.92–2.31)	1.30 (0.81–2.09)	**1.71 (1.06–2.77)**	
	Current	1	1.43 (0.46–4.47)	**4.26 (1.29–14.1)**	3.46 (0.98–12.2)	
BMI	≤25	1	1.30 (0.71–2.37)	1.14 (0.61–2.13)	**2.26 (1.13–4.49)**	0.14
	25.1–30	1	1.94 (1.18–3.16)	1.27 (0.78–2.07)	**2.18 (1.33–3.58)**	
	>30	1	1.47 (0.81–2.64)	**2.10 (1.12–3.94)**	1.26 (0.69–2.28)	
Blood draw time	6–9 am	1	1.38 (0.87–2.17)	1.22 (0.76–1.94)	1.28 (0.90–2.04)	0.14
	10 am–noon	1	**1.79 (1.01–3.17)**	1.59 (0.90–2.79)	**2.31 (1.29–4.15)**	
	1–4 pm	1	1.90 (0.84–3.42)	1.72 (0.82–3.62)	**2.61 (1.22–5.58)**	

[1]OR adjusted for age at T_0 screen (four categories), year of blood draw (continuous), gender, ethnic, study center, BMI (four categories, including missing class), smoking status (four categories), and education (four categories). [2]$p_{interaction}$: IGF1quartile was treated as categorical variable for the interaction model. [3]Analyses stratified by hormone replacement treatment was restricted to females only.

highest quartile significantly associated with risk. Further adjustment for other covariates in the full model did not modify the results significantly. We also observed a trend of increasing risk with increasing quartile of IGF2/IGFBP3, but the association was weaker and only reached statistical significance in the full model ($p_{trend} = 0.04$). When molar ratios of IGF1 and IGF2 were considered simultaneously, an increased risk of adenoma was observed with higher (IGF1 + IGF2)/IGFBP3 levels ($p_{trend} = 0.007$ in full model); the highest quartile was associated with a 1.5-fold increased risk (95%CI = 1.11–2.03).

The three IGF analyte levels were highly correlated among controls: Pearson correlation coefficients = 0.46 ($p < 0.001$) for IGF1 and IGF2, 0.60 ($p < 0.0001$) for IGF1 and IGFBP3 and 0.88 ($p < 0.0001$) for IGF2 and IGFBP3. To determine whether the associations observed with IGF1 and IGF2 were independent from each other and from IGFBP3, we conducted analyses in which we conditioned the other IGF levels measured (Supporting Information Table 1). Additional adjustment for IGF2 and IGFBP3 did not significantly affect the association between IGF1 levels and adenoma risk ($p_{trend} = 0.01$). However, the association with IGF2 was attenuated with adjustment for IGF1 and IGFBP3 levels, but not with adjustment for the IGF1/IGFBP3 molar ratio. The IGF2/IGFBP3 molar ratio remained associated with risk after adjustment for IGF1/IGFBP3 or IGF1 ($p_{trend} = 0.02$), suggesting that IGF2 levels may still play a role in colorectal adenoma susceptibility beyond what is expected due to their correlation with IGF1 levels. No association was observed between IGFBP3 levels and adenoma risk after adjustment for IGF1 and IGF2 levels.

To determine if the association between IGF1 levels and adenoma risk was modified by other factors, we conducted stratified analyses by age, gender, family history, hormone replacement treatment, smoking status and body mass index (Table 3). Although we observed stronger associations among older participants compared with younger subjects, and among males compared with females, neither interaction was significant (age: $p_{interaction} = 0.16$; gender: $p_{interaction} = 0.60$). Similarly, among women who never used hormone replacement therapy the association between IGF1 levels and adenoma risk was stronger than among those who ever used hormone therapy; however the interaction was not significant ($p_{interaction} = 0.82$). No significant patterns between strata were observed for family history, smoking status, or body mass index. Exclusion of nonwhites did not modify the results (data not shown).

Because circadian variations in serum levels of IGF1 have been hypothesized to influence cancer development,[24] we also explored the effect of time of blood draw on the association between IGF1 levels and adenoma risk (Table 3). In stratified analysis, the associations between serum IGF1 and adenoma risk appeared stronger when drawn in late morning or afternoon compared with early morning for each quartile of IGF1

Table 4. Risk of adenoma for IGF1 by quartile stratified by select clinical characteristics in the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial[1]

		Quartiles				Homogeneity
Measure		**1**	**2**	**3**	**4**	***P*[2]**
Sites	Left colon ($n = 383$)	76	105	91	111	0.99
		1	**1.64 (1.13–2.39)**	1.40 (0.95–2.05)	**1.80 (1.22–2.65)**	
	Rectum ($n = 108$)	26	25	24	33	
		1	1.29 (0.62–2.07)	1.11 (0.60–2.06)	1.68 (0.92–3.09)	
	Both ($n = 265$)	51	70	67	77	
		1	**1.68 (1.08–2.61)**	1.55 (0.995–2.42)	**1.79 (1.14–2.80)**	
Histology	Villous ($n = 126$)	23	31	31	41	0.16
		1	1.48 (0.82–2.70)	1.51 (0.82–2.75)	**2.12 (1.17–3.85)**	
	Tubular-villous ($n = 305$)	59	75	80	91	
		1	**1.54 (1.02–2.33)**	**1.70 (1.12–2.57)**	**2.08 (1.36–3.17)**	
	Tubular ($n = 215$)	47	65	47	56	
		1	**1.68 (1.08–2.62)**	1.14 (0.71–1.82)	1.39 (0.86–2.23)	
	NOS[3] ($n = 115$)	24	31	25	35	
		1	1.54 (0.85–2.82)	1.05 (0.56–1.97)	1.52 (0.83–2.80)	
Size	$\geq$1 cm ($n = 571$)	113	151	141	166	0.78
		1	**1.61 (1.15–2.25)**	**1.46 (1.04–2.04)**	**1.78 (1.26–2.51)**	
	<1 cm ($n = 191$)	40	51	43	57	
		1	1.52 (0.94–2.44)	1.25 (0.76–2.06)	**1.82 (1.12–2.98)**	
Multiplicity	Multiple ($n = 371$)	74	97	89	111	0.77
		1	**1.60 (1.09–2.36)**	1.40 (0.94–2.08)	**1.76 (1.19–2.62)**	
	Single ($n = 393$)	79	105	96	113	
		1	**1.57 (1.08–2.27)**	1.41 (0.96–2.06)	**1.83 (1.25–2.70)**	

[1]OR adjusted for age at T_0 screen (four categories), year of blood draw (continuous), gender, ethnicity, study center, BMI (four categories, including missing class), smoking status (four categories), and education (four categories). Control groups are as the overall control groups. [2]Homogeneity between specific clinical characteristics was assessed using the Wald test. [3]NOS: not otherwise specified.

level, suggesting a greater separation in the distribution of levels between cases and controls in the afternoon; however, the interaction was not significant ($p_{\text{interaction}} = 0.14$). Similar patterns were observed for IGF2 with a $p_{\text{interaction}} = 0.10$ (data not shown).

We examined whether the association between IGF1 levels and adenoma risk differed by specific adenoma features (Table 4). No significant heterogeneity was observed between different anatomic sites, histologies, sizes or multiplicity.

Discussion

This large case-control study showed that higher absolute IGF1 levels and higher IGF1molar ratios were associated with an increased risk of advanced colorectal adenoma, an association that was not substantially modified with further adjustment for IGF2 and/or IGFBP3. Further adjustment for BMI, smoking status, and education in the full model strengthened all the associations. In stratified analyses, no consistent or significant effects of age, gender, family history, hormone replacement treatment, smoking status and body mass index were observed. An elevated adenoma risk was also observed with highest IGF2 quartile.

At least seven epidemiological studies have examined the relationship between circulating IGF1 levels and colorectal adenoma risk.[15–21] With the exception of one prospective study,[15] all other published reports were based on prevalent cases identified at the time of an initial screening test.[16,18–21,25] Four of these studies focused on advanced adenoma[15,18,20,21] and three included both nonadvanced and advanced adenoma.[16,19,25] All four studies of advanced adenoma observed an increased risk of adenoma with higher IGF1 levels;[15,18,20,21] however, the increased risk was only statistically significant in one study with wide confidence intervals surrounding the risk estimates.[18] The inconsistency in the findings is likely due to the small sample sizes (ranging from 42 to 92 cases) of all four of these studies.[15,18,20,21] Among the three studies that examined both nonadvanced and advanced adenoma,[16,19,25] only one showed a significant association with IGF1 levels.[19] Studies examining the association between IGF1 levels and risk of adenoma recurrence have not observed a positive association;[26,27] however, these

studies only include subjects with adenoma, who are likely to have higher than average levels of IGF1.

We have demonstrated a significant association between higher IGF1 levels and risk of advanced colorectal adenoma, which was not modified by further adjustment for IGF2 and/or IGFBP3, and which was observed when molar ratios of IGF1/IGFBP3 and (IGF1+IGF2)/IGFBP3 were used instead of absolute serum levels. These results, together with previous studies,[15–21] suggest that IGF1 is involved in either the initiation of adenoma or the progression from nonadvanced adenoma to advanced adenoma, with the latter having a higher risk of progress to adenocarcinoma. Larger studies with nonadvanced adenoma are needed to conclusively identify where in the adenoma-to-carcinogenesis sequence IGF1 pays a role. However, we cannot draw a definitive conclusion on this question, given our study design, *i.e.*, a cross-sectional analysis of prevalent adenomas. The design of the PLCO study would permit testing this hypothesis with incident adenomas and prediagnostic serum samples.

We found a moderately increased risk for advanced adenoma in subjects with highest level of IGF2 comparing to the lowest level. Although the association was largely attenuated when further adjustment for IGF1 and/or IGFBP3 was made, the association with the molar ratio IGF2/IGFBP3 remained, suggesting that IGF2 may still play a role in colorectal neoplasia. Prospective studies of colorectal cancer also reported increased cancer risk among subjects with highest quartile of IGF2 levels compared with the lowest quartile.[28–31] There are two prior studies that analyzed IGF2 levels in relation to colorectal adenoma risk: a small study (52 adenoma cases) reported a strong association (OR = 3.05, 95% CI = 2.04–4.57),[19] but the larger study with (239 cases) did not find significant association (OR = 1.0).[16] However, neither of these studies differentiated between advanced and nonadvanced adenomas.

Most circulating IGF1 and IGF2 proteins are bound to IGFBP3 in blood; however, IGF1 binds to the IGF1-receptor on cell surface to initiate its cellular effects, causing cell proliferation and apoptosis.[32] This suggests that free/unbound IGF1 is its biologically active component. Hence, our observation that the IGF1/adenoma association persists when molar ratio is used as the measure of level adds to the robustness of our findings. It is worth noting that the molar ratios represent an approximation of the free fractions of IGFs. Because of competitive protein binding, as well as other mechanisms, assays which directly measure the free and physiologically active molecules might provide additional insight into this issue.

IGF2 is an imprinted gene, and loss of imprinting, typically a result of hypomethylation in the *IGF2* promoter region, has been detected in colorectal cancer tumor tissue.[33] Furthermore, overexpression of *IGF2* mRNA[34] has been observed in colorectal cancer tumors compared with normal tissue. A cross-sectional study found that loss of *IGF2* imprinting in blood lymphocytes was associated with a three-fold increased risk of adenoma and a 22-fold increased risk of colorectal cancer.[35] These results suggest that elevated serum IGF2 levels and/or loss of IGF2 imprinting may play a role in colorectal neoplasia susceptibility, but the effect, at least for IGF2 serum levels, is probably less than that for IGF1.

Association studies of serum IGFBP3 and colorectal cancer risk have reported inconsistent findings.[12,29,36,37] Results from the seven epidemiological studies of IGFBP3 and adenoma risk were also inconclusive.[15–21,38] All other studies did not report significant findings,[16,17,19–21] except for two small studies of advanced adenomas[15,18] that observed a significant inverse association between high IGFBP3 serum levels and adenoma risk. We did not observe any significant associations between IGFBP3 and colorectal adenoma risk, a finding which was consistent with most previous studies.

The underlying biological mechanism for involvement of the IGF signaling pathway in carcinogenesis has been reviewed by others.[1] Increased IGF1 levels are thought to increase IGF1 bioavailability and bioactivity. The binding of IGF1 proteins to their receptors, which are abundant in colorectal cell lines,[39,40] are hypothesized to activate a cascade of biological reactions leading to cell proliferation and inhibition of apoptosis, each of which could contribute to tumor development.[41] However, increased IGFBP3 levels may sequester IGF1, blocking its ability to bind to its cognate receptors, thereby reducing the activation of downstream reactions. However, our data failed to demonstrate a role for IGFBP3 in advanced adenoma formation.

Analyses of potential modification effect on the association between IGF1 and adenoma risk yielded no statistically significant interactions, perhaps due to the limited statistical power. We did observe stronger associations among older ($\geq$60 years) or male subjects. Although there are reports suggesting IGF follows a circadian pattern in mouse,[42] we did not observe a significant difference in the association between early morning and afternoon, which is consistent with the lack of circadian rhythm has been reported for IGF1 during the photoperiod,[43–45] the reason for this association was unclear and warrants further research. Future studies might benefit from matching the time of blood draw, and monitor the IGF1 change within 24 hr among same individuals.

Our study has several advantages and limitations. First, it is the largest study to date to investigate serum levels of IGF1, IGF2 and IGFBP3 and colorectal adenoma risk. Second, our study included only advanced adenoma cases, which are more likely to progress to colorectal cancer, and are therefore clinically more relevant. However, the results may not be generalizable to nonadvanced adenoma, and additional studies are needed to determine if insulin-like growth factors are associated with nonadvanced adenoma. Alternatively, insulin like growth factors may stimulate advancement from nonadvanced to advanced adenoma.[20] Furthermore, we did not examine the colon area above the sigmoid, which could induce some misclassification. This case-control study was

nested within a randomized population-based colorectal cancer screening trial, which reduces potential selection bias often present in typical clinic-based case-control studies of adenoma. However, our study population was predominantly white, more educated, less likely to smoke, and more physically active than the general population.[46] Although this may reduce the study's generalizability, we carefully controlled for these factors in our analysis and it is unlikely that these factors would cause significant bias in the results of our study. Finally, blood samples in the current report were collected at the time of sigmoidoscopic detection of prevalent adenomas (like most of previous studies[16,18–21,25]) using cases detected at the time of first screening examination. Thus, the cases were not prospectively diagnosed, and the serum was obtained in the presence of adenoma. It is theoretically possible that the disease process could have contributed to the elevated levels observed. Though the one prospective study of this association did not observe differentially elevated IGF1 among cases diagnosed earlier during follow-up rather than later,[15] it was a relatively small series. Further analysis of prospectively diagnosed adenomas and IGF measurements in prediagnostic sera is warranted to further test our observations.

In summary, in the largest study to date of serum IGF1, IGF2 and IGFBP3 levels, and advanced colorectal adenoma risk, we found that elevated levels of IGF1 were associated with a significant 1.5-fold increase in adenoma risk. A possible role for IGF2 was suggested, but this associated was lost when IGF1 levels were simultaneously taken into account; no association with IGFBP3 was detected. These results suggest that IGF1 plays a role in the early stages of colorectal carcinogenesis. Further prospective studies are needed to confirm our findings, and to determine if IGF1 serum levels are associated with nonadvanced adenomas, which are less likely to progress to full-fledged malignancy. Such knowledge could inform clinical assessment of risk, and provide clues as to where/when in the etiologic pathway IGF1 contributes to colorectal cancer susceptibility.

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