Serum Insulin-like Growth Factor (IGF) and IGF-binding Protein Levels and Risk of Lung Cancer: A Case-Control Study Nested in the β-Carotene and Retinol Efficacy Trial Cohort[1]

Margaret R. Spitz,[2] Matt J. Barnett, Gary E. Goodman, Mark D. Thornquist, Xifeng Wu, and Michael Pollak

Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030 [M. R. S., X. W.]; Fred Hutchinson Cancer Research Center, Public Health Sciences Division, Seattle, Washington 98109 [M. J. B., G. E. G., M. D. T.]; and Department of Oncology, McGill University H3A2TS and Jewish General Hospital, Montreal, Quebec, Canada [M. P.]

Abstract

Recent prospective studies have suggested that insulin-like growth factor (IGF)-I levels are related to risk of some epithelial cancers. We previously reported in a case-control study a significant association between IGF-I level and lung cancer risk, with a 2-fold increased risk in the highest quartile. We now report the results of a lung cancer case-control study nested in the placebo arm of the β-Carotene and Retinol Efficacy Trial in heavy smokers. We identified 159 cases for whom sera had been collected at least 3 years before diagnosis and for whom 2 suitable controls/case (final number, 297) could be matched from the same study arm on age (within 5-year intervals), sex, ethnicity, year of enrollment into the β-Carotene and Retinol Efficacy Trial, year of blood draw, and exposure category (smoking or asbestos). The cases were significantly heavier smokers than the controls (mean pack-years, 58.7 and 45.9, respectively; $P < 0.001$). An inverse relationship between IGF-I level and age was evident only for former smokers, and not for those who were current smokers at the time of blood draw. Both IGF-I and IGF-binding protein (IGFBP)-3 levels were higher in cases than in controls, but none of the differences achieved statistical significance. The odds ratios for IGF-I were around unity, except for subsets of heaviest smokers and those who had quit smoking for the longest period of time, in whom there were elevated risks in the second to fourth quartiles of IGF-I relative to the first quartile (odds ratios, 2.21–2.91), although again, none achieved statistical significance. For younger subjects, IGF-I was inversely associated with lung cancer risk in the models that also controlled for IGFBP-3. Elevated risks for lung cancer were noted in the highest quartile of IGFBP-3 level, and these tended to be higher in current smokers and more recent quitters. These results do not support the conclusions of our prior case-control study. It is possible that current smoking or recent cessation may exert a suppressive effect on IGF-I levels (notably in younger subjects with higher baseline levels) that may obscure a relatively modest association between IGF-I level and lung cancer risk. On the other hand, risks associated with elevated IGFBP-3 level tended to be higher in current smokers and more recent quitters. This trend toward a positive association with IGFBP-3 level is unexpected and requires further investigation. Finally, from these data, we cannot exclude the possibility that risk of lung cancer in nonsmokers may be related to IGF-I levels.

Introduction

IGFs[3] are peptide hormones with mitogenic and antiapoptotic properties that are involved in the regulation of proliferation and differentiation of many cell types, including normal and transformed bronchial epithelial cells (1, 2). The bulk of IGFs and IGFBPs found in the circulation are synthesized in the liver. Tissue IGF bioactivity is not only a function of circulating level of IGFs and their binding proteins but also of local production of IGFs and IGFBPs. There is considerable variability between normal individuals with respect to circulating levels of IGF-I and its principal circulating binding protein, IGFBP-3 (3, 4). Recent prospective studies have provided evidence that these levels are related to risk of certain cancers (5–14).

We previously reported in a hospital-based case-control study of 204 lung cancer cases and 218 matched control subjects that there was a relation between IGF-I level and lung cancer risk, with levels in the highest quartile range associated with a 2-fold increased risk ($P = 0.01$; Ref. 15). Without adjustment, IGFBP-3 was not associated with risk. However, when adjusted for IGF-I level, IGFBP-3 level in the highest quartile was associated with a 52% reduction in risk ($P = 0.03$). We also reported that IGF-I level was higher among those patients with distant *versus* localized disease and in patients with poorly differentiated compared with well-differentiated tumor phenotypes (15). Because our findings were derived from retrospective analysis, we were unable to rule out the impact of disease status on the association, and we noted the need to confirm these findings in prospective studies using prediagnostic measurements (15).

[1] Funded by NIH Grants CA86390 (to M. R. S.) and CA63673 (to G. E. G.) and by a grant from the National Cancer Institute of Canada (to M. P.).

[2] To whom requests for reprints should be addressed, at Department of Epidemiology, Box 189, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-3020; Fax: (713) 792-0807; E-mail: mspitz@mdanderson.org.

[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF-binding protein; CARET, β-Carotene and Retinol Efficacy Trial; OR, odds ratio; CI, confidence interval.

We now report the results of a case-control study nested in the placebo arm of a large chemoprevention intervention in heavy smokers, the CARET trial. In this study, we evaluated IGF-I and IGFBP-3 serum levels in lung cancer cases and matched controls free from lung cancer, using samples drawn at least 3 years before the lung cancer diagnosis.

Materials and Methods

Study Population. The CARET is a randomized, double-blinded, placebo-controlled chemoprevention trial to test the effect of the daily combination of β-carotene (30 mg) and retinyl palmitate (25,000 units) on the incidence of lung cancer in high-risk men and women smokers ($n = 14{,}254$) and male asbestos workers ($n = 4{,}060$; Ref. 16). The trial was initiated with a pilot phase and then expanded 10-fold at six study centers located in New Haven, Connecticut, Baltimore, Maryland, San Francisco, California, Seattle, Washington, Portland, Oregon, and Irvine, California. Individuals eligible for the high-risk smoker population included current and recent ex-cigarette smokers, aged 50–69 years, with smoking histories of >20 pack-years. Individuals eligible for the asbestos-exposed population were men aged 45–69 years who were current or former smokers with either substantial occupational exposure to asbestos or chest X-rays positive for asbestos-related changes. There were 133 never-smokers among the asbestos-exposed population, all randomized during the pilot study before the smoking eligibility criterion was added. The primary method of recruitment was by mailing study information and eligibility questionnaires to age-selected health insurance subscribers. Seventy-five percent of participants who enrolled in the 3-month placebo run-in were randomized.

Cigarette smoking history and status were assessed through participant self-report. Height and weight were measured by CARET study center staff during participant visits to the study centers. Serum was collected from the participants at baseline and periodically after randomization to be analyzed for micronutrient concentration. Serum samples were stored at −25°C for up to 48 h and then placed in long-term storage at −80°C. An End Points Review Committee evaluated end point reports, including pathologic review of tissue specimens. The CARET intervention was stopped 21 months early because of clear evidence of no benefit and substantial evidence of possible harm; there were 28% more lung cancers and 17% more deaths in the active intervention group (17).

From the placebo arm of the trial we selected all histologically confirmed lung cancer cases for whom serum collected at least 3 years before diagnosis was available and to whom a suitable control could be matched. Controls were randomly selected from the same study arm, matched to the cases on age within 5-year intervals (*i.e.*, 45–49 years, 50–54 years, and so forth), gender, ethnicity, year of enrollment into the CARET trial, and year of blood draw in a control:case ratio of 2:1. Controls had to have had a follow-up time at least as long as that of time to diagnosis of their matched case.

IGF-I and IGFBP-3 Measurement. The blood was centrifuged at 1500 rpm for 10 min at room temperature to separate the serum. The collected sera were stored at −80°C. The levels of IGF-I and IGFBP-3 were determined for all of the participants by ELISA using reagents from Diagnostic Systems Laboratories (Webster, TX), as described previously (6). Duplicate assays were performed on each sample, and the assay performance data provided are derived from control pooled sera run in triplicate on each assay plate. The intra- and interassay precision is between 4.5% and 8.6% and 3.3% and 6.8% of the coefficient of variation for the IGF-I assay and between 7.3% and 9.6% and 8.2% and 11.4% for the IGFBP-3 assay.

Table 1 Distribution of participant characteristics by case/control status (159 cases, 297 controls)

Variable	Cases *N* (%)	Controls *N* (%)
Sex[a]		
Male	122 (77)	223 (75)
Female	37 (23)	74 (25)
Race[a]		
White	152 (96)	283 (95)
Black	6 (4)	12 (4)
Asian/Pacific islander	1 (1)	2 (1)
Enrollment year[a]		
1985–1989	62 (39.0)	121 (40.7)
1990–1993	97 (61.0)	176 (59.3)
Year of blood draw[a]		
1986–1989	24 (15.1)	46 (15.5)
1990–1992	68 (42.8)	128 (43.1)
1993–1996	67 (42.1)	123 (41.4)
Exposure population[a]		
Asbestos-exposed	46 (29)	91 (31)
Smoker	113 (71)	206 (69)
Current smoker		
Yes	104 (65)	145 (49)[b]
No	55 (35)	152 (51)
Age (mean years ± SD)[a]	60.0 ± 5.6	59.9 ± 5.8
Pack-years (mean ± SD)	58.7 ± 24.3	45.9 ± 22.0[b]
BMI[c] (mean ± SD)	27.5 ± 4.6	28.1 ± 5.3

[a] Matching variables.
[b] $P < 0.001$.
[c] BMI, body mass index.

Statistical Analysis. We used paired *t* tests to compare the mean levels of IGF-I and IGFBP-3 between the cases and controls and the signed rank test to compare percentile distributions. The molar ratio between IGF-I and IGFBP-3 was calculated because it has been proposed that this may be a surrogate for free IGF-I. Conditional logistic regression, stratified on the matched sets, was used to analyze the association between the IGF levels and lung cancer, after adjustment for other possible confounders (smoking status at time of blood draw, pack-years of smoking, body mass index, and asbestos exposure). There were 55 cases and 152 controls who were not currently smoking at the time of the blood draw used for this study. Among those participants, the median number of years since quitting smoking was 6. This was used as the cut point to split the former smokers into two groups for analysis. We examined postdiagnosis survival by quartiles of IGF-I and IGFBP-3 using Kaplan-Meier estimates; the log-rank test was used to test for differences in survival curves. Multivariate linear regression was used to calculate and compare adjusted mean levels of IGF-I, IGFBP-3, and the ratio IGF-I:IGFBP-3 by smoking status and age groups.

Results

As summarized in Table 1, efficient matching was achieved for the 159 cases and 297 controls for all of the matching variables (age, gender, ethnicity, enrollment year, and year of blood draw). The mean ages for the cases and controls were 60.0 and 59.9 years, respectively. About three-quarters of the study population were male, and 95% were white. Twenty-nine percent of the cases and 31% of the controls were asbestos-exposed. The prevalence of current smokers was 65% for the cases and 49% for the controls, ($P < 0.001$). The cases were also heavier

Table 2 Adjusted[a] mean serum concentrations (SE) of IGF-I and IGFBP-3 by smoking and age subgroups or lung cancer cases and controls ($N = 456$)

	N (%)	IGF-I Mean ng/mL (SE)	IGFBP-3 μg/mL	IGF-I:IGFBP-3
Former/never-smokers	207 (45.4)	155.0 (3.8)	29.8 (0.6)	5.3 (0.1)
Age (yrs)				
<60	54 (26.1)	169.3 (6.9)	31.0 (1.1)	5.6 (0.2)
60–65	65 (31.4)	155.0 (6.2)	28.6 (1.0)	5.6 (0.2)
65+	88 (42.5)	149.5 (5.3)	29.0 (0.8)	5.3 (0.2)
P for trend		0.03	0.20	0.17
Current smokers	249 (54.6)	154.4 (3.5)	29.8 (0.5)	5.3 (0.1)
Age (yrs)				
<60	97 (39.0)	155.3 (5.9)	30.0 (0.8)	5.3 (0.2)
60–65	80 (32.1)	153.3 (6.4)	30.9 (0.9)	5.1 (0.2)
65+	72 (28.9)	150.3 (6.8)	29.6 (1.0)	5.2 (0.2)
P for trend across age groups		0.58	0.81	0.73
P for current *vs.* former smokers		0.92	1.00	0.71

[a] Means are adjusted for sex and pack-years of smoking.

Table 3 Serum IGF-I and IGFBP-3 levels by case/control status

	Mean (SD)	P^a	Percentiles					
			10%	25%	50%	75%	90%	P^b
IGF-I (ng/mL)								
Cases	158 (56)		92	118	153	190	243	
Controls	153 (54)	0.52	92	115	148	184	221	0.50
IGFBP-3 (μg/mL)								
Cases	30.7 (8.2)		20.1	25.1	30.0	36.1	41.2	
Controls	29.4 (7.9)	0.17	19.1	24.1	29.1	33.9	39.1	0.16

[a] Paired *t* test.
[b] Signed rank test for difference between cases and controls.

smokers, reporting a mean of 58.7 pack-years compared with 45.9 pack-years for the controls ($P < 0.001$).

In the present sample set, the expected positive correlation between IGF-I and IGFBP-3 levels was observed ($r = 0.53$, $P < 0.0001$; data not shown). It is recognized (4, 18, 19) that IGF-I levels gradually decline with age, and it has been suggested (12) that verification of the age-IGF-I relationship for sample sets under study may be useful in establishing that sample sets were obtained, stored, and assayed appropriately. However, the expected relationship between age and IGF-I level, after adjusting for sex and pack-years, was not seen ($r = -0.08$, $P = 0.07$; data not shown), raising possibilities of suboptimal sample storage or collection for the particular IGF-I assay used, poor assay performance, or a factor present in our study population that modified the relation between age and IGF-I level observed in other cohorts. Specifically, a 10-year increase in age was associated with an average decrease in IGF-I of 7.6 μg/liter (95% CI, −0.6 to 15.8), a weaker association than has been reported previously. However, participants in CARET are much heavier smokers than those in studies in which the age-IGF-I relation was originally defined. Because some studies (for example, Ref. 18) have provided evidence that smoking is associated with a reduction in IGF-I level, we reexamined the age-IGF-I relation separately in current smokers and former/never-smokers (Table 2) Only a modest and statistically nonsignificant inverse association between IGF-I and age was observed among current smokers. Among nonsmokers, the average decrease in IGF-I associated with a 10-year increase in age was 13.1 μg/liter (95% CI, 1.6–24.6); this finding is consistent with data from participants in the same age range presented in plots by Goodman-Gruen and Barrett-Connor (20), whose study population was predominately nonsmokers (<7% current smokers). The mean levels of both IGF-I and IGFBP-3 were higher in cases than in controls (158 ng/mL *versus* 153 ng/mL for IGF-I; 30.7 μg/mL *versus* 29.4 μg/mL for IGFPB-3; Table 3). However, none of these differences was statistically significant. This pattern also held up when the data were analyzed by percentile distribution.

Table 4 presents the ORs, with adjustment for selected risk factors, by quartiles of IGF-I, IGFBP-3, and the ratio of IGF-I:IGFBP-3. All analyses account for the matched design of the study. The ORs for IGF-I were generally around or below unity. For both models evaluated, an elevated risk was observed in the fourth quartile of IGFBP-3 level, with ORs of 1.67 and 2.35 (Table 4). The ORs for the molar ratio ranged from 0.67 to 0.86, and none were statistically significant.

When restricting the analyses to those <60 years of age, IGF-I was inversely associated with lung cancer risk in model 2 that also controlled for IGFBP-3, but no association was observed without control for IGFBP-3 (model 1; data not shown).

Table 5 summarizes the OR estimates for three smoking groups (current smokers, those who quit fewer than 6 years before their blood draw, and those having quit 6 or more years before their blood draw). Only among those who had quit smoking for the longest period of time was an elevated risk of lung cancer observed among subjects in the second to fourth quartiles of IGF-I relative to the first quartile (ORs, 2.21–2.91), although none achieved statistical significance. This may represent a chance finding, but it brings to mind the possibility that current smoking or recent cessation may obscure a modest relation between IGF-I level and lung cancer risk. On the other

Table 4 Lung cancer risks by quartiles of serum IGF-I and IGFBP-3

	N (no. of cases/controls)	Model 1[a]			Model 2[b]		
		OR	CI	*P*	OR	CI	*P*
IGF-I							
Q1 (<115)	38/73	1.00			1.00		
Q2 (115–149.9)	36/80	0.85	0.49–1.49		0.79	0.42–1.49	
Q3 (150–184.9)	42/73	1.11	0.65–1.91		0.83	0.43–1.61	
Q4 (≥185)	43/71	1.11	0.64–1.93		0.64	0.31–1.33	
Trend		1.06	0.89–1.26		1.02	0.71–1.11	0.29
IGFBP-3							
Q1 (<2,400)	30/72	1.00			1.00		
Q2 (2400–2899.9)	39/75	1.26	0.71–2.23		1.36	0.69–2.65	
Q3 (2900–3399.9)	37/76	1.20	0.66–2.17		1.40	0.68–2.89	
Q4 (≥3400)	53/74	1.67	0.96–2.92		2.35	1.13–4.92	
Trend		1.17	0.98–1.40		1.22	1.03–1.66	0.03
IGF-I:IGFBP-3							
Q1 (<0.042)	46/74	1.00					
Q2 (0.042–0.051)	35/70	0.74	0.42–1.32				
Q3 (0.052–0.061)	34/75	0.67	0.37–1.22				
Q4 (≥0.062)	44/78	0.86	0.49–1.50				
Trend		0.95	0.79–1.14	0.59			

[a] Model 1, conditional logistic regression model, controlling for matching variables: age, sex, race, year of enrollment, and year of blood draw.
[b] Model 2, conditional logistic regression model with adjustment for body mass index, smoking status, pack-years of smoking, exposure population, and IGF-I or IGFBP-3.

Table 5 Interactions between smoking status and quartiles of serum IGF-I, IGFBP-3, and IGF-1/IGFBP-3 on odds of lung cancer[a]

Quartile	Smoking status (*N* = number of cases/number of controls)											
	Quit 6+ years ago (*N* = 25/84)				Quit < 6 years ago (*N* = 30/68)				Current smokers (*N* = 104/145)			
	N	OR	CI	*P*	*N*	OR	CI	*P*	*N*	OR	CI	*P*
IGF-I												
Q1	2/21	1.00			7/15	1.00			29/37	1.00		
Q2	9/20	2.91	0.51–16.66	0.23	6/19	0.84	0.20–3.59	0.81	21/41	0.66	0.30–1.46	0.31
Q3	5/21	2.21	0.35–14.08	0.40	8/18	0.74	0.18–3.00	0.67	29/34	0.91	0.41–2.01	0.82
Q4	9/22	2.21	0.38–12.84	0.38	9/16	0.99	0.22–4.36	0.99	25/33	0.87	0.41–1.86	0.72
Trend		1.12	0.70–1.78	0.64		0.98	0.61–1.58	0.94		0.99	0.78–1.26	0.92
IGFBP-3												
Q1	5/23	1.00			5/16	1.00			20/33	1.00		
Q2	6/17	1.58	0.38–6.56	0.53	4/15	1.27	0.21–7.52	0.79	29/43	1.26	0.55–2.88	0.58
Q3	8/26	0.92	0.22–3.83	0.91	6/18	0.85	0.17–4.24	0.85	23/32	1.55	0.62–3.89	0.35
Q4	6/18	0.93	0.21–4.01	0.92	15/19	3.22	0.82–12.70	0.09	32/37	1.91	0.82–4.43	0.13
Trend		0.94	0.59–1.49	0.78		1.47	0.95–2.29	0.08		1.24	0.95–1.61	0.11
IGF-I:IGFBP-3												
Q1	4/15	1.00			10/19	1.00			32/40	1.00		
Q2	7/21	0.93	0.20–4.24	0.92	7/15	0.63	0.16–2.43	0.50	21/34	0.68	0.30–1.51	0.34
Q3	5/21	0.76	0.15–3.85	0.74	4/15	0.39	0.09–1.78	0.23	25/39	0.58	0.25–1.36	0.21
Q4	9/27	1.01	0.23–4.45	0.99	9/19	0.56	0.15–2.05	0.38	26/32	0.64	0.27–1.48	0.29
Trend		1.00	0.64–1.57	1.00		0.82	0.54–1.25	0.35		0.86	0.65–1.13	0.26

[a] Conditional logistic regression models with adjustment for body mass index, pack-years of smoking, and exposure population.

hand, risks associated with elevated IGFBP-3 level tended to be higher in current smokers and more recent quitters.

We also evaluated risk by three categories of cigarette pack-years (data not shown). There was no effect of IGF-I level in the two lower pack-year strata. However, in the heaviest smokers, the point estimate for the OR in the fourth quartile was 2.03, although it was not statistically significant.

Time between diagnosis and death of cases did not differ between subjects grouped by quartiles of IGF-I, IGFBP-3, or the IGF-I:IGFBP-3 ratio. The median survival for the quartiles (Q1, Q2, Q3, and Q4, respectively) is as follows: 10, 9, 9, and 7 months for IGF-I ($P = 0.31$); 10, 8, 11, and 8 months for IGFBP-3 ($P = 0.50$); and 10, 10, 9, and 7 months for the IGF-I:IGFBP-3 ratio ($P = 0.65$).

Discussion

At least seven prospective studies of common epithelial cancers have shown a relation between circulating IGF-I level before diagnosis and risk (6–14). Similar findings have been reported in some (but not all) case-control studies. Because the associations of IGF-I and IGFBP-3 with these cancers were similar to those we observed in our previous lung cancer case-control study, it seemed reasonable to assume that elevated IGF-I levels might antedate the development of lung cancer as well.

Recently, a nested case-control study of lung cancer in women ($n = 93$ cases, 186 controls; Ref. 20) demonstrated no significant association between IGF-I level or any of the binding proteins and cancer risk. London *et al.* (21) published the

results of their nested case-control study of 230 lung cancer patients and 740 control subjects drawn from the Shanghai cohort in China. They also did not detect a positive association between IGF-I level and lung cancer risk.

Unlike lung cancer, in none of the cancers where the association between IGF physiology and risk was noted is tobacco use a major predisposing factor. Therefore, our inability to detect any substantial association between lung cancer risk and IGF:IGBP-3 levels in this cohort may be because (*a*) in tobacco-related cancers, heterogeneity between subjects with respect to growth factors becomes an unimportant variable in the presence of substantive carcinogen exposure or (*b*) there is indeed an association with IGF physiology, which is obscured by the influence of smoking on the serum analyte levels.

There are several possible reasons for the discrepancy between the prospective results reported here and those from our previous case-control study (15). First, we cannot exclude the possibility that our prior observation was a spurious association based on chance. Second, although the cachexia of advanced cancer is associated with low IGF-I levels, blood samples from the cancer cases in our prior case-control study were obtained at the time of clinical presentation, when it is possible that circulating IGF-I levels might be elevated as a consequence of ectopic production of IGF-I by neoplastic cells, a phenomenon that has been observed in laboratory studies (3, 5, 22–26).

The effect of smoking on IGF-I levels requires further study, as reviewed recently. Available data are cross-sectional, and a rigorous investigation of this topic would require serial measurements in individual subjects before, during, and after smoking behavior. Some reports (for example, Ref. 19) suggest a suppressive effect of smoking on IGF-I levels, but others (for example, Refs. 20 and 27) find no evidence for this relationship. Careful review of controls from our prior case control study (15) reveals a trend toward decreasing IGF-I level with increasing pack-years among current and former smokers controlling for age ($<$32.5 pack-years, mean IGF-I = 172.5 ng/ml; 32.5–56.25 pack-years, mean IGF-I = 162.2 ng/ml; and $>$56.25 pack-years, mean IGF-I = 157.6 ng/ml; $P = 0.10$)

The effect of smoking on IGF-I level may be modified by age. In the data reported in Table 2, the expected age-IGF-I level relationship was obscured in current smokers but still evident in former smokers. Any suppressive effect of smoking on IGF-I levels (*e.g.*, in younger subjects with higher baseline levels) could obscure a relation between IGF-I levels and lung cancer risk. Under this scenario, heavy smoking would increase lung cancer risk by increasing carcinogen exposure while simultaneously lowering IGF-I level. In keeping with this speculation, data in Table 5 show a trend for increasing IGF-I levels to be associated with increased lung cancer risk only among longer-term quitters. However, we also noted that there was a trend of increasing risk with increasing IGF-I level among the subgroup of heaviest smokers, a finding that seems inconsistent with the data from Table 5.

It is certainly plausible that the overwhelming effects of tobacco carcinogen exposure would mask more subtle effects of IGFs on carcinogenesis. Although the present results do not support the conclusions of our prior case-control study (15), it is of interest that in that study the fold increase in risk associated with higher IGF-I level was consistently lower than the fold increase associated with mutagen sensitivity (28). This may represent a clue that in the case of lung cancer, carcinogenesis is driven more by carcinogen-induced mutations in somatic cells than by their growth factor environment.

The finding of a trend toward a positive association between IGFBP-3 levels and risk was unexpected in view of prior studies generally showing opposite results. London *et al.* (21) reported a reduced risk of lung cancer associated with higher levels of IGFBP-3, a pattern that we also observed in our previous case-control analysis (16). However, as other published studies have reported, as do we in this analysis, a positive association between the binding protein level and risk of both colorectal and breast cancer (8, 9). The reasons for this inconsistency require further study. One possibility concerns ectopic production of IGFBP-3 by a subset of cancers that may vary between studies. Another plausible explanation involves interacting analytic and biological factors: some cancers may be associated with proteolytic activity resulting in fragmentation of IGFBP-3 into several immunoreactive peptides, and under certain assay conditions, such fragments may be recognized as IGFBP-3. This could result in the observed trend toward higher IGFBP-3 readings for cases than controls in nonprospective studies. Multiple analyses performed might have resulted in some chance observations that are not biologically robust.

Prior circumstantial evidence is consistent with the possibility that there is no important relation between IGF-I level and risk of tobacco-related cancers. Childhood energy intake, leg length, and height are all crude surrogates for IGF-I levels early in life. Each of these surrogates has been linked to risk of cancers unrelated to tobacco use, but not to tobacco-related cancers (29–33). Our prospective data for lung cancer provide direct evidence that circulating levels of IGF-I and IGFBP-3 are not significantly related to lung cancer risk in populations with a strong smoking history. However, from these data, we cannot rule out the possibility that risk of lung cancer in nonsmokers may be related to IGF- I levels.

References

1. Jones, J. I., and Clemmons, D. R. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev., *16:* 3–34, 1995.

2. LeRoith, D., Werner, H., Beitner-Johnson, D., and Roberts, C. T. Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr. Rev., *16:* 143–163, 1995.

3. Stiles, A. D., and D'Ercole, A. J. The insulin-like growth factors and the lung. Am. J. Respir. Cell. Mol. Biol., *3:* 93–100, 1990.

4. Juul, A., Dalgaard, P., Blum, W. F., Bang, P., Hall, K., Michaelsen, K. F., Muller, J., and Skakkebaek, N. E. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. J. Clin. Endocrinol. Metab., *80:* 2534–2542, 1995.

5. Reeve, J. G., Brinkman, A., Hughes, S., Mitchell, J., Schwander, J., and Bleehen, N. M. Expression of insulin-like growth factor (IGF) and IGF-binding protein genes in human lung tumor cell lines. J. Natl. Cancer Inst. (Bethesda), *84:* 628–634, 1992.

6. Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostrate cancer risk: a prospective study. Science (Wash. DC), *279:* 563–566, 1998.

7. Hankinson, S. E., Willett, W. C., Colditz, G. A., Hunter, D. J., Michaud, D. S., Deroo, B., Rosner, B., Speizer, F. E., and Pollak, M. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet, *351:* 1393–1396, 1998.

8. Kaaks, R., Toniolo, P., Akhmedkhanov, A., Lukanova, A., Biessy, C., Dechaud, H., Rinaldi, S., Zeleniuch-Jacquotte, A., Shore, R. E., and Riboli, E. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J. Natl. Cancer Inst. (Bethesda), *92:* 1592–1600, 2000.

9. Toniolo, P., Bruning, P. F., Akhmedkhanov, A., Bonfrer, J. M., Koenig, K. L., Lukanova, A., Shore, R. E., and Zeleniuch-Jacquotte, A. Serum insulin-like growth factor-I and breast cancer. Int. J. Cancer, *88:* 828–832, 2000.

10. Ma, J., Pollak, M. N., Giovannucci, E., Chan, J. M., Tao, Y., Hennekens, C. H., and Stampfer, M. J. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J. Natl. Cancer Inst. (Bethesda), *91:* 620–625, 1999.

11. Pollak, M. Insulin-like growth factor physiology and cancer risk. Eur. J. Cancer, *36:* 1224–1228, 2000.

12. Pollak, M. Insulin-like growth factors (IGFs) and prostate cancer. Epidemiol. Rev., *23:* 59–66, 2001.

13. Yu, H., and Rohan, T. Role of the insulin-like growth factor family in cancer development and progression. J. Natl. Cancer Inst. (Bethesda), *92:* 1472–1489, 2000.

14. Giovannucci, E., Pollak, M., Platz, E., Willett, W., Stampfer, M., Majeed, N., Colditz, G., Speizer, F., and Hankinson, S. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol. Biomark. Prev., *9:* 345–349, 2000.

15. Yu, H., Spitz, M. R., Mistry, J., Gu, J., Hong, W. K., and Wu, X. Plasma levels of insulin-like growth factor-I and lung cancer risk: case-control analysis. J. Natl. Cancer Inst., *91:* 151–156, 1999.

16. Thornquist, M. D., Omenn, G. S., Goodman, G. E., Grizzle, J. E., Rosenstock, L., Barnhart, S., Anderson, G. L., Hammar, S., Balmes, J., Cherniack, M., Cone, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L., Jr., Valanis, B., and Williams, J.H., Jr. Statistical design and monitoring of the Carotene and Retinol Efficacy Trial (CARET). Controlled Clin. Trials, *14:* 308–324, 1993.

17. Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L., Valanis, B., Williams, J. H., Barnhart, S., and Hammar, S. Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. N. Engl. J. Med., *334:* 1150–1155, 1996.

18. Landin-Wilhelmsen, P. K., Wilhelmsen, L., Lappas, G., Rosen, T., Lindstedt, G., Lundberg, P. A., and Bengtsson, B. A. Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin. Endocrinol., *41:* 351–357, 1994.

19. Lukanova, A., Toniolo, P., Akhmedkhanov, A., Biessy, C., Haley, N. J., Shore, R. E., Riboli, E., Rinaldi, S., and Kaaks, R. A prospective study of insulin-like growth factor-I, IGF-binding proteins-1, -2 and -3 and lung cancer risk in women, Int. J. Cancer, *92:* 888–892, 2001.

20. Goodman-Gruen, D., and Barrett-Connor, E. Epidemiology of insulin-like growth factor-1 in elderly men and women. The Rancho Bernardo Study. Am. J. Epidemiol., *145:* 970–976, 1997.

21. London, S. J., Yuan, J-M., Travlos, G. S., Gao, Y-T., Wilson, R. E., Ross, R. K., and Yu, M. C. Insulin-like growth factor I, IGF-binding protein 3, and lung cancer risk in a prospective study of men in China. J. Natl. Cancer Inst. (Bethesda), *94:* 749–754, 2002.

22. Favoni, R. E., de Cupis, A., Ravera, F., Cantoni, C., Pirani, P., Ardizzoni, A., Noonan, D., and Biassoni, R. Expression and function of the insulin-like growth factor I system in human non-small-cell lung cancer and normal lung cell lines. Int. J. Cancer, *56:* 858–866, 1994.

23. Quinn, K. A., Treston, A. M., Unsworth, E. J., Miller, M. J., Vos, M., Grimley, C., Battey, J., Mulshine, J. L., and Cuttitta, F. Insulin-like growth factor expression in human cancer cell lines. J. Biol. Chem., *271:* 11477–11483, 1996.

24. Jaques, G., Rotsch, M., Wegmann, C., Worsch, U., Maasberg, M., and Havemann, K. Production of immunoreactive insulin-like growth factor I and response to exogenous IGF-I in small cell lung cell lines. Exp. Cell Res., *176:* 336–343, 1988.

25. Kaiser, U., Schardt, C., Brandscheidt, D., Wollmer, E., and Havemann, K. Expression of insulin-like growth factor receptors I and II in normal human lung and in lung cancer. J. Cancer Res. Clin. Oncol., *119:* 665–668, 1993.

26. Lee, C. T., Wu, S., Gabrilovich, D., Chen, H., Nadaf-Rahrov, S., Ciernik, I. F., and Carbone, D. P. Antitumor effects of an adenovirus expressing antisense insulin-like growth factor I receptor on human lung cancer cells. Cancer Res., *56:* 3038–3041, 2001.

27. Jernstrom, H., Deal, C., Wilkin, F., Chu, W., Tao, Y., Majeed, N., Hudson, T., Narod, S. A., and Pollak, M. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. Cancer Epidemiol. Biomark. Prev., *10:* 377–384, 2001.

28. Wu, X., Yu, H., Amos, C. I., Hong, W. K., and Spitz, M. R. Joint effect of insulin-like growth actors and mutagen sensitivity in lung cancer risk. J. Natl. Cancer Inst. (Bethesda), *92:* 737–743, 2000.

29. Hebert, P. R., Ajani, U., Cook, N. R., Lee, I. M., Chan, K. S., and Hennekens, C. H. Adult height and incidence of cancer in male physicians (United States). Cancer Causes Control, *8:* 591–597, 1997.

30. Davey Smith, G., Shipley, M., and Leon, D. A. Height and mortality from cancer among men: prospective observational study. Br. Med. J., *317:* 1351–1352, 1998.

31. Gunnell, D. J., Smith, G. D., Holly, J. M. P., and Frankel, S. Leg length and risk of cancer in the Boyd Orr cohort. Br. Med. J., *317:* 1350–1351, 1998.

32. Frankel, S., Gunnell, D. J., Peters, T. J., Maynard, M., and Davey Smith, G. Childhood energy intake and adult mortality from cancer: the Boyd Orr cohort study. Br. Med. J., *316:* 499–504, 1998.

33. Albanes, D., Jones, D. Y., Schatzkin, A., Micozzi, M. S., and Taylor, P. R. Adult stature and risk of cancer. Cancer Res., *48:* 1658–1662, 1988.