Circulating Insulin-Like Growth Factor-I and Binding Protein-3 and Risk of Prostate Cancer

Gianluca Severi,1,2 Howard A. Morris,3 Robert J. MacInnis,1,2 Dallas R. English,1,2 Wayne D. Tilley,3,4 John L. Hopper,2 Peter Boyle,5 and Graham G. Giles1,2

' Cancer Epidemiology Centre, The Cancer Council Victoria; 'Centre for Molecular, Environmental, Genetic and Analytical Epidemiology, University of Melbourne, Melbourne, Victoria, Australia; ‘Hanson Institute; ‘Dame Roma Mitchell Cancer Research Laboratories, Department of Medicine, University of Adelaide, Adelaide, South Australia, Australia; and ‘IARC, Lyon, France

Abstract

Some recent epidemiologic studies have failed to confirm positive associations between insulin-like growth factor-I (IGF-I) and the risk of prostate cancer observed in earlier studies but have reported suggestive evidence for a positive association between IGF-binding protein-3 (IGFBP-3) and prostate cancer risk, a result contradicting the earlier assumption that high levels of IGFBP-3 would be protective against prostate cancer. We tested the association between IGF-I and IGFBP-3 and prostate cancer risk by measuring the two peptides in plasma samples collected at baseline in a prospective cohort study of 17,049 men. We used a case-cohort design, including 524 cases diagnosed during a mean of 8.7 years follow-up and a randomly sampled subcohort of 1,826 men. The association between each peptide level and prostate cancer risk was tested using Cox models adjusted for country of birth and alcohol consumption. The risk of prostate cancer was not associated with baseline levels of IGF-I or the molar ratio IGF-I/IGFBP-3 (all odds ratios are between 0.82 and 1.08; P<sub>trend</sub> ≥ 0.2), whereas the risk increased with baseline levels of IGFBP-3 (P<sub>trend</sub> = 0.008), the hazard ratio (HR) associated with a doubling of the concentration of IGFBP-3 being 1.70 (95% confidence interval, 1.15-2.52). The HR for quartile 4 relative to quartile 1 of IGFBP-3 was 1.49 (95% confidence interval, 1.11-2.00). The HRs did not differ by tumor aggressiveness or age at onset (all Ps ≥ 0.4). In our study, high levels of IGFBP-3 but not IGF-I were associated with an increased risk of prostate cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1137–41)

Introduction

Insulin-like growth factor-I (IGF-I) is a peptide involved in cell regulation that is mainly produced in the liver but also in other organs like the prostate. It is present in large concentrations in the circulation, mostly bound to the principal IGF-binding protein, IGFBP-3, and the concentration of both peptides varies greatly between individuals. With IGF-I being both mitogenic and antiapoptotic (1, 2), it has been hypothesized that IGF-I increases the risk of various types of cancer, including prostate. Similarly, IGFBP-3 might decrease the risk of cancer by decreasing the availability of free IGF-I and maybe through antiproliferative and proapoptotic actions independent of IGF-I as shown in cell lines (3, 4). In vitro studies have shown that prostate epithelial cells respond to IGFs (5), and in animal models, administration of IGF-I promotes prostate growth (6). Prostate cancer progression is associated with increased IGF-I expression (7).

A recent review of six epidemiologic studies that investigated the association of IGF-I and IGFBP-3 levels with cancer risk reported a pooled odds ratio for prostate cancer of 1.83 [95% confidence interval (95% CI), 1.03-3.26] for highest versus lowest IGF-I category and 0.88 (95% CI, 0.61-1.28) for highest versus lowest category of IGFBP-3 (8). Five new studies (9-13) and two updates of previous reports (14, 15) have been published since. These studies, generally with larger sample sizes than those included in the review, found little evidence of an association between IGF-I and the overall risk of prostate cancer. Subgroup analyses by tumor aggressiveness provided largely inconsistent results. In addition, some of these studies found a positive association between IGFBP-3 and prostate cancer risk either overall or for subgroups (11-14), a result in direct contrast with the earlier observations.

We tested for associations between IGF-I and IGFBP-3 levels and prostate cancer risk by measuring levels in blood samples taken at baseline from men enrolled in the Melbourne Collaborative Cohort Study.

Materials and Methods

Subjects and Case-Cohort Design. The Melbourne Collaborative Cohort Study is a prospective cohort study of 41,528 people (17,049 men) ages between 27 and 75 years at baseline (99.3% of whom were ages 40-69 years). Recruitment occurred between 1990 and 1994 in the Melbourne metropolitan area. Details of the study have been published elsewhere (16, 17). The Cancer Council Victoria’s Human Research Ethics Committee approved the study protocol. Subjects gave written consent to participate and for the investigators to obtain access to their medical records.

A case-cohort design was used for studies that included plasma analyses. All men first diagnosed with prostate cancer between baseline and June 30, 2002 were eligible as was a random sample (hereafter called the subcohort) of 2,167 men from the cohort. The study was designed to have the same power as a nested case-control study with two controls per case; preliminary analysis based on a method by Wacholder et al. (18), suggesting that the subcohort needed to have 3.6 times as many members as there were cases of prostate cancer. For this analysis, men were excluded if they had a confirmed diagnosis of prostate cancer before baseline (n = 106 in the full cohort and 9 in the subcohort).

Case Ascertainment. Addresses and vital status of the subjects were determined by record linkage to the electoral register and death records. Deaths were confirmed by cause via the Registry of Births, Deaths, and Marriages, Victoria. Subjects were excluded if they died before baseline (n = 31 in the full cohort and 3 in the subcohort) or had no linkage data (n = 22 in the full cohort and 2 in the subcohort).

A case-cohort design was used for studies that included plasma analyses. All men first diagnosed with prostate cancer between baseline and June 30, 2002 were eligible as was a random sample (hereafter called the subcohort) of 2,167 men from the cohort. The study was designed to have the same power as a nested case-control study with two controls per case; preliminary analysis based on a method by Wacholder et al. (18), suggesting that the subcohort needed to have 3.6 times as many members as there were cases of prostate cancer. For this analysis, men were excluded if they had a confirmed diagnosis of prostate cancer before baseline (n = 106 in the full cohort and 9 in the subcohort).

Case Ascertainment. Addresses and vital status of the subjects were determined by record linkage to the electoral register and death records. Deaths were confirmed by cause via the Registry of Births, Deaths, and Marriages, Victoria. Subjects were excluded if they died before baseline (n = 31 in the full cohort and 3 in the subcohort) or had no linkage data (n = 22 in the full cohort and 2 in the subcohort).

Received 10/24/05; revised 3/20/06; accepted 4/25/06.

Grant support: National Health and Medical Research Council grants 251533, 220907, and 126402 and Cancer Council Victoria. Cohort recruitment was funded by VicHealth and Cancer Council Victoria.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

Requests for reprints: Gianluca Severi, Cancer Epidemiology Centre, Cancer Council Victoria, 1 Rathdowne Street, Carlton, Victoria 3053, Australia. Phone: 61-3-9635-5412; Fax: 61-3-9635-5320. E-mail: gianluca.severi@cancervic.org.au

Copyright © 2006 American Association for Cancer Research.
Assessment of Circulating Levels of IGF-I and IGFBP-3.

Psychometric characteristics of the cases and the subcohort. About 72% of men in the subcohort were born in Australia, New Zealand, or the United Kingdom and 27% in Italy or Greece.

IGF-I and IGFBP-3 levels were categorized into quartiles according to the distribution of the values for the subcohort. Quartiles were assigned within each laboratory batch to adjust for any variation between batches. Tests for linear trend were based on pseudo-continuous variables under the assumption that all subjects within each quartile had the same concentrations equal to the within-quartile median. The pseudo-continuous variables were log$_2$ transformed before inclusion in the models so that the hazard ratio (HR) would represent the relative difference in risk associated with a doubling of the concentration.

Cox proportional hazards regression models, with age as the time axis (19), were used to estimate HRs and 95% CIs. The model was adjusted for left censoring at the age at baseline. We used the Prentice method to take the case-cohort sampling into account and the robust method was used to calculate the variance-covariance matrix (20, 21). Follow-up for a subcohort member began at baseline and ended at diagnosis of prostate cancer or cancer of unknown primary site, death, the date last known to be in Victoria, or June 30, 2002, whichever came first. To estimate HRs for nonaggressive and aggressive cases and for early-onset and late-onset cases and to test the difference, we fitted stratified Cox models based on competing risks using a data duplication method (22).

Tests based on Schoenfeld residuals (23) showed no evidence that proportional hazard assumptions were violated for any of the two analytes. To investigate the possible effect of prevalent prostate cancers, we tested whether the HRs differed by excluding the first 2 years of follow-up.

Analyses were adjusted for country of birth (Australia/New Zealand, United Kingdom, Italy, or Greece) and alcohol consumption. Adjustments for smoking status, education, body mass index, and energy intake did not appreciably change the HRs, so these variables were not included in final analyses. To study the influence of simultaneous adjustment for both analytes, we included them in a single model.

Statistical analyses were done using Stata/SE 8.2 (Stata Corp., College Station, TX). Because the robust method was used to calculate the variance-covariance matrix, the Wald test, not the likelihood ratio test, was used to test hypotheses. All $P$s were two sided, and $P < 0.05$ was considered as statistically significant.
for aggressive and nonaggressive prostate cancer ($P_{\text{trend}} = 0.05$ and 0.04, respectively), the HRs (95% CIs) associated with a doubling of the concentration of IGFBP-3 being 2.31 (1.00-5.31) for aggressive prostate cancer and 1.57 (1.03-2.38) for nonaggressive prostate cancer. Similarly, HRs did not differ significantly between early-onset and late-onset prostate cancer (all $P_s \geq 0.5$).

The HRs for IGF-I and the molar ratio IGF-I/IGFBP-3 changed only marginally after further adjustment for baseline prostate-specific antigen (PSA) values, whereas the HR for quartile 4 relative to quartile 1 of IGF-I and the linear trend in the HRs slightly decreased to 1.38 (95% CI, 0.98-1.97) and 1.51 (95% CI, 0.96-2.37), respectively.

The proportion of T1 tumors (i.e., incidental histologic findings from transurethral resection of the prostate or from needle biopsy because of elevated PSA levels) in the cases diagnosed in the first 3 years of follow-up (~25% of all cases) was 61% and increased to 79% in the cases diagnosed in the last 3 years of follow-up (~25% of all cases). The proportion of high-grade, Gleason score 8-10 tumors decreased from 19% to 13% and the median PSA levels decreased from 6.1 to 2.5 ng/mL. Despite this change in the case pool over time, there was no significant difference in the HRs for IGF-I and IGFBP-3 between the first 2 years and the rest of the follow-up (all $P_s \geq 0.5$).

The inclusion of both IGF-I and IGFBP-3 in the same model did not materially change the estimates for IGF-I, whereas the HRs (95% CIs) for quartiles 2 to 4 relative to quartile 1 of IGFBP-3 slightly increased to 1.08 (0.79-1.46), 1.27 (0.92-1.76), and 1.61 (1.15-2.26), respectively. The HR (95% CI) associated with a doubling of the concentration of IGFBP-3 increased to 1.89 (1.20-2.98; $P_{\text{trend}} = 0.006$).

Reliability and Quality Control. From the reliability study, the intraclasc correlation (95% CI) was 0.39 (0.27-0.51) for IGF-I, 0.78 (0.73-0.84) for IGFBP-3, and 0.56 (0.35-0.76) for PSA. For the pooled plasma samples, the overall coefficient of variation was 12% for IGF-I (9% within batches and 7% between batches), 9% for IGFBP-3 (8% and 3%), and 12% for PSA (8% and 10%). The correlation between IGF-I and IGFBP-3 was 0.61.

**Discussion**

We found that prediagnostic circulating levels of IGF-I were not associated with the risk of prostate cancer, whereas high levels of IGFBP-3 were associated with an increased risk of prostate cancer. Our findings are not consistent with the hypothesis generated by earlier studies (8) that high levels of circulating IGF-I are associated with an increased risk of prostate cancer and high levels of IGFBP-3 are associated with a decreased risk. They are, however, consistent with some more recent reports (13).

The main strengths of our study are its large sample size and high level of follow-up. Our study is thus far the largest among those that have investigated the possible association between IGF-I and IGFBP-3 and prostate cancer risk. Another strength is its large number of aggressive cases relative to other studies. To increase phenotype specificity compared with the other two studies that considered tumor aggressiveness (9, 13), we did not classify T3 cases with Gleason scores >7 as aggressive cases. This case-cohort study was designed back in 2002 when cancer cases that were diagnosed within the Melbourne Collaborative Cohort Study were identified through linkage to the Victorian Cancer Registry, while now they are identified through linkage to all the Australian Cancer Registries. However, during the follow-up to June 30, 2002, only four cases were diagnosed outside Victoria and therefore were not included in the analysis. Another strength of our study is the quality of the measurement of IGFBP-3 as evidenced by high intraclass correlation and low coefficient of variation for the pooled plasma samples. The reliability of the IGF-I measurements was much lower as evidenced by the low intraclass correlation. Previous studies have shown that the reliability of IGF-I measurements taken at different times for the same individuals can be high (24). A single measure of IGF-I, therefore, might not be reliable enough for association studies (24). The variability in the measurements of IGF-I might partly explain the lack of association between IGF-I levels and prostate cancer risk in some recent studies, including ours (9-13, 15), but does not explain the contrasting results.
between these studies and the earlier ones (25-27). Some authors have proposed that the increasingly widespread use of PSA testing in recent years might have influenced the association between IGF-I and IGFBP-3 and prostate cancer (13). IGF-I would be associated with aggressive tumors and not with tumors with low metastatic potential that form a significant proportion of the prostate cancers currently being diagnosed. The lack of difference in the HRs for aggressive and nonaggressive tumors and the inconsistent differences by tumor aggressiveness observed in the studies published thus far do not support this explanation. In an update of the Northern Sweden Health and Disease Cohort study, Stattin et al. found that the risk of prostate cancer increased with levels of IGF-I only for cases diagnosed at age <59 years (HR for quartile 4 relative to the first quartile of IGF-I, 1.41; 95% CI, 1.01-1.67; \( P_{\text{trend}} = 0.002 \)) and not for cases diagnosed at age \( \geq 59 \) years (HR, 1.41; 95% CI, 0.83-2.40; \( P_{\text{trend}} = 0.5 \)). This finding was not confirmed in our study. In a case-control study nested within the 

<table>
<thead>
<tr>
<th>Table 2. Relative risk of prostate cancer by quartile of hormone levels and by tumor aggressiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1*</td>
</tr>
<tr>
<td>All prostate cancer</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Nonaggressive prostate cancer</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Aggressive prostate cancer</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Late-onset prostate cancer (&gt;64 y)</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Early-onset prostate cancer (&lt;64 y)</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Follow-up from 2 y onwards</td>
</tr>
<tr>
<td>IGF-I</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
<tr>
<td>IGFBP-3</td>
</tr>
</tbody>
</table>

NOTE: A tumor was classified as aggressive if Gleason score was \( \geq 7 \) or stage was advanced (T4 or N+ or M+). We were not able to define aggressiveness for six cases because Gleason score and tumor stage were not available (clinical diagnoses only).

*Quartiles were assigned within each laboratory batch to adjust for any variation between batches.

1HRs from Cox regression models adjusted for country of birth (Australia/New Zealand, United Kingdom, Italy, or Greece) and alcohol consumption. The Prentice method has been used to take into account the case-cohort sampling (see Materials and Methods). HRs by tumor aggressiveness and age at diagnosis were obtained from Cox regression models fitted using competing risks methods.

2The hypothesis of a linear trend in the HR was tested, including in the model a pseudo-continuous variable computed assigning the median level of the specific hormone for each quartile.

3Test for difference in the estimates for the pseudo-continuous variables (i.e., linear trend) between aggressive and nonaggressive cases and between early-onset and late-onset cases.

4Test for difference in the estimates for the pseudo-continuous variables (i.e., linear trend) between the first 2 years and the rest of the follow-up.

the proliferative effects of IGFs (1). The original hypothesis that IGFBP-3 is associated with a decreased risk of prostate cancer is clearly contradicted by our results that are remarkably similar to those reported in the recent report from the Health Professionals Follow-up Study (13). In that study, the HR (95% CI) for quartile 4 relative to quartile 1 of IGFBP-3 was 1.62 (1.07-2.46), which was similar to the HR (95% CI) obtained in our study [1.49 (1.11-2.00)]. Comparing the highest and lowest categories of IGFBP-3, some of the earlier studies observed relative risks that ranged between 0.41 and 0.76 (25-27), but none of these estimates was statistically significant.

At least two mechanisms have been described for IGFBP-3 to stimulate proliferation of human breast cancer cell lines independently of IGFs. The \textit{in vivo} growth of the human breast cancer cell line T47D is stimulated by expression of IGFBP-3 via a mechanism involving the enhanced responsiveness to the proliferative effects of epidermal growth factor (28). IGFBP-3 has also been found to interact directly with the retinoic X receptor and therefore inhibit the all-\textit{trans}-retinoic acid action to maintain normal growth and differentiation of epithelial cells (29). Of potential relevance is the finding that IGFBP-3 is expressed by both normal and several types of abnormal prostate cells, including nodular hyperplasia, cancer, and metastatic cancer cells (30).

In conclusion, the results of our study provide good evidence that IGFBP-3 levels are positively associated with prostate cancer risk. Although the effect is moderate, this result might be important for the prevention of a disease whose etiology is still largely unknown. A previous longitudinal study has shown that IGFBP-3 levels decrease with increasing levels of physical activity and are therefore potentially modifiable (31).
Acknowledgments

We thank the original investigators, the Program Manager, Georgina Marr, and the diligent team who recruited the participants and continue working on follow-up, the many thousands of Melbourne residents who continue to participate in the study, and Sonia Dunn for assistance with the plasma measurements.

References

Circulating Insulin-Like Growth Factor-I and Binding Protein-3 and Risk of Prostate Cancer

Gianluca Severi, Howard A. Morris, Robert J. MacInnis, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/15/6/1137

Cited articles
This article cites 30 articles, 11 of which you can access for free at:
http://cebp.aacrjournals.org/content/15/6/1137.full.html#ref-list-1

Citing articles
This article has been cited by 16 HighWire-hosted articles. Access the articles at:
/content/15/6/1137.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.