Insulin-like Growth Factors and Prostate Cancer: A Population-based Case-Control Study in China

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Abstract

Insulin-like growth factors (IGFs) have potent mitogenic and antiapoptotic effects on prostate epithelial cells. Through modulation of IGF bioactivity and other mechanisms, IGF-binding proteins (IGFBPs) also have growth-regulatory effects on prostate cells. Recently, IGF-I and IGFBP-3 have been implicated in prostate cancer risk among Western populations. To assess whether IGF-I, IGF-II, IGFBP-1, or IGFBP-3 are also associated with prostate cancer in a low-risk population, we measured plasma levels of these factors among 128 newly diagnosed prostate cancer cases and 306 randomly selected population controls in Shanghai, China. Relative to the lowest quartile of IGF-I levels, men in the highest quartile had a 2.6-fold higher prostate cancer risk, with a significant trend [odds ratio (OR) = 2.63; 95% confidence interval (95% CI) = 1.19–5.79; P_{trend} = 0.01]. In contrast, men in the highest quartile of IGFBP-3 levels had a 46% decreased risk relative to the lowest quartile (OR = 0.54; 95% CI = 0.26–1.15; P_{trend} = 0.08). A similar but less distinct result was observed for IGFBP-1 (OR = 0.60; 95% CI = 0.31–1.17; P_{trend} = 0.25). Men in the highest quartile for the IGF-I:IGFBP-3 molar ratio (an indirect measure of free IGF-I) had a 2.5-fold higher risk compared with the lowest quartile (OR = 2.51; 95% CI = 1.32–4.75, P_{trend} < 0.001). These associations were more pronounced after adjustment for serum 5α-androstane-3α,17β-diol glucuronide and sex hormone-binding globulin levels. There was no significant association with IGF-II levels. Our findings in a low-risk population provide evidence that IGF-I, IGFBP-3, and IGFBP-1 are determinants of prostate cancer and indicate that additional studies are needed to evaluate their effects on ethnic and geographic incidence differentials and to elucidate carcinogenic mechanisms.

Introduction

IGF-I[2] and IGF-II are polypeptides functioning as both tissue growth factors and endocrine hormones (1, 2). Whereas IGF-I is a principal regulator of childhood and adult growth, IGF-II functions primarily during prenatal life (3). In addition to being regulators of breast, lung, and colon cancer cells (3, 4), IGF-I and -II have strong mitogenic and antiapoptotic effects on both normal and transformed prostate epithelial cells *in vitro* and *in vivo* (5–7), suggesting that IGFs may promote the development and growth of prostate cancer in humans.

The bioavailability of IGFs is modulated by IGFBPs. By binding to IGFs, IGFBPs limit the availability of free IGFs, which are required to activate the type I IGF receptor, the principal mediator of IGF regulatory effects (8). Of the IGFBPs identified to date, IGFBP-3 is present in the highest concentrations in the circulating blood (9), with >90% of circulating IGFs forming ternary complexes with IGFBP-3 and a protein known as acid-labile subunit (4). Although IGFBP-3 may promote cellular apoptosis through reduction of free IGF-I (4), its apoptotic effects are not necessarily dependent on blocking the binding of IGFs to the type I IGF receptor (1, 10); thus, IGFBP-3 may inhibit growth by both IGF-dependent and -independent mechanisms. IGFBP-1, present at much lower concentrations in the circulation than IGFBP-3, may be able to cross capillary walls because it does not circulate as part of a large protein complex; it therefore may regulate IGF bioavailability through transcapillary transport to the extravascular space (11).

Four recent epidemiological studies, two retrospective (12, 13) and two prospective (14, 15), conducted in Greece, Sweden, and the United States revealed that circulating levels of IGF-I are associated with prostate cancer risk, despite differences in laboratory methods (RIA, immunoradiometric assay, and ELISA) and biological specimens used (serum *versus* plasma). In one of the three studies assessing levels of IGFBP-3 (14), there was an inverse association with prostate cancer risk. In contrast, in the only study of IGFBP-1 levels, there was a positive association with prostate cancer risk (16). IGF-II was associated with prostate cancer risk in one study (15), but not another (14).

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[2] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF-binding protein; DSL, Diagnostic Systems Laboratories; 3α-diol G, 5α-androstane-3α,17β-diol glucuronide; SHBG, sex hormone-binding globulin; BMI, body mass index; OR, odds ratio; CI confidence interval; PSA, prostate-specific antigen.

Because the previous epidemiological studies of IGFs and IGFBPs in relation to prostate cancer were conducted in high-risk Western populations, we investigated the effects of plasma IGF-I, IGF-II, IGFBP-1, and IGFBP-3 levels as part of a population-based case-control study of prostate cancer in China, where incidence rates are among the lowest in the world (17).

Materials and Methods

Study Population. Details of this study have been described previously[3] (18, 19). Briefly, cases of primary prostate cancer newly diagnosed in urban Shanghai between 1993 and 1995 were identified through a rapid reporting system established by the Shanghai Cancer Registry. Because prostate screening is not widespread in China, cases were patients with clinically significant prostate cancer who presented with symptoms. Cases were classified as having localized (American Urological Association stage A or B) or advanced (stage C or D) disease. On the basis of a regional registry of all persons over age 18 in urban Shanghai, male population controls were selected randomly from the 6.5 million permanent residents of the region and frequency-matched to the expected age distribution (by 5-year age categories) of the cases. Using a structured questionnaire, trained interviewers elicited information on epidemiological risk factors from cases and controls within 30 days after selection. Anthropometric measures were taken during the interview. This study was approved by the Office of Human Subjects Research, NIH (Bethesda, MD), and the Institutional Review Board, Shanghai Cancer Institute (Shanghai, China).

Blood Collection and Laboratory Assays. Cases and controls provided 20 ml of fasting blood for the study. Samples for cases were collected at the hospital prior to treatment, whereas those for controls were collected at the time of interview. Samples were processed within 3 h of collection at a central laboratory in Shanghai, and the plasma fractions were stored at −70°C before being shipped frozen to the United States on dry ice.

Laboratory personnel were masked to case-control status. Samples were physically arranged in case-control pairs or triplets to minimize day-to-day laboratory variation. Plasma levels of IGF-I and IGF-II were determined using assay kits based on ELISA preceded by IGFBP removal via acid-ethanol extraction (DSL, Webster, TX). The lower limits of detection of the IGF-I and IGF-II assays are 0.03 and 2.4 ng/ml, respectively. IGFBP-1 and IGFBP-3 were also quantified using ELISA assays from DSL; the lower limit of detection of both assays is 0.04 ng/ml. For all four analytes, each sample was assayed twice, and the mean of the two determinations was used for data analysis. Samples for which the relative difference between the two determinations exceeded 10% were repeated. Split samples ($n = 45$) from a single individual were included among the study samples and used to assess laboratory reproducibility. For IGF-I, IGF-II, IGFBP-1, and IGFBP-3, the coefficients of variation for these split samples were 11.2, 13.8, 16.4, and 17.3%, respectively. At a separate laboratory, we compared IGF-I results from the ELISA kit (DSL) with those from a RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA), using a subset of the samples ($n = 34$), and found similar results: the difference in means was 3.4%, and the Spearman correlation coefficient was 0.77.

RIA was used to measure serum 3α-diol G and SHBG.

Table 1 Selected characteristics of 128 prostate cancer cases and 306 population controls in a Chinese population

Characteristic	Cases ($n = 128$)	Population controls ($n = 306$)
Age[a] (years)	71.9 (7.5)	72.0 (7.0)
Married,[b] *n* (%)	112 (87.5%)	281 (91.8%)
Education ≥ middle school, *n* (%)	48 (37.5%)	78 (25.5%)
Ever used alcohol, *n* (%)	41 (32.0%)	131 (42.8%)
Ever smoked, *n* (%)	69 (53.9%)	202 (66.0%)
Height[a] (cm)	167.6 (5.9)	167.5 (5.9)
Weight[a] (kg)	60.8 (8.0)	61.4 (10.1)
BMI[a] (kg/m^2)	21.7 (3.0)	21.9 (3.3)
Waist-to-hip ratio[a]	0.91 (0.05)	0.89 (0.06)
PSA[b] (ng/ml)	89.5	1.6
Total daily dietary intake[a,c] (kcal)	2434 (674)	2337 (726)

[a] Mean (SD).
[b] Median.
[c] Does not include calories from alcohol intake.

Statistical Analysis. To avoid a potential treatment effect among cases, only cases whose samples were collected at least 1 day prior to treatment were included in this analysis. Using *t* tests, we compared the mean plasma levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 between cases and controls. We examined among controls the Spearman correlations of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 with each other and with other potential risk factors, including serum 3α-diol G and SHBG, as well as weight, height, BMI, and waist-to-hip ratio (a measure of abdominal adiposity). Stratified analyses were used to identify potential confounding factors. Unconditional logistic regression was used to generate ORs and 95% CIs estimating the association of IGFs with prostate cancer after adjustment for other potential risk factors (20). For logistic regression analyses, IGF-I, IGF-II, IGFBP-1, and IGFBP-3 levels among cases and controls were categorized according to quartiles defined by the distributions among controls. Tests for linear trend were performed using quartile levels as continuous variables. All presented *P*s are two-sided.

Results

Selected characteristics of the 128 cases and 306 controls included in this analysis are presented in Table 1. Compared with controls, cases tended to be more educated, less likely to be married, and less likely to smoke or consume alcohol.

The mean IGF-I level among cases was significantly higher than among controls (138.6 *versus* 123.7 ng/ml; $P = 0.006$; Table 2). In contrast, the age-adjusted mean IGFBP-1 level was lower among cases than controls, although not significantly ($P = 0.39$). The age-adjusted mean IGF-II and IGFBP-3 levels did not differ significantly between cases and controls ($P = 0.84$ and 0.85, respectively). Among the 306 controls, levels of IGF-I, IGF-II, and IGFBP-3 were all positively correlated with one another, whereas IGFBP-1 was inversely correlated with the others (Table 3). SHBG and 3α-diol G levels were correlated with all of the IGFs and IGFBPs, as were all of the anthropometric factors, including height, weight, BMI, and waist-to-hip ratio. Interestingly, the correlations with IGFBP-1 were almost all inverse.

The ORs associated with prostate cancer risk by IGF-I, IGF-II, IGFBP-1, and IGFBP-3 level, as well as the IGF-I:

[3] A. W. Hsing, Y-T. Gao, G. Wu, X. Wang, A. P. Chokkalingam, J. Deng, J. Cheng, I. A. Sesterhenn, F. K. Mostofi, and C. Chang. Polymorphic CAG/CAA repeat lengths in the *AIB1/SRC-3* gene and prostate cancer risk: a population-based case-control study, submitted for publication.

Table 2 Means of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 by case/control status

Variable	Cases (n = 128)		Controls (n = 306)		P
	Mean	95% CI	Mean	95% CI	
IGF-I (ng/ml)	138.6	129.1–148.0	123.7	118.9–128.4	0.005
IGF-II (ng/ml)	438.1	416.2–459.9	440.6	426.3–454.9	0.84
IGFBP-1 (ng/ml)	103.6	92.4–114.9	109.5	102.2–116.7	0.39
IGFBP-3 (ng/ml)	2775.5	2634.8–2916.2	2792.0	2701.0–2883.1	0.85

Table 3 Spearman correlation coefficients of selected variables among population controls

Variable	IGF-I	IGF-II	IGFBP-1	IGFBP-3
IGF-I	1.00			
IGF-II	0.67[a]	1.00		
IGFBP-1	−0.38[a]	−0.38[a]	1.00	
IGFBP-3	0.66[a]	0.72[a]	−0.36[a]	1.00
SHBG	−0.46[a]	−0.43[a]	0.41[a]	−0.41[a]
3α-diol G	0.16[b]	0.21[a]	−0.23[a]	0.09
Weight	0.27[a]	0.18[b]	−0.33[a]	0.15[b]
Height	0.21[a]	0.12[c]	−0.08	0.11[c]
BMI	0.20[a]	0.13[c]	−0.31[a]	0.13[c]
Waist-to-hip ratio	0.11[c]	0.14[c]	−0.32[a]	0.13[c]

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

IGFBP-3 molar ratio are presented in Table 4. In the age-adjusted model, men in the highest quartile of plasma IGF-I had a non-significantly increased risk. Although increased risks were not observed for men in the second and third quartiles, there was a borderline significant positive trend (P_{trend} = 0.09). Risks were unrelated to plasma IGF-II, IGFBP-1, or IGFBP-3 levels after adjustment for age alone. Further adjustment for IGFBP-1 and IGFBP-3 increased the strength of the association between IGF-I and prostate cancer risk; relative to the lowest quartile of IGF-I levels, men in the highest quartile had a 2.6-fold higher risk, with a significant positive trend (OR = 2.63; 95% CI = 1.19–5.79; P_{trend} = 0.01). Similar risks were associated with the highest quartile of the IGF-I:IGFBP-3 molar ratio, which estimates the proportion of free IGF-I in the circulation (OR = 2.51; 95% CI = 1.32–4.75; $P_{trend} < 0.001$). In contrast, IGF-II was not associated with prostate cancer risk after adjustment for age, IGFBP-3, and IGFBP-1.

Although inclusion of IGFBP-1 among the adjustment factors improved model fit for both IGF-I and IGF-II, it did not materially alter their point estimates. When we adjusted only for age and IGFBP-3, the highest quartile of IGF-I was associated with a 2.4-fold higher prostate cancer risk (OR = 2.35; 95% CI = 1.11–4.97; P_{trend} = 0.01), whereas that of IGF-II remained unassociated with risk (OR = 1.25; 95% CI = 0.55–2.86; P_{trend} = 0.60).

Adjustment for both age and IGF-I revealed a nonsignificant inverse association between IGFBP-1 and prostate cancer risk (OR = 0.60; 95% CI = 0.31–1.17; P_{trend} = 0.25). A stronger inverse association was observed between IGFBP-3 and risk after adjustment for age and IGF-I; men in the highest quartile of IGFBP-3 levels had a 46% decreased risk cancer relative to the lowest quartile, with a borderline significant inverse trend (OR = 0.54; 95% CI = 0.26–1.15; P_{trend} = 0.08).

Except for 3α-diol G and SHBG, no other measured factors, including estradiol, height, weight, BMI, and waist-to-hip ratio, were found to materially change the ORs or improve model fit for any of the IGFs and IGFBPs. Adjustment for 3α-diol G, believed to be a good indicator of intraprostatic androgenicity (21), together with the binding protein SHBG in multivariate models increased the magnitude of all of the IGF and IGFBP associations (Table 4). In these models, the OR comparing the highest to lowest quartiles of IGF-I increased from 2.63 to 3.92 (95% CI = 1.58–9.70; P_{trend} = 0.003), with a similar effect on the risk estimate for the IGF-I:IGFBP-3 molar ratio. The OR comparing the highest to lowest quartiles of IGFBP-1 decreased from 0.60 to 0.40 with a significant inverse trend (95% CI = 0.19–0.85; P_{trend} = 0.03). A similar but less pronounced effect was observed for the association with IGFBP-3 (OR = 0.47; 95% CI = 0.21–1.05; P_{trend} = 0.06). Adjustment for 3α-diol G and SHBG also increased the risk estimate for IGF-II, although not significantly (OR = 2.22; 95% CI = 0.83–5.93; P_{trend} = 0.16).

The multivariate results stratified by clinical stage of prostate cancer (localized *versus* regional/remote) are shown in Table 5. IGF-I was significantly associated with prostate cancer risk in both stages, but was more pronounced for localized disease. The ORs comparing highest to lowest quartiles of IGF-I were 15.73 (95% CI = 3.04–81.94) for localized disease and 2.17 (95% CI = 0.78–6.01) for regional/remote disease. This result was reflected in the IGF-I:IGFBP-3 molar ratio findings, which showed a higher OR for localized (OR = 6.30; 95% CI = 1.96–20.24) than for regional/remote stage disease (OR = 2.53; 95% CI = 1.11–5.78). The ORs comparing the highest to lowest quartiles of IGFBP-1 were somewhat higher for localized than for advanced stage disease, whereas for IGFBP-3 and IGF-II, the ORs were slightly higher for advanced than for localized stage disease.

Although there was no significant effect modification by any of the other potential risk factors studied, increasing levels of IGF-I were not associated with prostate cancer risk among men with below-median levels of 3α-diol G (P_{trend} = 0.21; Table 6). In contrast, among men with 3α-diol G levels at or above the median, increasing IGF-I levels were strongly related to increases in risk (P_{trend} = 0.004). In addition, we found that the slope of the risks associated with increasing quartiles of IGF-I among those with 3α-diol G levels at or above the median was significantly different from that among men with 3α-diol G levels below the median (P = 0.03).

Discussion

In this case-control study conducted in a low-risk population in China, we found a strong dose-dependent association between increasing plasma levels of total IGF-I and prostate cancer risk, as well as inverse associations with levels of IGFBP-1 and IGFBP-3. In contrast, we found no association between levels of IGF-II and prostate cancer risk.

The effects of IGF-I, IGFBP-1, and IGFBP-3 on prostate cancer risk are biologically plausible, as demonstrated by numerous *in vitro* and *in vivo* studies (1, 9). It has been suggested that total plasma IGF-I may be a surrogate for tissue IGF-I bioactivity (4, 9), which is supported by our finding that the prostate cancer risk associated with the IGF-I:IGFBP-3 molar ratio (an indirect measure of free IGF-I) was similar to that for plasma IGF-I alone. In this manner, increasing levels of circulating IGF-I may indicate increasing activation of the type I IGF receptor and thus increasing growth of prostate cancer. In contrast, increasing levels of circulating IGFBPs, including IGFBP-1 and IGFBP-3, lower the amount of free IGF-I, indicating a reduction in activation of the type I IGF receptor and

Table 4 ORs of prostate cancer in relation to plasma levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 in a Chinese population

	Quartile	Adjusted for age only[a]		Adjusted for age, IGFs[b]		Adjusted for age, IGFs, SHBG, 3α-diol G[c]	
		n (cases/controls)	OR (95% CI)	*n* (cases/controls)	OR (95% CI)	*n* (cases/controls)	OR (95% CI)
IGF-I	Q1 (<93.2 ng/ml)	29/76	1.00	26/73	1.00	26/73	1.00
	Q2 (93.2–123.2 ng/ml)	24/77	0.79 (0.41–1.52)	24/76	1.00 (0.49–2.03)	24/76	1.52 (0.69–3.38)
	Q3 (123.3–151.7 ng/ml)	27/76	0.93 (0.49–1.77)	27/76	1.39 (0.66–2.93)	27/75	1.81 (0.77–4.23)
	Q4 (>151.7 ng/ml)	48/77	1.58 (0.87–2.88)	48/75	2.63 (1.19–5.79)	47/75	3.92 (1.58–9.70)
			P_{trend} = 0.09		P_{trend} = 0.01		P_{trend} = 0.003
IGF-II	Q1 (<343.5 ng/ml)	29/76	1.00	28/71	1.00	28/71	1.00
	Q2 (343.5–433.2 ng/ml)	34/77	1.13 (0.61–2.09)	33/77	1.16 (0.56–2.38)	33/77	1.84 (0.81–4.17)
	Q3 (433.3–521.9 ng/ml)	35/76	1.20 (0.65–2.21)	34/76	1.21 (0.56–2.62)	33/75	1.95 (0.82–4.67)
	Q4 (>521.9 ng/ml)	30/77	1.01 (0.53–1.90)	30/76	1.13 (0.48–2.68)	30/76	2.22 (0.83–5.93)
			P_{trend} = 0.94		P_{trend} = 0.80		P_{trend} = 0.16
IGFBP-1	Q1 (<61.7 ng/ml)	41/75	1.00	41/75	1.00	41/75	1.00
	Q2 (61.7–94.8 ng/ml)	25/75	0.61 (0.33–1.13)	25/75	0.62 (0.34–1.16)	24/75	0.53 (0.27–1.04)
	Q3 (94.9–149.0 ng/ml)	33/75	0.80 (0.45–1.43)	33/75	0.88 (0.48–1.60)	33/75	0.62 (0.31–1.21)
	Q4 (>149.0 ng/ml)	26/75	0.54 (0.29–1.02)	26/75	0.60 (0.31–1.17)	26/74	0.40 (0.19–0.85)
			P_{trend} = 0.62		P_{trend} = 0.25		P_{trend} = 0.03
IGFBP-3	Q1 (<2238.6 ng/ml)	36/76	1.00	36/76	1.00	35/76	1.00
	Q2 (2238.6–2813.0 ng/ml)	31/76	0.97 (0.53–1.76)	31/76	0.84 (0.45–1.58)	31/76	0.70 (0.35–1.41)
	Q3 (2813.1–3248.0 ng/ml)	28/76	0.77 (0.42–1.42)	28/76	0.55 (0.27–1.13)	28/75	0.50 (0.23–1.11)
	Q4 (>3248.0 ng/ml)	33/77	0.90 (0.49–1.66)	33/77	0.54 (0.26–1.15)	33/77	0.47 (0.21–1.05)
			P_{trend} = 0.59		P_{trend} = 0.08		P_{trend} = 0.06
IGF-I:IGFBP-3[d]	Q1 (<0.0362)	24/76	1.00	21/74	1.00	21/74	1.00
	Q2 (0.0362–0.0432)	16/77	0.61 (0.29–1.26)	16/76	0.71 (0.33–1.51)	16/76	0.71 (0.31–1.61)
	Q3 (0.0433–0.0519)	31/75	1.24 (0.66–2.34)	31/74	1.44 (0.74–2.80)	31/74	1.91 (0.92–3.96)
	Q4 (>0.0519)	57/76	2.19 (1.21–3.96)	57/76	2.51 (1.32–4.75)	56/75	3.58 (1.74–7.35)
			P_{trend} = 0.001		P_{trend} < 0.001		P_{trend} < 0.001

[a] Adjusted for age (<60, 60–64, 65–69, 70–74, 75–79, 80–84, >84 years).
[b] Same as age, but IGF-I, IGF-II, and IGF-I:IGFBP-3 ratio adjusted for IGFBP-1 (quartiles) and IGFBP-3 (quartiles); IGFBP-1 and IGFBP-3 adjusted for IGF-I (quartiles).
[c] Same as (age plus IGFs) but further adjusted for SHBG (<319, 319–517, 518–742, >742 nmol/l) and 3α-diol G (<28, 28–36, 37–48, >48 ng/dl).
[d] Indirect measure of free IGF-I.

thus a reduction in growth of prostate cancer. In addition, the IGF-I-independent association between IGFBP-3 and prostate cancer risk is consistent with the observation that IGFBP-3 can induce apoptosis independent of its IGF-sequestering action (10).

The positive association between circulating levels of IGF-I and prostate cancer risk among Chinese men is consistent with findings in high-risk Western populations in countries such as Greece, Sweden, and the United States (12–15). In addition, the magnitude of IGF-I-associated risk observed in all these populations is similar. The consistent findings from three retrospective studies (this study and Refs. 12, 13) and two prospective studies (14, 15) in various racial/ethnic populations, using different assay methods, support the etiological role of IGF-I in prostate cancer. In our study, the excess risk was confined largely to the highest quartile. In contrast, a prospective study in the United States noted a somewhat linear increase in risk across quartiles (14). The reasons for this difference are unclear, but may be related to the much higher IGF-I levels observed in the study performed in the United States.

Given the case-control design of our study, it is possible that the presence of cancer may have affected the measurements of IGF levels, particularly because malignant prostate epithelial cells are capable of expressing IGF-I. Such an effect, however, should be minimal in our study because the mean plasma level of IGF-I was lower among cases with advanced *versus* localized tumors (135.5 *versus* 143.7 ng/ml).

Because weight loss reduces IGF-I levels in laboratory animals (22), it is possible that weight loss attributable to illness may have affected the IGF-I levels and thus the association observed in this study. However, cases had only slightly lower mean adult weight relative to controls (<1 kg difference), and the age-adjusted mean adult weight between localized and advanced stage cases was similar (61.0 and 60.7 kg, respectively). Furthermore, decreased IGF-I levels attributable to tumor-related weight loss would tend to reduce the magnitude of the association between IGF-I and prostate cancer, so that our results may actually underestimate the true relationship. In fact, the slightly lower IGF-I levels among patients with regional/remote cancer compared with those with localized tumors suggest that advancing disease may contribute to some of the reductions in circulating IGF-I, which may explain in part the stronger association with IGF-I observed for localized cancer than for advanced stage disease.

Accumulating evidence suggests the involvement of androgens in IGF-mediated cellular regulation. Androgens promote IGF-I and IGF-I receptor expression *in vivo* (23), and antiandrogen treatment for prostate cancer often leads to increases in IGFBP expression, which reduces free IGF-I (24, 25). Thus, increasing androgen levels may increase IGF expression, bioavailability, and activity. In our study, multivariate adjustment for 3α-diol G and SHBG, to which androgens are bound to prevent degradation in circulation, substantially increased the magnitude of the IGF-I association with prostate cancer. In addition, the relationship between IGF-I and prostate cancer risk was significantly more pronounced among men with higher 3α-diol G levels, suggesting a significant interaction. These findings support the role of androgens in the IGF-mediated regulation of growth, although further studies are needed to clarify the mechanisms involved.

Although IGF-I has been shown to be correlated with height in our study and in previous studies (26), we observed no

Table 5 Stage-specific ORs for prostate cancer in relation to plasma levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 in a Chinese population

IGF quartiles[a]	Localized disease		Advanced disease	
	n (cases/controls)	OR (95% CI)	*n* (cases/controls)	OR (95% CI)
IGF-I[b]				
Q1	5/73	1.00	21/73	1.00
Q2	9/76	4.77 (1.10–20.62)	15/76	1.05 (0.43–2.59)
Q3	13/75	7.63 (1.65–35.39)	14/75	0.95 (0.35–2.53)
Q4	20/75	15.73 (3.04–81.94)	27/75	2.17 (0.78–6.01)
		$P_{trend} = 0.001$		$P_{trend} = 0.14$
IGF-II[b]				
Q1	8/71	1.00	20/71	1.00
Q2	17/77	3.90 (1.12–13.53)	16/77	1.13 (0.42–3.04)
Q3	14/75	2.81 (0.71–11.12)	19/75	1.38 (0.48–3.92)
Q4	8/76	1.88 (0.38–9.42)	22/76	2.56 (0.78–8.42)
		$P_{trend} = 0.83$		$P_{trend} = 0.11$
IGFBP-1[c]				
Q1	15/75	1.00	26/75	1.00
Q2	8/75	0.49 (0.17–1.39)	16/75	0.58 (0.27–1.27)
Q3	11/75	0.55 (0.19–1.61)	22/75	0.67 (0.31–1.45)
Q4	13/74	0.48 (0.15–1.49)	13/74	0.32 (0.13–0.81)
		$P_{trend} = 0.21$		$P_{trend} = 0.03$
IGFBP-3[c]				
Q1	12/76	1.00	24/76	1.00
Q2	10/76	0.29 (0.09–1.00)	21/76	0.86 (0.38–1.92)
Q3	13/75	0.40 (0.12–1.36)	15/75	0.52 (0.20–1.36)
Q4	13/77	0.31 (0.09–1.05)	20/77	0.49 (0.18–1.33)
		$P_{trend} = 0.20$		$P_{trend} = 0.12$
IGF-I:IGFBP-3[b]				
Q1	6/74	1.00	15/74	1.00
Q2	6/76	0.83 (0.22–3.10)	10/76	0.60 (0.22–1.61)
Q3	9/74	1.34 (0.39–4.65)	22/74	2.12 (0.92–4.87)
Q4	26/75	6.30 (1.96–20.24)	30/75	2.53 (1.11–5.78)
		$P_{trend} < 0.001$		$P_{trend} = 0.003$

[a] See Table 4 for quartile ranges.
[b] Adjusted for IGFBP-1 and IGFBP-3 (quartiles), age, SHBG, and 3α-diol G (categories specified in Table 4).
[c] Adjusted for IGF-I (quartiles), age, SHBG, and 3α-diol G (categories specified in Table 4).

Table 6 IGF-I multivariate ORs for prostate cancer by serum 3α-diol G level

n	Quartiles of IGF-I[a]				P_{trend}
	Q1	Q2	Q3	Q4	
3α-diol G <518 ng/dl					
OR (95% CI)[a,b]	1.00	0.88 (0.37–2.09)	1.00 (0.40–2.48)	1.82 (0.67–4.95)	0.21
n (cases/controls)	24/39	20/39	21/39	27/32	
3α-diol G ≥518 ng/dl					
OR (95% CI)[a,b]	1.00	1.93 (0.30–12.28)	5.20 (0.80–33.69)	12.29 (1.83–82.39)	0.004
n (cases/controls)	2/34	4/37	6/36	20/43	

[a] See Table 4 for IGF-I, IGFBP-1, and IGFBP-3 quartile ranges.
[b] Adjusted for age (categories specified in Table 4), IGFBP-1 (quartiles), and IGFBP-3 (quartiles).

strong influence of height on the association between IGF-I and prostate cancer. Similarly, we found no evidence that obesity (BMI) or abdominal adiposity (waist-to-hip ratio) influenced the observed association between total IGF-I level and prostate cancer risk, although these measures were risk factors in our study (19) and have been reported to influence IGF-I levels (3).

Our results for IGFBP-3 support those of the United States prospective study by Chan *et al.* (14), which found a borderline significant inverse trend after adjusting for age and circulating IGF-I, and are consistent with laboratory evidence of an IGF-independent effect of IGFBP-3 on cell growth (10). Biologically, it is unclear whether the effect of IGFBP-3 is independent of total or free IGF-I. Future studies should evaluate the IGFBP-3 association in conjunction with free IGF-I, for which an assay has only recently been developed and which has not yet been incorporated into epidemiological studies.

The IGFBP-3 findings are not likely to have been affected by the presence of disease. Despite being an IGFBP-3 protease, PSA (an early marker of prostate cancer) is enzymatically inactive in circulation because of the presence of circulating protease inhibitors (27), so that the elevated PSA levels among cases would have minimal impact on our findings. Furthermore, an inverse association between prostate cancer and IGFBP-3 levels that is not subject to disease effects was also found in a prospective study (14), and the magnitude of the risk reduction in that study resembled the reduction in our study.

The observed inverse association between IGFBP-1 and prostate cancer risk differs from the findings of Signorello *et al.* (16), who found a significant positive association among Swedish men. The reasons for this inconsistency are unclear. Although the two studies differ in blood collection and adjustment [the current study used fasting bloods and adjusted for both serum 3α-diol G and SHBG levels, whereas the Swedish study did not (16)] these factors alone are unlikely to fully explain the inconsistency in findings. In addition, the role of IGFBP-1 in IGF bioactivity is not well understood at present. Although IGFBP-1 may decrease IGF bioactivity by sequestering IGFs and thus prevent activation of the type I IGF receptor, IGFBP-1 may also increase IGF bioactivity by transporting IGFs out of the vascular space and into target tissues (11). Further investigations, including studies with direct free IGF-I determinations, are needed to clarify the mechanism by which IGFBP-1 affects prostate cancer risk.

IGF-II has been found to act in an autocrine manner to increase prostate cancer cell proliferation *in vitro* (5, 6). Consistent with one other epidemiological study to address this question (14), we found no significant association between levels of IGF-II and prostate cancer in our study, even after adjustment for IGFBP-1, IGFBP-3, SHBG, and 3α-diol G, although adjustment for the androgens elevated the point estimates considerably. It is unclear why our results and those of the previous study (14) differ from the other study (15), which found IGF-II to be significantly inversely associated with prostate cancer risk. As the point estimates for increasing plasma IGF-II quartiles were all above one and increased with adjustment, it is possible that with a larger sample size we might have seen a significant effect of IGF-II on prostate cancer risk, although the risks would be smaller than for IGF-I.

Biases arising from selection and survival in our study should be minimal because >90% of the eligible cases participated in the study and because most blood samples were collected within 30 days after diagnosis. Although 70–80% of the cases and controls consented to the blood draw, we attempted to minimize treatment effects by including in the analysis only cases whose blood samples were taken at least 1 day prior to therapy. The excluded cases ($n = 71$) were similar to the included cases with regard to demographic characteristics and anthropometric measures (data not shown), and the percentage of each group with advanced stage disease was also similar (69 and 63%, respectively).

In summary, our case-control study of prostate cancer conducted in a low-risk population (China) revealed that plasma IGF-I, IGFBP-1, and IGFBP-3 are associated with prostate cancer risk. Our findings, together with epidemiological studies conducted in high-risk Western populations, provide strong evidence of a role for IGFs in prostate cancer. Future investigations should include prospective studies of men from ethnic groups at varying risk of prostate cancer, including African Americans, who have the highest incidence rates in the world. In addition, epidemiological and laboratory studies are needed to elucidate underlying mechanisms, including the role played by the type I IGF receptor, circulating androgens and free IGF-I, and IGF bioactivity levels within prostatic tissue.

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References

1. Grimberg, A., and Cohen, P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J. Cell. Physiol., *183:* 1–9, 2000.

2. Burroughs, K. D., Dunn, S. E., Barrett, J. C., and Taylor, J. A. Insulin-like growth factor-I: a key regulator of human cancer risk? J. Natl. Cancer Inst. (Bethesda), *91:* 579–581, 1999.

3. Rosen, C. J. Serum insulin-like growth factors and insulin-like growth factor-binding proteins: clinical implications. Clin. Chem., *45:* 1384–1390, 1999.

4. Pollak, M., Beamer, W., and Zhang, J. C. Insulin-like growth factors and prostate cancer. Cancer Metastasis Rev., *17:* 383–390, 1999.

5. Cohen, P., Peehl, D. M., Lamson, G., and Rosenfeld, R. G. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins in primary cultures of prostate epithelial cells. J. Clin. Endocrinol. Metab., *73:* 401–407, 1991.

6. Angelloz-Nicoud, P., and Binoux, M. Autocrine regulation of cell proliferation by the insulin-like growth factor (IGF) and IGF binding protein-3 protease system in a human prostate carcinoma cell line (PC-3). Endocrinology, *136:* 5485–5492, 1995.

7. Torring, N., Vinter-Jensen, L., Pedersen, S. B., Sorensen, F. B., Flyvbjerg, A., and Nexo, E. Systemic administration of insulin-like growth factor I (IGF-I) causes growth of the rat prostate. J. Urol., *158:* 222–227, 1997.

8. Grimberg, A., and Cohen, P. Growth hormone and prostate cancer: guilty by association? J. Endocrinol. Investig., *22:* 64–73, 1999.

9. Rosen, C. J., and Pollak, M. Circulating IGF-I. New perspectives for a new century. Trends Endocrinol. Metab., *10:* 136–141, 1999.

10. Rajah, R., Valentinis, B., and 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., *272:* 12181–12188, 1997.

11. Kelley, K. M., Oh, Y., Gargosky, S. E., Gucev, Z., Matsumoto, T., Hwa, V., Ng, L., Simpson, D. M., and Rosenfeld, R. G. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int. J. Biochem. Cell Biol., *28:* 619–637, 1996.

12. Mantzoros, C. S., Tzonou, A., Signorello, L. B., Stampfer, M., Trichopoulos, D., and Adami, H. O. Insulin-like growth factor 1 in relation to prostate cancer and benign prostatic hyperplasia. Br. J. Cancer, *76:* 1115–1118, 1997.

13. Wolk, A., Mantzoros, C. S., Andersson, S. O., Bergstrom, R., Signorello, L. B., Lagiou, P., Adami, H. O., and Trichopoulos, D. Insulin-like growth factor 1 and prostate cancer risk: a population- based, case-control study. J. Natl. Cancer Inst. (Bethesda), *90:* 911–915, 1998.

14. 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 prostate cancer risk: a prospective study. Science (Wash. DC), *279:* 563–566, 1998.

15. Harman, S. M., Metter, E. J., Blackman, M. R., Landis, P. K., and Carter, H. B. 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., *85:* 4258–4265, 2000.

16. Signorello, L. B., Brismar, K., Bergstrom, R., Andersson, S. O., Wolk, A., Trichopoulos, D., and Adami, H. O. Insulin-like growth factor-binding protein-1 and prostate cancer. J. Natl. Cancer Inst. (Bethesda), *91:* 1965–1967, 1999.

17. Hsing, A. W., Tsao, L., and Devesa, S. S. International trends and patterns of prostate cancer incidence and mortality. Int. J. Cancer, *85:* 60–67, 2000.

18. Hsing, A. W., Gao, Y-T., Wu, G., Wang, X., Deng, J., Chen, Y-L., Sesterhenn, I. A., Mostofi, F. K., Benichou, J., and Chang, C. Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res., *60:* 5111–5116, 2000.

19. Hsing, A. W., Deng, J., Stanczyk, F. Z., Benichou, J., Sesterhenn, I. A., Mostofi, F. K., Xie, T., and Gao, Y-T. Body size and prostate cancer: a population-based case-control study in Shanghai, China. Cancer Epidemiol. Biomark. Prev., *9:* 1335–1341, 2000.

20. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research. I. The Analysis of Case-Control Studies, IARC Scientific Publ. No. 32, pp. 5–338. Lyon, France: IARC, 1980.

21. Guess, H. A., Friedman, G. D., Sadler, M. C., Stanczyk, F. Z., Vogelman, J. H., Imperato-McGinley, J., Lobo, R. A., and Orentreich, N. 5α-Reductase activity and prostate cancer: a case-control study using stored sera. Cancer Epidemiol. Biomark. Prev., *6:* 21–24, 1997.

22. Skjaerbaek, C., Frystyk, J., Grofte, T., Flyvbjerg, A., Lewitt, M. S., Baxter, R. C., and Orskov, H. Serum free insulin-like growth factor-I is dose-dependently decreased by methylprednisolone and related to body weight changes in rats. Growth Horm. IGF Res., *9:* 74–80, 1999.

23. Vendola, K., Zhou, J., Wang, J., and Bondy, A. Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. Hum. Reprod., *14:* 2328–2332, 1999.

24. Nickerson, T., Miyake, H., Gleave, M. E., and Pollak, M. Castration-induced apoptosis of androgen-dependent Shionogi carcinoma is associated with increased expression of genes encoding insulin-like growth factor-binding proteins. Cancer Res., *59:* 3392–3395, 1999.

25. Nickerson, T., and Pollak, M. Bicalutamide (Casodex)-induced prostate regression involves increased expression of genes encoding insulin-like growth factor binding proteins. Urology, *54:* 1120–1125, 1999.

26. Juul, A., Bang, P., Hertel, N. T., Main, K., Dalgaard, P., Jorgensen, K., Muller, J., Hall, K., and Skakkebaek, N. E. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J. Clin. Endocrinol. Metab., *78:* 744–752, 1994.

27. Cohen, P., Peehl, D. M., Graves, H. C., and Rosenfeld, R. G. Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. J. Endocrinol., *142:* 407–415, 1994.

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