Insulin-Like Growth Factor-I (IGF-I) and IGF Binding Protein-3 as Predictors of Advanced-Stage Prostate Cancer

June M. Chan, Meir J. Stampfer, Jing Ma, Peter Gann, J. Michael Gaziano, Michael Pollak, Edward Giovannucci

Background: Plasma levels of insulin-like growth factor-I (IGF-I) have been associated with the risk of prostate cancer. However, the association of IGF-I with specific tumor stage and grade at diagnosis, which correlate with risk of recurrence and mortality, has not been studied rigorously. To determine whether plasma levels of IGF-I and its main circulating binding protein, IGF binding protein-3 (IGFBP-3), predict more aggressive forms of prostate cancer, we investigated the association between plasma levels of each and specific stages and grades of prostate cancer. *Methods:* We examined 530 case patients and 534 control subjects in a nested case–control study in the prospective Physicians' Health Study. Patients with prostate cancer diagnosed from 1982 through 1995 were matched to control subjects by age and smoking status. IGF-I and IGFBP-3 were measured in prospectively collected plasma samples. Conditional logistic regression models were used to estimate the relative risks (RRs) for prostate cancer associated with IGF-I and IGFBP-3, stratified on grade (Gleason score $\geq$7 versus <7) and stage (early = stage A or B prostate cancer versus advanced = stage C or D prostate cancer). All statistical tests were two-sided. *Results:* Plasma levels of IGF-I and IGFBP-3 were predictors of advanced-stage prostate cancer (RR = 5.1, 95% confidence interval [CI] = 2.0 to 13.2 for highest versus lowest quartiles of IGF-I; RR = 0.2, 95% CI = 0.1 to 0.6 for highest versus lowest quartiles of IGFBP-3) but not of early-stage prostate cancer. Neither was differentially associated with Gleason score. Men with high IGF-I levels and low IGFBP-3 levels had an RR for advanced-stage prostate cancer of 9.5 (95% CI = 1.9 to 48.4) compared with men with low levels of both. Combining IGF-I and IGFPB-3 measurements with a standard prostate-specific antigen (PSA) mea-surement for prostate cancer screening increased the speci-ficity (from 91% to 93%) but decreased sensitivity (from 40% to 36%) compared with measurement of PSA alone. *Conclusions:* Circulating levels of IGF-I and IGFBP-3 may predict the risk of developing advanced-stage prostate can-cer, but their utility for screening patients with incident prostate cancer may be limited.

Insulin-like growth factor-I (IGF-I) is a potent stimulator of normal and neoplastic cell growth and has antiapoptotic actions on prostate epithelial cells *(1–7).* The major circulating binding

Affiliatons of authors: J. M. Chan, Department of Epidemiology and Biosta-tistics and Urology, University of California at San Francisco, Channing Labo-ratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, and Department of Epidemiology, Harvard School of Public Health, Boston; M. J. Stampfer, E. Giovannucci, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston; J. Ma, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; P. Gann, Depart-ment of Preventive Medicine, Northwestern University Medical School, Chi-cago, IL; J. M. Gaziano, Division of Preventive Medicine, Brigham and Wom-en's Hospital, and Massachusetts Veterans Epidemiology Research and Information Center, Veterans Administration Boston Health Care System, Bos-ton; M. Pollak, Cancer Prevention Research Unit, Departments of Medicine and Oncology, Lady Davis Research Institute of the Jewish General Hospital and McGill University, Montreal, Canada.

Correspondence to: June M. Chan, Sc.D., Department of Epidemiology and Biostatistics and Urology, UCSF, 3333 California St., Suite 280, San Francisco, CA 94118 (e-mail: Jchan@epi.ucsf.edu).

See "Notes" following "References."

protein for IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), influences the bioavailability of IGF-I (2,3,7,8) and has independent proapoptotic activity in the PC-3 prostate cancer cell line (6). Normal prostate epithelium cells contain type I IGF receptors and depend on IGF-I for growth (2). In vitro and in vivo experiments demonstrate that IGF-I increases proliferation of both androgen-dependent and androgen-independent prostate cancer cell lines (2), and IGFBP-3 can decrease the growth-stimulating effects of IGF-I (3).

An earlier report on a subset of 151 patients with prostate cancer from the current study population (9) and the work of other investigators (10–15) indicate a positive association between plasma levels of IGF-I and the risk of prostate cancer, with relative risks (RRs) of 2–4 when extreme quartiles were compared (9–15). Two other small prospective investigations of 30 patients (16) and 45 patients (17) reported no association for IGF-I, but the statistical power of these studies was limited. IGFBP-3 has also been inversely linked to the risk of prostate cancer in three studies (9,13,15), including our earlier investigation on a subset of the current study population. Overall, the association of IGFBP-3 with the risk of prostate cancer has been less consistently documented. All of these studies had limited power to assess associations of IGF-I and IGFBP-3 specifically with advanced stage or grade of prostate cancer.

With the shift toward earlier stages at diagnosis because of prostate-specific antigen (PSA) screening, it is becoming increasingly important to understand the etiology of prostate cancer recurrence and progression. Tumor stage and grade at diagnosis predict prostate cancer progression; identifying risk factors for advanced-stage or high-grade tumors could facilitate the development of novel therapies and regimens to prevent recurrence. Therefore, we examined the associations between plasma levels of IGF-I and IGFBP-3 and specific grades and stages of prostate cancer among 530 case patients and 534 control subjects in a nested case–control study in the prospective Physicians' Health Study, which included the 151 case–control pairs in our earlier report (9). With this large sample, we also considered potential interactions among IGF-I and IGFBP-3 and prostate cancer and assessed whether IGF-I and IGFBP-3 measurements could improve the screening capabilities of PSA testing.

METHODS

The Physicians' Health Study was a randomized placebo-controlled trial of aspirin and β-carotene conducted among 22 071 U.S. male physicians, aged 40–84 years in 1982. Men were excluded if they 1) had a history of myocardial infarction, stroke, transient ischemic attack, or cancer (except nonmelanoma skin cancer); 2) had current renal or liver disease, peptic ulcer, or gout; or 3) currently used aspirin, vitamin A, or β-carotene supplements. At enrollment and annually thereafter through 1995, men completed short, mailed questionnaires on diet, lifestyle, and medical history. Through 1995, follow-up of this cohort for morbidity and mortality was more than 99% complete (18).

Before randomization, we sent blood kits to all participants with instructions to have their blood drawn into vacutainer tubes containing EDTA. The samples were centrifuged, and the plasma was sent by overnight courier, in cryopreservation vials with cold packs, to the laboratory, where the samples were separated into aliquots and stored at –82 °C. Precautions were taken to prevent thawing or warming of specimens during storage.

Blood samples were provided by 14 916 men (68%), and these men form the base population for this nested case–control study.

Selection of Case Patients and Control Subjects

As of December 31, 1995, 786 incident cases of prostate cancer were ascertained among the 14 916 men who provided blood samples. Among these patients, 530 had sufficient plasma available for analysis of IGF-I and IGFBP-3. Choosing case patients on the basis of sufficient plasma volume was unlikely to have introduced selection bias because men could not have provided blood on the basis of any relationship between their IGF levels and future risk of prostate cancer. On average, assays were performed in samples collected 9 years before the time of diagnosis (minimum = 6 months and maximum = 13 years).

When a participant reported a new diagnosis of prostate cancer, we requested medical records, which were reviewed by study investigators. We defined advanced-stage tumors as stage C (extraprostatic, but no evidence of distant metastases) or stage D (distant metastatic or fatal) at diagnosis; early-stage tumors were defined as stage A (asymptomatic, incidentally detected lesions) or stage B (palpable tumors confined to the prostate gland). We also examined high-grade (Gleason score of ≥7) and low-grade (Gleason score of <7) tumors separately. Among the 530 cases of prostate cancer in this study, 142 cases were advanced (88 = stage C and 54 = stage D), 325 were early stage (64 = stage A and 261 = stage B), and 63 were missing data on tumor stage. There were 140 cases with Gleason scores of 7 or more and 266 with Gleason scores of less than 7; 124 cases were missing data on Gleason scores at diagnosis. For comparison with our previous report, we also considered a combined high-grade or advanced-stage classification, which was defined as advanced stage or high grade at diagnosis.

Control subjects were randomly selected from the pool of men who gave blood at baseline, who had a sufficient volume of stored blood for IGF analysis, and who had not had a total or partial prostatectomy or prostate cancer by the date of diagnosis of the case patient. The rationale for requiring that control subjects did not have a total or partial prostatectomy was to ensure that the control subjects had an intact prostate gland and, therefore, had the opportunity to develop prostate cancer. To maximize the number of case subjects that we could select from the population, we did not restrict case subjects to those men without a partial prostatectomy at the time of their prostate cancer diagnosis. The number of case subjects who might have been excluded based on this criterion would have been small and, therefore, unlikely to substantially influence the results. Furthermore, there would only be a potential for selection bias if having a partial prostatectomy differentially affects the association of serum levels of IGF-I and IGFBP-3 and risk of prostate cancer among the case subjects compared with that of the control subjects. This effect seems unlikely, because the majority of serum IGF-I is produced by the liver.

We matched one control subject to each case patient on the basis of age (within 1 year) and smoking status (never, past, or current). In four instances, a control subject later became a case patient, and an additional control subject was selected for comparison with the original case patient. Thus, there was a total of 534 control subjects in this study.

Measurement of IGF-I and IGFBP-3

Plasma samples were assayed in the laboratory of Dr. Michael Pollak, which used an enzyme-linked immunosorbent

assay (ELISA), with kits provided by Diagnostics Systems Laboratory (Webster, TX). Previous studies have shown IGF-I and IGFBP-3 in plasma to be relatively stable over time, and levels of IGF-I and IGFBP-3 measured by laboratory methods similar to those used in this study have been linked to cancer diagnoses in other investigations (9,19,20).

We previously reported our findings from 151 case–control pairs assayed in 1996 and 1997; these case patients were diagnosed from 1982 through 1992 (9). In the current study, we combined data from those 151 pairs (designated as wave 1) with 379 additional case patients and their 383 control subjects (designated as wave 2), who were diagnosed from 1982 through 1995 and whose samples were assayed in 1998. The intra-assay coefficients of variation were 4.9% for IGF-I and 9.0% for IGFBP-3 in the wave 1 samples and 6.5% for IGF-I and 7.0% for IGFBP-3 in the wave 2 samples.

Although assay reagents for the present study and our prior study (9) were from the same supplier, the assays were carried out 2 years apart with different production lots of ELISA reagents (IGF-I = lots 02117 and 12098c; IGFBP-3 = lots 07266d and 021019a), and we noted a drift in absolute values between assays. Among the control subjects, the range for IGF-I in wave 1 was 99–500 ng/mL and the median was 237 ng/mL; the range for IGF-I in wave 2 was 35–352 ng/mL and the median was 160 ng/mL. For the control subjects, the range for IGFBP-3 in wave 1 was 1144–5388 ng/mL and the median was 2879 ng/mL; the range for IGFBP-3 in wave 2 was 1005–5292 ng/mL and the median was 3092 ng/mL. Control work with pooled serum samples assayed with reagents from different production lots confirmed the presence of drift in absolute values between different production lots. To combine the data, we categorized the men into quartiles or tertiles based on the distributions of their respective waves in all analyses, thereby avoiding any influence of interassay variability.

There were no data available to directly test the hypothesis that a single measurement of IGF-I (or IGFBP-3) levels in adulthood correlates with levels much earlier in life; however, the short-term reproducibility of circulating IGF-I and IGFBP-3 levels in adult men was investigated by our group. The correlation coefficients for IGF-I and IGFBP-3 measurements taken twice over a 5-year interval on a sample of 49 men from the Physicians' Health Study cohort ranged between 0.70 and 0.75 (Pollak M, Ma J: unpublished results). Similar correlation coefficients were reported for a sample of 149 men from the Health Professionals Follow-up Study (21).

As part of a previous independent investigation, the archived blood samples of 468 case patients and 463 control subjects, collected in 1982, were assayed for PSA levels in 1993. The mean within-pair coefficient of variation was 8.6%, and the intraclass correlation coefficient was .96 (22).

Statistical Analysis

To examine the associations between IGF-I and IGFBP-3 and risk of prostate cancer, we used conditional logistic regression models to calculate odds ratios (and 95% confidence intervals [CIs]) as estimates of the RRs. All statistical tests were two-sided. We analyzed IGF-I and IGFBP-3 simultaneously in the same model to investigate the independent effects of each; the two are highly correlated ($r = .62$ and $P<.001$ in wave 1; $r = .64$ and $P<.001$ in wave 2). Biologic evidence and previous epidemiologic studies also support the hypothesis that the binding of

IGF-I to IGFBP-3 influences their bioavailability (3,9). To test for linear trend, we used the medians of the quartiles from the wave-specific distributions of IGF-I and IGFBP-3 and the molar ratio of IGF/IGFBP-3. A primary hypothesis was that IGF-I and IGFBP-3 might be differently associated with the risk of clinically important (aggressive) prostate cancer compared with those prostate cancers with an inherently less aggressive phenotype. We therefore examined the levels of IGF-I and IGFBP-3 and the risk of advanced-stage versus early-stage prostate cancer and risk of high versus low Gleason score prostate cancer. In our previous report on just the 151 case–control pairs in wave 1, we observed no substantial difference in risks for high-grade/high-stage versus low-grade/low-stage disease for IGF-I and IGFBP-3. However, with the small number of case patients (n = 67 with high-grade/high-stage disease), statistical power was limited, and we did not examine stage independent of grade.

To evaluate potential interactive effects between IGF-I and IGFBP-3, we cross-classified tertiles of IGF-I and IGFBP-3 and analyzed the association with the risk of advanced-stage and non-advanced-stage prostate cancer. We also considered whether the molar ratio of IGF-I/IGFBP-3 was associated with the risk of prostate cancer. We used the following equivalents for conversion: IGF-I at 1 ng/mL = 0.130 nM IGF-I and IGFBP-3 at 1 ng/mL = 0.036 nM IGFBP-3.

We considered potential confounding by body mass index but found that body mass index was not an independent predictor of prostate cancer risk in the study population and that inclusion of body mass index in the statistical model did not alter the main results for IGF-I or IGFBP-3. Thus, we did not retain body mass index in the final multivariable analyses.

When we previously reported the strong RRs for IGF-I and IGFBP-3, it was speculated that these blood measurements might be useful for prostate cancer screening. We constructed several theoretical screening tests and assessed their sensitivity and specificity among the 468 case patients and 463 control subjects who had measurements for PSA, IGF-I, and IGFBP-3. We compared tests incorporating the molar ratio of IGF-I/IGFBP-3 and PSA levels against the often used clinical definition of a positive test of PSA as 4 ng/mL or more. We did not routinely assess information on digital rectal exams. Because of interassay variability, we were unable to use the combined data to calculate absolute cutoffs for the IGF-I/IGFBP-3 molar ratio. Instead, we were restricted to the use of quantile cutoffs. We considered alternative tests that incorporated IGF-I or IGFBP-3 individually and a cross-classified variable combining categories of IGF-I and IGFBP-3. The molar ratio, however, produced the highest sensitivities and specificities, and the other results are not shown.

RESULTS

We examined IGF-I and IGFBP-3 and the risk of total prostate cancer in the new sample of 379 case patients and 383 control subjects (wave 2) to confirm the results of our first report [wave 1 (9)]. Unexpectedly, there was no association between IGF-I ($P = .39$) or IGFBP-3 ($P = .47$) and total prostate cancer risk in the wave 2 sample (results not shown). However, when we proceeded to examine stage-specific prostate cancer outcomes in the wave 2 sample, IGF-I and IGFBP-3 were statistically significantly associated with risk of advanced-stage (stage C or D) prostate cancer but not with early-stage (stage A or B) disease (Table 1). Although there was limited statistical power,

Table 1. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) and the relative risk (RR) and 95% confidence interval (CI) of early-stage and advanced-stage prostate cancer* in the Physicians' Health Study, stratified by wave

	RR (95% CI)† of prostate cancer by quartiles of IGF-I and IGFBP-3	
	Wave 1	Wave 2
Stage A or B		
	(n = 90 case patients)	(n = 235 case patients)
Quartiles of IGF-I		
1	1.0 (referent)	1.0 (referent)
2	1.8 (0.6 to 5.2)	0.9 (0.5 to 1.5)
3	2.5 (0.9 to 6.6)	0.6 (0.3 to 1.1)
4	3.5 (1.1 to 11.1)	0.8 (0.4 to 1.6)
P_{trend}‡	.03	.40
Quartiles of IGFBP-3		
1	1.0 (referent)	1.0 (referent)
2	0.4 (0.1 to 1.1)	1.2 (0.7 to 2.2)
3	0.3 (0.1 to 0.8)	1.1 (0.6 to 2.0)
4	0.4 (0.1 to 1.3)	1.4 (0.7 to 2.9)
P_{trend}‡	.21	.42
Stage C or D		
	(n = 49 case patients)	(n = 93 case patients)
Quartiles of IGF-I		
1	1.0 (referent)	1.0 (referent)
2	4.7 (0.6 to 34.9)	3.4 (1.3 to 8.8)
3	3.1 (0.6 to 15.0)	4.5 (1.6 to 12.8)
4	10.0 (1.4 to 74.5)	4.7 (1.5 to 14.9)
P_{trend}‡	.05	.01
Quartiles of IGFBP-3		
1	1.0 (referent)	1.0 (referent)
2	0.1 (0.02 to 1.0)	0.6 (0.3 to 1.5)
3	0.6 (0.1 to 3.4)	0.5 (0.2 to 1.3)
4	0.2 (0.02 to 1.2)	0.2 (0.07 to 0.8)
P_{trend}‡	.36	.02

*Case stage is based on the Whitmore–Jewett classification scheme; 63 case patients did not have sufficient medical chart information to assign a stage and were excluded from this analysis.

†Model is simultaneously adjusted for IGF-I and IGFBP-3, and cases and controls are matched on age and smoking status (never, past, current, data missing). All statistical tests were two-sided.

‡Test for trend was based on the medians of quartiles of IGF-I and IGFBP-3.

the wave 1 sample reflected positive associations for IGF-I and inverse associations for IGFBP-3 and risk of both early-stage and advanced-stage prostate cancer; the associations were stronger for advanced-stage disease.

Our main interest in this new investigation was to examine stage- and grade-specific prostate cancer outcomes. Because IGF-I and IGFBP-3 were more strongly associated with advanced prostate cancer in both waves 1 and 2, to maximize statistical power in these stratified analyses, we combined the two waves. With this larger sample, men in the highest quartile of IGF-I had an RR for prostate cancer of 5.1 (95% CI = 2.0 to 13.2) compared with that of men in the lowest quartile; for IGFBP-3, the corresponding RR was 0.2 (95% CI = 0.1 to 0.6) (Table 2). Finer stratification of the outcome variable revealed that this stronger association existed both for stage C and stage D cancers.

In contrast, IGF-I and IGFBP-3 were not as clearly differentially associated with prostate tumors that had high or low Gleason scores (Table 3); there was a statistically significant positive association between IGF-I and the risk of tumors with low Gleason scores. As expected, a combination of high-grade/high-stage prostate cancer yielded results intermediate between those depicted in Tables 2 and 3 and was not considered further (results not shown). Because of the stark contrast in associations for prostate cancer stage but not Gleason score, we continued to stratify by stage at diagnosis in subsequent analyses.

Given the results presented in Tables 2 and 3, we hypothesized that high-grade tumors are more autonomous in their growth and less influenced by host factors, such as circulating IGF-I. In contrast, tumors with lower Gleason scores might be more influenced by such factors. In an analysis restricted to case patients who had a Gleason score of 2–7, the association between IGF-I and IGFBP-3 and risk of advanced-stage prostate cancer was more pronounced (there were 66 advanced-stage case patients with a Gleason score <8). The RRs for stage C or D prostate cancer for quartiles of IGF-I were 1.0 (referent), 4.4 (95% CI = 1.2 to 16.8), 7.2 (95% CI = 1.7 to 29.8), and 9.6 (95% CI = 2.0 to 45.6). The corresponding RRs for quartiles of IGFBP-3 were 1.0 (referent), 0.7 (95% CI = 0.2 to 2.1), 0.5 (95% CI = 0.1 to 1.9), and 0.2 (95% CI = 0.0 to 0.8) (IGF-I and IGFBP-3 were mutually adjusted for in this analysis). There was no association between IGF-I and IGFBP-3 and risk of

Table 2. Relative risk (RR) and 95% confidence interval (CI) of advanced-stage and early-stage prostate cancer associated with quartiles of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) in the Physicians' Health Study

	Quartiles				
	1	2	3	4	P_{trend}*
RR (95% CI) of stage C or D† prostate cancer (n = 142 case patients)‡					
IGF-I	1.0 (referent)	3.2 (1.4 to 7.4)	3.5 (1.5 to 8.0)	5.1 (2.0 to 13.2)	.002
IGFBP-3	1.0 (referent)	0.5 (0.2 to 1.0)	0.5 (0.2 to 1.2)	0.2 (0.1 to 0.6)	.01
RR (95% CI) of stage A or B prostate cancer (n = 325 case patients)‡					
IGF-I	1.0 (referent)	1.0 (0.6 to 1.7)	0.9 (0.5 to 1.5)	1.2 (0.7 to 2.2)	.27
IGFBP-3	1.0 (referent)	0.9 (0.6 to 1.5)	0.8 (0.5 to 1.3)	1.0 (0.6 to 1.8)	.80

*Test for trend was based on the medians of quartiles of IGF-I and IGFBP-3.

†Case stage is based on the Whitmore–Jewett classification scheme; 63 case patients did not have sufficient medical chart information to assign a stage and were excluded from this analysis.

‡Model is simultaneously adjusted for IGF-I and IGFBP-3, and cases and controls are matched on age and smoking status (never, past, current, data missing). All statistical tests were two-sided.

Table 3. Relative risk (RR) and 95% confidence interval (CI) of high-grade and low-grade prostate cancer associated with quartiles of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) in the Physicians' Health Study*

| | Quartiles | | | | P_{trend}† |
	1	2	3	4	
	RR (95% CI) of Gleason score ≥7 prostate cancer (n = 140 case patients)‡				
IGF-I	1.0 (referent)	2.3 (1.1 to 4.7)	1.8 (0.8 to 3.9)	1.6 (0.6 to 4.2)	.45
IGFBP-3	1.0 (referent)	0.6 (0.3 to 1.3)	0.5 (0.2 to 1.2)	0.8 (0.3 to 2.1)	.72
	RR (95% CI) of Gleason score <7 prostate cancer (n = 266 case patients)‡				
IGF-I	1.0 (referent)	1.1 (0.6 to 1.8)	1.2 (0.6 to 2.1)	2.1 (1.1 to 4.1)	.002
IGFBP-3	1.0 (referent)	0.9 (0.5 to 1.5)	0.8 (0.4 to 1.4)	0.6 (0.3 to 1.2)	.14

*Data on Gleason score were missing from 124 case patients, who were excluded from this analysis.

†Test for trend was based on the medians of quartiles of IGF-I and IGFBP-3.

‡Model is simultaneously adjusted for IGF-I and IGFBP-3, and cases and controls are matched on age and smoking status (never, past, current, data missing). All statistical tests are two-sided.

early-stage tumors among this subgroup of men with Gleason scores of less than 8.

To examine whether the results by stage were affected by undiagnosed prostate cancers at baseline, we further stratified by year of diagnosis (Table 4). The positive association for IGF-I and the inverse association for IGFBP-3 were evident for advanced-stage prostate cancers diagnosed both before and after 1991, and the associations were actually stronger for advanced-stage tumors diagnosed during the later years (RR for the fourth versus the first quartile of IGF-I = 9.2, 95% CI = 1.5 to 55.7; RR for the fourth versus the first quartile of IGFBP-3 = 0.2, 95% CI = 0.04 to 1.0). IGF-I, but not IGFBP-3, was also associated with early-stage tumors diagnosed before 1991. Despite the small number of case patients (60), the strong and statistically significant associations for advanced-stage tumors diagnosed more than 9 years after blood was drawn argue against the possibility that the associations were the result of IGF production by the tumors themselves.

Table 5 shows cross-classified tertiles of IGF-I and IGFBP-3 and risk of advanced-stage prostate cancer. As expected, men in the highest tertile of IGF-I and lowest tertile of IGFBP-3 had the greatest risk compared with that of men in the lowest tertiles of each (RR = 9.5, 95% CI = 1.9 to 48.4). A test for interaction between IGF-I and IGFBP-3 and risk of advanced-stage prostate cancer yielded a P value of .06. The highest risk was among men in the highest tertile of IGF-I and lowest tertile of IGFBP-3 compared with men in the lowest tertile of IGF-I and highest tertile of IGFBP-3 (RR = 42.1, 95% CI = 3.0 to 587). This estimate was unstable, however, because there was only one case patient and six control subjects in the reference group. We found no association for cross-classified tertiles of IGF-I and IGFBP-3 and the risk of early-stage prostate cancer (P for interaction = .20) (results not shown).

The molar ratio of IGF-I/IGFBP-3 was associated with risk, but the relation was not as strong as for quartiles of IGF-I, adjusted for IGFBP-3 (as published in our first report *(9)* and as seen in Table 2). Men in the fourth versus the first quartile of IGF-I/IGFBP-3 had an RR for advanced-stage prostate cancer of 2.50 (95% CI = 1.19 to 5.23; P_{trend} = .03). The molar ratio was not statistically significantly associated with risk of early-stage prostate cancer (for being in the fourth versus the first quartile of IGF-I/IGFBP-3, RR = 1.35, 95% CI = 0.86 to 2.13, P_{trend} = .14).

The sensitivity and specificity for the standard clinical definition of a positive PSA test (PSA≥4 ng/mL) were 40% and 91%, respectively. We explored whether additional measurement of the IGF-I/IGFBP-3 molar ratio could help increase specificity—and thus decrease the number of false-positive tests and unnecessary biopsy examinations—without too much loss in sensitivity by considering the following modified definitions of a positive test: 1) either PSA of 10 or more ng/mL or PSA of 4–10 ng/mL and IGF-I/IGFBP-3 in the top 25th percentile; 2) either PSA of 10 or more ng/mL or PSA of 4–10 ng/mL and IGF-I/IGFBP-3 in the top 50th percentile; and 3) either PSA of 10 or more ng/mL or PSA of 4–10 ng/mL and IGF-I/IGFBP-3 in the top 75th percentile. The sensitivity and specificity for these three tests were as follows: test 1 = 22% and 97%, respectively; test 2 = 28% and 95%, respectively; and test 3 = 36% and 93%, respectively. Compared with the standard test, only test 3 approached the same level of sensitivity with a slight increase in specificity.

DISCUSSION

We observed a strong positive association for plasma IGF-I and an inverse association for plasma IGFBP-3 and risk of extraprostatic and distant metastatic prostate cancer, but little association with overall risk of tumors confined to the prostate gland, except those tumors diagnosed early in the follow-up (which includes the majority of wave 1 case patients from our first report). This apparent discrepancy may partially be explained by the increasing use of PSA screening in the early 1990s. With PSA screening, for any given stage, tumors are more likely to be diagnosed when they are smaller or less aggressive. The diagnosis of larger or more virulent stage A or B tumors, before the common use of PSA screening, may somewhat account for the positive associations of IGF-I with total, early-stage, and advanced-stage prostate cancer during the beginning years of follow-up. In contrast, IGF-I and IGFBP-3 only poorly differentially predicted high-grade versus low-grade tumors. Overall, these results suggest that IGF-I and IGFBP-3 not only stimulate tumor initiation and growth but also may facilitate invasion and metastases. One can also speculate that the actions of circulating IGF-I may not be confined to the prostate gland but may also prime the bone microenvironment for metastatic lesions.

Table 4. Relative risk (RR) and 95% confidence interval (CI) of advanced-stage and early-stage prostate cancer by year of diagnosis, associated with quartiles of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) in the Physicians' Health Study

| | Quartiles | | | | |
	1	2	3	4	P_{trend}*
	Diagnosis before January 1, 1991				
	RR (95% CI) of stage C or D† prostate cancer (n = 82 case patients)‡				
IGF-I	1.0 (referent)	2.9 (1.1 to 7.9)	2.3 (0.8 to 6.3)	5.1 (1.5 to 17.5)	.02
IGFBP-3	1.0 (referent)	0.6 (0.2 to 1.7)	0.5 (0.2 to 1.5)	0.2 (0.1 to 0.8)	.03
	RR (95% CI) of stage A or B prostate cancer (n = 118 case patients)‡				
IGF-I	1.0 (referent)	1.7 (0.8 to 4.0)	2.0 (0.9 to 4.6)	3.1 (1.1 to 8.5)	.02
IGFBP-3	1.0 (referent)	1.3 (0.6 to 2.7)	0.5 (0.2 to 1.3)	0.8 (0.3 to 2.0)	.23
	Diagnosis on or after January 1, 1991				
	RR (95% CI) of stage C or D prostate cancer (n = 60 case patients)‡				
IGF-I	1.0 (referent)	5.7 (1.1 to 30.0)	8.2 (1.6 to 42.3)	9.2 (1.5 to 55.7)	.03
IGFBP-3	1.0 (referent)	0.3 (0.1 to 1.1)	0.4 (0.1 to 1.7)	0.2 (0.04 to 1.0)	.11
	RR (95% CI) of stage A or B prostate cancer (n = 207 case patients)‡				
IGF-I	1.0 (referent)	0.8 (0.4 to 1.5)	0.5 (0.3 to 1.0)	0.7 (0.3 to 1.6)	.46
IGFBP-3	1.0 (referent)	0.8 (0.4 to 1.4)	0.9 (0.5 to 1.7)	1.1 (0.5 to 2.4)	.55

*Test for trend was based on the medians of quartiles of IGF-I and IGFBP-3.

†Case stage is based on the Whitmore–Jewett classification scheme; 63 case patients did not have sufficient medical chart information to assign a stage and were excluded from this analysis.

‡Model is simultaneously adjusted for IGF-I and IGFBP-3, and cases and controls are matched on age and smoking status (never, past, current, data missing). All statistical tests are two-sided.

Table 5. Cross-classified tertiles of insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3) and the relative risk (RR) and 95% confidence interval (CI) of stage C and D prostate cancer in the Physicians' Health Study

| | RR (95% CI) of advanced-stage prostate cancer (142 case patients and 144 control subjects) by tertiles of IGFBP-3* | | |
Tertiles of IGF-I	1	2	3
1	1.0 (referent) [22/37]	1.8 (0.6 to 4.9) [13/13]	0.2 (0.03 to 2.0 [1/6]
2	3.4 (1.2 to 10.0) [14/8]	2.3 (0.9 to 5.4) [23/18]	1.6 (0.6 to 4.2) [16/18]
3	9.5 (1.9 to 48.4) [12/3]	2.8 (1.1 to 7.3) [18/13]	1.7 (0.7 to 4.2) [23/28]

*Test for interaction using tertile indicators of IGF-I and IGFBP-3, P = .06. Number in brackets = No. of case patients/No. of control subjects. All statistical tests are two-sided.

The association of IGF-I and IGFBP-3 with risk of advanced-stage tumors was especially strong among men with lower-grade tumors (Gleason scores of <8); these men had a greater than ninefold elevation in risk of advanced-stage prostate cancer associated with higher IGF levels and a corresponding 85% reduction in risk associated with higher IGFBP-3 levels. This finding supports the hypothesis that the invasion and metastasis of low-grade tumors are more influenced by circulating IGF-I and IGFBP-3 levels, whereas the invasion and metastasis of high-grade tumors are more autonomous.

Men at greatest risk of advanced prostate cancer were those with simultaneously high levels of IGF-I and low levels of IGFBP-3. These men had a greater than ninefold elevation in risk of advanced-stage prostate cancer compared with men with the lowest IGF-I and IGFBP-3 levels. It should be noted that only 2% of the control population had simultaneously high levels of IGF-I and low levels of IGFBP-3; similarly, 3% of the control subjects had the opposing extreme profile of low levels of IGF-I and high levels of IGFBP-3. The test for a multiplicative interaction between IGF-I and IGFBP-3 and risk of advanced-stage prostate cancer was not statistically significant. This possible interaction should be evaluated in future investigations of IGF-I and IGFBP-3, and the physiologic explanation for this association requires elucidation.

We cannot exclude the possibility that tumor production of IGF-I and IGFBP-3 or a tumor's influence on their levels may in part account for the observed associations. However, the prospective design of this study and the stronger association with advanced disease diagnosed later rather than earlier in follow-up implicate IGF-I as a predictor of subsequent risk rather than a direct marker of tumor presence. If we speculate that tumors presenting at an advanced stage at diagnosis have a more aggressive phenotype than do tumors that are caught at an earlier stage, then the following model can be considered: IGF-I and IGFBP-3 predict risk of aggressive prostate cancer several years in advance of clinical detection but are predictive of more indolent tumors only over a shorter time span. The etiologies of aggressive and indolent tumors may differ, and IGF-I and IGFBP-3 may play roles of different magnitude and timing in the initiation and progression of these two tumor phenotypes.

Most other studies have not addressed the impact of IGF on specific tumor grade and stage (11,14,16,17). In a retrospective analysis, Wolk et al. (10) did not observe any marked difference in association by tumor stage, although there was some suggestion that IGF-I was a stronger predictor of stage C than it was of stage A or B tumors. Harman et al. (12) found no correlation between IGF and tumor volume in a small prospective study (n = 72), but they did not consider stage or grade. Stattin et al.

(13) did not directly contrast prostate cancers of low stage or grade versus high stage or grade but reported that results were similar for total prostate cancer and for organ-confined tumors and tumors that were well to moderately differentiated. Chokkalingam et al. *(15)* reported similar results for IGF-I and IGFBP-3 and risk of total prostate cancer; however, they found a stronger association between IGF-I and risk of localized prostate cancer than between IGF-I and risk of advanced-stage disease (they did not consider grade). This apparent discrepancy from our results might partially be the result of differences in the study design and populations. Their study was a smaller retrospective case–control study incorporating blood samples taken at the time of diagnosis from 47 patients with localized disease and 77 patients with advanced-stage disease. We examined IGF levels in 142 patients with advanced-stage cancer and in 325 patients with early-stage cancer an average of 9 years before clinical diagnosis. Furthermore, their study examined a low-risk population of Chinese men in China, whereas our study was conducted primarily among Caucasian men in the United States.

Our findings suggest that the critical window of time for influencing metastatic potential may be years in advance of clinical detection, even with PSA screening. In our cohort, only 60 patients with advanced-stage prostate cancer were diagnosed from 1991 through 1995, and our differential results by stage and follow-up time should be confirmed in current settings where PSA screening is more routine.

Recent studies *(12,23–27)* examined whether IGF-I and IGFBP-3 may improve the sensitivity or specificity of current screening methods (e.g., PSA test) for prostate cancer and urged further study on this topic *(23)*. It is important to distinguish between the utility of IGF-I and IGFBP-3 as risk factors for subsequent prostate cancer and their potential as screening tools for identifying individuals with prevalent prostate cancer. PSA is not a risk factor for prostate cancer, but it is a direct marker of tumor presence. In contrast, IGF-I and IGFBP-3 may predict risk of developing prostate cancer far in advance of any clinical indicators of tumor. Compared with a PSA test alone (positive test = PSA of ≥4 ng/mL), a combined PSA and IGF-I/IGFBP-3 test increased specificity only slightly (from 91% to 93%); it is unlikely that this test would be applied in clinical practice as a screening tool, given the corresponding drop in sensitivity (40% to 36%). However, given the strong association between IGF-I and IGFBP-3 and advanced-stage disease, future studies could address whether these serum measurements at the time of diagnosis might aid prognostication and, therefore, improve decision making about treatment. Furthermore, it will be of interest to determine whether any of the potential health benefits of PSA screening for prostate cancer vary by circulating IGF-I or IGFBP-3 levels.

A few limitations of this study should be considered. Although we did not detect a differential association between IGF-I and IGFBP-3 and the risk of low- versus high-grade tumors, we must acknowledge the potential for measurement error in assessing grade. We determined grade on the basis of the Gleason scores reported in the medical charts of the case patients. Some tumors were graded on review of a biopsy examination, whereas others were graded on review of surgical prostatectomy specimens, and grades from biopsy examinations often underestimate prostatectomy grades. Furthermore, there is likely to be inter-pathologist variability in grading and staging tumors. Thus, additional investigations with more uniform re-

porting of grade would be helpful to confirm the lack of differential association for Gleason score and IGF-I and IGFBP-3.

In conclusion, this study provides further strong evidence that circulating IGF-I and IGFBP-3 may predict the risk of advanced-stage prostate cancer.

REFERENCES

(1) Pollack M. Insulin-like growth factors and prostate cancer. Epidemiol Rev 2001;23:59–66.

(2) Cohen P, Peehl DM, Lamson G, Rosenfeld RG. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. J Clin Endocrinol Metab 1991;73:401–7.

(3) Cohen P, Peehl DM, Rosenfeld RG. The IGF axis in the prostate. Horm Metab Res 1994;26:81–4.

(4) LeRoith D, Baserga R, Helman L, Roberts CT Jr. Insulin-like growth factors and cancer. Ann Intern Med 1995;122:54–9.

(5) Iwamura M, Sluss PM, Casamento JB, Cockett AT. Insulin-like growth factor I: action and receptor characterization in human prostate cancer cell lines. Prostate 1993;22:243–52.

(6) Rajah R, Valentinis B, Cohen P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-β1 on programmed cell death through a p53 and IGF-independent mechanism. J Biol Chem 1997;272:12181–8.

(7) Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 2000;92:1472–89.

(8) LeRoith D. Insulin-like growth factor receptors and binding proteins. Baillieres Clin Endocrinol Metab 1996;10:49–73.

(9) Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563–6.

(10) Wolk A, Mantzoros CS, Andersson SO, Bergstrom R, Signorello LB, Lagiou P, et al. Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. J Natl Cancer Inst 1998;90:911–5.

(11) Mantzoros CS, Tzonou A, Signorello LB, Stampfer M, Trichopoulos D, Adami HO. Insulin-like growth factor 1 in relation to prostate cancer and benign prostatic hyperplasia. Br J Cancer 1997;76:1115–8.

(12) Harman SM, Metter EJ, Blackman MR, Landis PK, Carter HB. Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. J Clin Endocrinol Metab 2000;85:4258–65.

(13) Stattin P, Bylund A, Rinaldi S, Biessy C, Dechaud H, Stenman UH, et al. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. J Natl Cancer Inst 2000;92:1910–7.

(14) Khosravi J, Diamandi A, Mistry J, Scorilas A. Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in benign prostatic hyperplasia and prostate cancer. J Clin Endocrinol Metab 2001;86:694–9.

(15) Chokkalingam AP, Pollak M, Fillmore CM, Gao YT, Stanczyk FZ, Deng J, et al. Insulin-like growth factors and prostate cancer: a population-based case-control study in china. Cancer Epidemiol Biomarkers Prev 2001;10: 421–7.

(16) Schaefer C, Friedman GD, Quesenberry CP Jr, Orentreich N, Vogelman JH, Chan JM, et al. IGF-I and prostate cancer. Science 1998;282:199a.

(17) Lacey JV, Hsing AW, Fillmore CM, Hoffman S, Helzlsouer KJ, Comstock GW. Null association between insulin-like growth factors, insulin-like growth factor-binding proteins, and prostate cancer in a prospective study. Cancer Epidemiol Biomarkers Prev 2001;10:1101–2.

(18) Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9.

(19) Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393–6.

(20) Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, et al. 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 1999;91:620–5.

(21) Platz EA, Pollak MN, Rimm EB, Majeed N, Tao Y, Willett WC, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999; 8:1107–10.

(22) Gann PH, Hennekens CH, Stampfer MJ. A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. JAMA 1995;273:289–94.

(23) Wolk A, Andersson SO, Mantzoros CS, Trichopoulos D, Adami HO. Can measurements of IGF-1 and IGFBP-3 improve the sensitivity of prostate-cancer screening? Lancet 2000;356:1902–3.

(24) Djavan B, Bursa B, Seitz C, Soeregi G, Remzi M, Basharkhah A, et al. Insulin-like growth factor 1 (IGF-1), IGF-1 density, and IGF-1/PSA ratio for prostate cancer detection. Urology 1999;54:603–6.

(25) Cutting CW, Hunt C, Nisbet JA, Bland JM, Dalgleish AG, Kirby RS. Serum insulin-like growth factor-1 is not a useful marker of prostate cancer. BJU Int 1999;83:996–9.

(26) Finne P, Auvinen A, Koistinen H, Zhang WM, Maattanen L, Rannikko S, et al. Insulin-like growth factor I is not a useful marker of prostate cancer in men with elevated levels of prostate-specific antigen. J Clin Endocrinol Metab 2000;85:2744–7.

(27) Baffa R, Reiss K, El-Gabry EA, Sedor J, Moy ML, Shupp-Byrne D, et al. Low serum insulin-like growth factor 1 (IGF-1): a significant association with prostate cancer. Tech Urol 2000;6:236–9.

NOTES