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

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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 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).

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\[ P < 0.03 \]
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-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 historically 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 g 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.

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

a Matching variables.
b P < 0.001.
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 (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 sample sets that modified the relation between age and IGF-I level, 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.

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
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 predosing factor. Therefore, our inability to detect any substantial association between lung cancer risk and IGF:IGFBP-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 carcogen 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 carcogen exposure would mask more subtle effects of IGFs on carcogenesis. 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, carcogenesis is driven more by carcogen-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 carcogenesisal 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


