Serum Insulin-like Growth Factor (IGF)-I and IGF-Binding Protein-3 Concentrations and Prostate Cancer Risk: Results from the European Prospective Investigation into Cancer and Nutrition

Naomi E. Allen,[1] Timothy J. Key,[1] Paul N. Appleby,[1] Ruth C. Travis,[1] Andrew W. Roddam,[1] Sabina Rinaldi,[2] Lars Egevad,[2] Sabine Rohrmann,[3] Jakob Linseisen,[3] Tobias Pischon,[4] Heiner Boeing,[4] Nina Føns Johnsen,[5] Anne Tjønneland,[5] Henning Grønbæk,[6] Kim Overvad,[7] Lambartus Kiemeney,[8] H. Bas Bueno-de-Mesquita,[9] Sheila Bingham,[10] Kay Tee Khaw,[11] Rosario Tumino,[12] Franco Berrino,[13] Amalia Mattiello,[14] Carlotta Sacerdote,[15] Domenico Palli,[16] José Ramón Quirós,[17] Eva Ardanaz,[18] Carmen Navarro,[19] Nerea Larrañaga,[20] Carlos Gonzalez,[21] Maria-José Sanchez,[22] Antonia Trichopoulou,[23] Cryssoula Travezea,[23] Dimitrios Trichopoulos,[24] Mazda Jenab,[2] Pietro Ferrari,[2] Elio Riboli,[25] and Rudolf Kaaks[3]

[1]Cancer Research UK Epidemiology Unit, University of Oxford, Oxford, United Kingdom; [2]IARC, Lyon, France; [3]Division of Cancer Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany; [4]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; [5]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark; [6]Medical Department V, Aarhus University Hospital, Aarhus, Denmark; [7]Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; [8]Departments of Epidemiology and Urology, University Medical Centre Nijmegen, Nijmegen, the Netherlands; [9]Centre for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, the Netherlands; [10]Medical Research Council Dunn Human Nutrition Unit and Medical Research Council Centre for Nutritional Epidemiology in Cancer Prevention and Survival, Department of Public Health and Primary Care, University of Cambridge; [11]Clinical Gerontology Unit, University of Cambridge, School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, United Kingdom; [12]Cancer Registry, Azienda Ospedaliera ''Civile M.P. Arezzo,'' Ragusa, Italy; [13]Epidemiology Unit, Istituto Nazionale dei Tumori, Milan, Italy; [14]Dipartimento di Medicina Clinica e Sperimentale, Università di Napoli Federico II, Naples, Italy; [15]Department of Biomedical Science and Human Oncology, Center for Cancer Prevention-Piemonte, Torino, Italy; [16]Molecular and Nutritional Epidemiology Unit, Center for Study and Prevention of Cancer, Scientific Institute of Tuscany, Florence, Italy; [17]Public Health and Health Planning Directorate, Asturias, Spain; [18]Public Health Institute of Navarra, Pamplona, Spain; [19]Department of Epidemiology, Murcia Health Council, Murcia, Spain; [20]Public Health Department of Gipuzkoa, Basque Government, San Sebastian, Spain; [21]Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain; [22]Escuela Andaluza de Salud Publica, Granada, Spain; [23]Department of Hygiene and Epidemiology, University of Athens Medical School; [24]Hellenic Health Foundation, Athens, Greece; and [25]Department of Epidemiology and Public Health, Imperial College, London, United Kingdom

Abstract

Background: **Some studies suggest that elevated serum insulin-like growth factor (IGF)-I concentrations are associated with an increased risk of prostate cancer and, in particular, with an increased risk of advanced-stage prostate cancer.**

Methods: **We analyzed the association between prediagnostic serum concentrations of IGF-I and IGF-binding protein-3 (IGFBP-3) and prostate cancer risk in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition. This study includes 630 incident prostate cancer cases and 630 matched control subjects. Odds ratios and their 95% confidence intervals (95% CI) were calculated for prostate cancer risk associated with increasing IGF-I and IGFBP-3 concentrations using conditional logistic regression.**

Results: **The risk of total prostate cancer in the highest versus the lowest third of serum peptide concentration was 1.35 (95% CI, 0.99-1.82; P_{trend} = 0.08) for IGF-I, 1.39 (95% CI, 1.02-1.89; P_{trend} = 0.12) for the IGF-I residuals after adjusting for IGFBP-3, 1.22 (95% CI, 0.92-1.64; P_{trend} = 0.38) for IGFBP-3, and 1.01 (95% CI, 0.74-1.37; P_{trend} = 0.75) for the IGFBP-3 residuals after adjusting for IGF-I. There was no significant difference in the association of peptide hormones and prostate cancer by stage of disease, although the association of serum IGF-I concentration with risk was slightly stronger for advanced-stage disease; the odds ratio for the highest versus the lowest third was 1.65 (95% CI, 0.88-3.08; P_{trend} = 0.21) for IGF-I and 1.76 (95% CI, 0.92-3.40; P_{trend} = 0.11) for IGF-I adjusted for IGFBP-3.**

Conclusions: **In this large nested case-control study, serum IGF-I concentration is not strongly associated with prostate cancer risk, although the results are compatible with a small increase in risk, particularly for advanced-stage disease; no association for IGFBP-3 was observed.** (Cancer Epidemiol Biomarkers Prev 2007;16(6):1121–7)

Received 12/18/06; revised 2/27/07; accepted 3/14/07.

Grant support: U.S. National Cancer Institute (contract no. U01 CA098216-01); Europe Against Cancer Programme of the European Commission (SANCO); Cancer Research UK; German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; ISCIII Red de Centros RCESP C03/09, Spain; Spanish Regional Governments of Andalusia, Basque Country, Murcia, Murcia and Navarra; Medical Research Council, United Kingdom; Stroke Association, United Kingdom; British Heart Foundation; Department of Health, United Kingdom; Food Standards Agency, United Kingdom; Wellcome Trust, United Kingdom; Greek Ministry of Health; Greek Ministry of Education; Italian Association for Research on Cancer; Italian National Research Council; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch Zorg Onderzoek Nederland; and World Cancer Research Fund.

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 1734 solely to indicate this fact.

Requests for reprints: Naomi E. Allen, Cancer Research UK Epidemiology Unit, University of Oxford, Oxford OX3 7LF, United Kingdom. Phone: 44-1865-289600; Fax: 44-1865-289610. E-mail: naomi.allen@ceu.ox.ac.uk

doi:10.1158/1055-9965.EPI-06-1062

Introduction

Prostate cancer is the most common cancer in men in many Western countries and is the second most common cause of cancer death (1). Despite the major effect of prostate cancer on public health, little is known about the etiology of the disease and the only established risk factors are age, ethnicity, and a family history of the disease (2).

Insulin-like growth factor (IGF)-I is a peptide growth factor that is primarily produced in the liver along with IGF-binding protein-3 (IGFBP-3). IGF-I is involved in multiple cellular responses related to growth, including synthesis of DNA, RNA, and cellular proteins (3). IGF-I stimulates cell proliferation and inhibits cell death in most tissue types (4), including normal and malignant prostate cancer cells (5, 6). The bioavailability of circulating IGF-I is complex; six IGFBPs have been identified, the most abundant of which is IGFBP-3. This protein binds approximately 75% to 90% of circulating IGF-I, together with an acid-labile subunit, and is therefore thought to be an important determinant of the amount of IGF-I available to enter target tissues and reach IGF-I cellular receptors (3). In addition to its effects on IGF-I bioactivity, IGFBP-3 has been shown to independently influence cell growth and survival and to promote apoptosis in prostate cancer cell lines (7).

There is considerable between-person variation in the circulating concentrations of IGF-I and their binding proteins that are determined by genetic and environmental factors (3, 8). This variation may be important because evidence from a meta-analysis of six epidemiologic studies suggests that elevated levels of serum IGF-I, expressed either as absolute concentrations or relative to levels of IGFBP-3, may be associated with an increased risk of prostate cancer (9), although findings from more recent prospective studies (10-17) and studies not included in the meta-analysis (18-20) are inconsistent. In particular, there is growing evidence that serum IGF-I levels are more strongly associated with advanced-stage disease than localized disease (12, 17, 18), suggesting that IGF-I may be associated with tumor aggressiveness. Data on the association of circulating IGFBP-3 levels with prostate cancer risk are conflicting; some prospective studies have found elevated IGFBP-3 concentrations to be associated with an increase in prostate cancer risk (13, 15, 16, 21), whereas other studies have reported null (19, 22) or nonsignificant reductions in risk with IGFBP-3 (10, 12, 14, 17, 23).

The aim of the present study is to investigate associations between prediagnostic serum concentrations of IGF-I and IGFBP-3 and prostate cancer risk among 630 men with incident prostate cancer and 630 matched controls in the European Prospective Investigation into Cancer and Nutrition (EPIC) and to evaluate these associations by stage and grade of disease.

Materials and Methods

Study Cohort. EPIC recruitment procedures and collection of questionnaire data, anthropometric measurements, and blood samples have been described in detail elsewhere (24). In brief, extensive standardized questionnaire data on dietary and nondietary variables were collected between 1991 and 2001 from about 370,000 women and 150,000 men across Europe, and a blood sample was collected from ~400,000 of these individuals (37% of whom were male). The present study includes prostate cancer cases occurring after blood collection and their matched control subjects from 7 of the 10 participating countries: Denmark, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom. France and Norway were not included in the present study because these cohorts only included women; Sweden was not included because prostate cancer cases in this country had been included in a previous study (21).

A 30 mL blood sample was collected according to a standardized protocol. Filled tubes were kept at 5°C to 10°C, protected from light, and transferred to a local laboratory for further processing and aliquoting, with the exception of subjects recruited through the Oxford center. Here, blood samples were collected throughout the United Kingdom and transported to the laboratory in Norfolk by mail at ambient temperature. Blood fractions (serum, plasma, red cells, and buffy coat for DNA extraction) were aliquoted into 0.5 mL straws, which were then heat sealed at both ends and stored in liquid nitrogen tanks at −196°C, except in Denmark where samples were stored in 1 mL tubes in nitrogen vapor at −150°C.

Follow-up for Cancer Incidence and Vital Status. In Denmark, Italy, the Netherlands, Spain, and the United Kingdom, incident cancer cases were identified through record linkage with regional cancer registries. In Germany and Greece, follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next of kin. Data on vital status in most EPIC study centers were collected from mortality registries at the regional or national level, in combination with data collected by active follow-up (Greece). For each EPIC study center, closure dates of the study period were defined as the latest dates of complete follow-up for both cancer incidence and vital status (dates varied between centers, from June 1999 to January 2003).

Selection of Case and Control Subjects. Case subjects were selected among men who developed prostate cancer after their recruitment into the EPIC study and before the end of the study period (defined for each study center by the latest end-date of follow-up). Men who had prevalent cancer (except nonmelanoma skin cancer) at the time of blood collection and who had missing information on the date of blood collection were excluded from the study. This analysis includes 630 case patients and 630 matched control subjects, who are also included in a collaborative analysis of genetic and hormonal factors on prostate cancer risk (25) and for whom IGF peptide measurements were available; results from analyses of steroid sex hormones in relation to prostate cancer risk are reported elsewhere.[26] These 630 cases included 79 men recruited in Denmark, 60 in Italy, 186 in Germany, 9 in Greece, 25 in the Netherlands, 93 in Spain, and 178 in the United Kingdom. For each case, one control subject was chosen at random among appropriate risk sets consisting of all cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. An incidence density sampling protocol for control selection was used, such that control subjects could include subjects who became a case later in time, whereas each control could also be sampled more than once. Matching criteria included study center, age at recruitment (±6 months), time of the day of blood collection (±1 h), and time between blood collection and last consumption of foods or drinks (<3, 3-6, >6 h). All participants gave written consent for future analyses of their blood samples, and the study was approved by the local ethics committees in the participating countries and the ethical review board of the IARC.

Data on stage and grade of disease were collected from each center, where possible. Four hundred and forty-one cases (70%) had information on tumor-node-metastasis staging, or

[26] R.C. Travis, submitted for publication.

equivalent; of these, 303 (69%) were classified as localized (tumor-node-metastasis staging score of T_1-T_2 and N_0 and M_0, or equivalent) and 138 (31%) were classified as advanced (T_3/T_4 and/or N_1:N_3 and/or M_1, or equivalent). However, for most cases, we were unable to determine whether these data were derived from clinical or pathologic records. Data on Gleason score (or equivalent) were available for 455 cases (72%); of these, 309 (68%) were classified as low grade (Gleason score <7, or equivalent) and 146 (32%) were classified as high grade (Gleason score 7+, or equivalent). For cases where information on both stage and grade were available, 76% of localized cases were also classified as low grade and 57% of advanced cases were also classified as high grade.

Laboratory Assays. Serum IGF-I and IGFBP-3 concentrations were measured by ELISAs from Diagnostic Systems Laboratories. IGF-I assays included an acid-ethanol precipitation of IGF-I–binding proteins to avoid interference of IGFBPs with the IGF-I assay. Cases were analyzed in the same batch as their matched control, and each batch contained three additional sera inserted for laboratory quality control. All assays were done at IARC by laboratory personnel who were blinded as to the case-control status of the blood samples. Mean intrabatch coefficients of variation were estimated to be 3.0% (at 5.72 nmol/L) for IGF-I and 5.3% (at 141 nmol/L) for IGFBP-3. Interbatch coefficients of variations were 13.7% for IGF-I and 9.4% for IGFBP-3. The lowest limits of detection, in terms of the lowest standard of the standard curve, were 1.18 nmol/L for IGF-I and 8.75 nmol/L for IGFBP-3.

Statistical Analyses. Differences in baseline characteristics between case and control subjects were compared using the paired *t* test for continuous variables and the χ^2 test for categorical variables. The correlations between the natural logarithm of hormone concentration and continuous variables (i.e., age, height, and weight) were assessed among the controls using Pearson's partial correlation coefficients, adjusted for age at blood collection, laboratory batch, and center. For categorical variables, analysis of covariance was used to investigate geometric mean differences in the hormone concentrations among the controls by the following: country (seven categories), smoking (never, past, current), alcohol intake (<8, 8-15, 16-39, 40+ g/d), body mass index (BMI; kg/m^2; in quartiles), physical activity (index of combined recreational, household, and occupational physical activity: inactive, moderately inactive, moderately active/active), marital status (married/cohabiting, not married/cohabiting), and educational level (less than secondary school, secondary school, university degree or higher).

Relative risks [odds ratios (OR)] for prostate cancer in relation to serum IGF-I and IGFBP-3 concentrations were estimated by conditional logistic regression models. Peptide hormone concentrations were categorized into thirds with tertile cutpoints based on the (untransformed) distribution of the hormones among the control subjects. Ninety-five percent confidence intervals (95% CI) were computed using the SEs of the pertinent regression coefficients, and linear trends were assessed using the log-transformed continuous variable. The effects of potential confounders, other than matching criteria, which are controlled for by design, were examined by including additional regression terms in the logistic regression models, both individually and combined. Potential confounders included smoking, alcohol, physical activity, education, and marital status. None of these variables substantially altered the risk estimates and were not included in the final models. However, results are shown for the association of prostate cancer risk with IGF-I and IGFBP-3, after adjustment for each other, using the residuals of the regression of log-transformed IGF-I on IGFBP-3 levels (and vice versa), which were then categorized into thirds.

The association between IGF-I and IGFBP-3 and prostate cancer risk was assessed separately by stage and histologic grade; χ^2 tests were used to examine heterogeneity of prostate cancer risk associated with a linear increase in peptide hormone concentration between these groups. We did not conduct analyses among the different combinations of stage and grade owing to the small numbers of cases in these subgroups. χ^2 tests were used to examine heterogeneity of prostate cancer risk by age at blood collection (<60, 60+ years), age at diagnosis (<60, 60+ years), BMI at recruitment (<26.6, 26.6+ kg/m^2), lag time between blood collection and diagnosis (<2, 2+ years), and country of recruitment (seven countries). All *P* values presented are two tailed and those below 0.05 were considered statistically significant. Analyses were done using STATA version 9 (STATA Corp.).

Results

This study includes 630 prostate cancer case patients diagnosed from recruitment until the end of follow-up and 630 matched control subjects. The median age at blood collection was 61 years (range, 43-76 years), the median time between blood collection and cancer diagnosis was 3.4 years (range, 0.1-8.5 years), and the median age at cancer diagnosis was 65 years (range, 47-82 years).

Basic characteristics of the study participants are shown in Table 1. There were no significant differences between cases and controls according to height, BMI, smoking, alcohol intake, physical activity, marital status, or education, although cases were, on average, 1.3 kg lighter than controls ($P = 0.05$). Case patients had slightly higher geometric mean serum concentrations of IGF-I and IGFBP-3 than their matched

Table 1. Characteristics of prostate cancer patients and control subjects in EPIC

Characteristic	Cases	Controls	*P*
N	630	630	
Anthropometry: mean (SD)			
Height (cm)	172.2 (6.7)	172.5 (7.0)	0.43
Weight (kg)	79.3 (11.2)	80.6 (12.2)	0.05
BMI (kg/m^2)	26.7 (3.5)	27.1 (3.6)	0.09
Smoking, *n* (%)*			
Never	197 (31.5)	168 (27.0)	
Former	282 (45.0)	281 (45.2)	
Current	147 (23.5)	173 (27.8)	0.11
Alcohol consumption, *n* (%)*			
<8 g/d	223 (35.5)	223 (35.5)	
8-15 g/d	121 (19.3)	134 (21.3)	
16-39 g/d	169 (26.9)	155 (24.7)	
≥40 g/d	115 (18.3)	116 (18.5)	0.74
Physical activity, *n* (%)*			
Inactive	124 (20.0)	107 (17.3)	
Moderately inactive	218 (35.1)	199 (32.1)	
Moderately active/active	279 (44.9)	314 (50.6)	0.12
Marital status, *n* (%)*			
Married or cohabiting	391 (87.5)	396 (89.2)	
Not married or cohabiting	56 (12.5)	48 (10.8)	0.43
Education, *n* (%)*			
Less than secondary school	232 (38.7)	231 (39.0)	
Secondary school	215 (35.8)	224 (37.8)	
University degree	153 (25.5)	137 (23.1)	0.60
Serum peptides (nmol/L†):			
Geometric mean (95% CI)			
IGF-I	22.0 (21.3-22.7)	21.2 (20.5-21.9)	0.08
IGFBP-3	129.9 (127.7-132.2)	128.6 (126.4-130.9)	0.38

*Denotes that the factor is unknown for some men—these men are excluded from the calculations of percentages and *P* values.
†To convert to ng/mL, divide by 0.1307 for IGF-I and 0.035 for IGFBP-3.

Table 2. OR (95% CI) of overall prostate cancer by thirds of serum IGF-I and IGFBP-3 concentrations in EPIC

Peptide	No. cases/controls	OR (95% CI)
IGF-I		
Low	169/212	1.00
Medium	248/208	1.54 (1.16-2.06)
High	213/210	1.35 (0.99-1.82)
P_{trend}*		0.08
Residuals of IGF-I after adjusting for IGFBP-3		
Low	169/212	1.00
Medium	248/208	1.29 (0.96-1.73)
High	213/210	1.39 (1.02-1.89)
P_{trend}*		0.12
IGFBP-3		
Low	193/212	1.00
Medium	211/210	1.12 (0.85-1.48)
High	226/208	1.22 (0.92-1.64)
P_{trend}*		0.38
Residuals of IGFBP-3 after adjusting for IGF-I		
Low	211/212	1.00
Medium	211/210	1.01 (0.76-1.35)
High	208/208	1.01 (0.74-1.37)
P_{trend}*		0.75

NOTE: Tertile cutpoints are 17.8 and 26.1 nmol/L for IGF-I and 119.5 and 141.7 nmol/L for IGFBP-3.
*P_{trend} based on the continuous (log transformed) variable.

controls, but the differences were not statistically significant (Table 1).

Among the control subjects, serum IGF-I concentrations were positively correlated with IGFBP-3 concentrations (r = 0.67; $P < 0.001$) and both serum IGF-I and IGFBP-3 concentrations were inversely correlated with age ($r = -0.16$ for IGF-I and $r = -0.15$ for IGFBP-3; both $P < 0.001$). Serum IGF-I concentrations were positively correlated with height (r = 0.12; P = 0.004), but height was not associated with IGFBP-3 concentrations. Serum IGF-I was associated with BMI in a nonlinear manner, with the highest IGF-I concentration in the second quartile of BMI (24.5-26.6 kg/m^2; $P_{heterogeneity}$ = 0.05 across quartiles of BMI); there was no association between BMI and IGFBP-3 concentrations (data not shown). Alcohol intake was not associated with IGF-I concentration but was positively associated with IGFBP-3; the geometric mean concentration was 9% higher in those consuming 40+ g/d alcohol compared with those consuming <8 g/d ($P_{heterogeneity}$ = 0.001 across four categories). Smoking, physical activity, marital status, education level, and country of recruitment were not significantly associated with serum IGF-I or IGFBP-3 concentrations (data not shown).

Conditional logistic regression analysis showed a weak positive association between IGF-I concentration and overall prostate cancer risk; the ORs for the middle and highest third versus the lowest third were 1.54 (95% CI, 1.16-2.06) and 1.35 (95% CI, 0.99-1.82), respectively (P_{trend} = 0.08; Table 2). The residuals of IGF-I, adjusted for IGFBP-3 through a linear regression model, showed a very similar association to that of IGF-I alone; the OR for the highest versus the lowest third was 1.39 (95% CI, 1.02-1.89; P_{trend} = 0.12). IGFBP-3 concentration was also weakly positively associated with prostate cancer, but this result was not statistically significant, with the OR for the highest versus the lowest third being 1.22 (95% CI, 0.92-1.64; P_{trend} = 0.38). The residuals of IGFBP-3, adjusted for IGF-I, showed no association with prostate cancer risk. Adjustment for smoking, alcohol intake, marital status, physical activity, or education, either individually or in combination, made no appreciable difference to the risk estimates or 95% CI for either IGF-I or IGFBP-3 (data not shown).

The associations between IGF-I and IGFBP-3 concentrations and risk of prostate cancer for localized and advanced disease are shown in Table 3. There was some suggestion that IGF-I and IGF-I residual concentration was more strongly associated with risk of advanced disease than localized disease; in advanced cases, the OR for the highest versus the lowest third was 1.65 (95% CI, 0.88-3.08; P_{trend} = 0.21) for IGF-I and 1.76 (95% CI, 0.92-3.40; P_{trend} = 0.11) for the IGF-I residuals. However, these associations were not statistically significant and there was no significant heterogeneity in the linear trends between localized and advanced disease ($P_{heterogeneity}$ = 0.53 for IGF-I and 0.28 for the IGF-I residuals). For IGFBP-3, no association was seen for either localized or advanced disease, although there was a weak negative association for the residuals of IGFBP-3 with risk of advanced-stage disease, with an OR for the highest versus the lowest third of 0.68 (95% CI, 0.35-1.33; P_{trend} = 0.39; Table 3).

Table 3. OR (95% CI) of localized and advanced prostate cancer by thirds of serum IGF-I and IGFBP-3 concentrations in EPIC

	Localized*		Advanced†		$P_{heterogeneity}$
	No. cases/controls	OR (95% CI)	No. cases/controls	OR (95% CI)	
IGF-I					
Low	87/102	1.00	37/49	1.00	
Medium	122/98	1.48 (0.98-2.23)	51/47	1.47 (0.81-2.66)	
High	94/103	1.11 (0.71-1.73)	50/42	1.65 (0.88-3.08)	
P_{trend}‡		0.48		0.21	0.53
Residuals of IGF-I after adjusting for IGFBP-3					
Low	87/102	1.00	37/49	1.00	
Medium	122/98	1.25 (0.81-1.93)	51/47	1.21 (0.68-2.16)	
High	94/103	1.30 (0.84-2.01)	50/42	1.76 (0.92-3.40)	
P_{trend}‡		0.64		0.11	0.26
IGFBP-3					
Low	99/101	1.00	42/50	1.00	
Medium	102/108	0.98 (0.65-1.47)	44/32	1.52 (0.86-2.70)	
High	102/94	1.13 (0.73-1.76)	52/56	1.10 (0.63-1.93)	
P_{trend}‡		0.62		0.86	0.88
Residuals of IGFBP-3 after adjusting for IGF-I					
Low	111/115	1.00	45/39	1.00	
Medium	94/93	1.06 (0.70-1.58)	46/44	0.84 (0.44-1.62)	
High	98/95	1.09 (0.71-1.66)	47/55	0.68 (0.35-1.33)	
P_{trend}‡		0.93		0.39	0.44

*Based on 303 case-control pairs where the case was diagnosed with localized disease (tumor-node-metastasis staging score of T_1-T_2 and N_0 and M_0, or equivalent).
† Based on 138 case-control pairs where the case was diagnosed with advanced disease (T_3/T_4 and/or N_1:N_3 and/or M_1, or equivalent).
‡ P_{trend} based on the continuous (log transformed) variable.

Table 4. Association of serum IGF-I and IGFBP-3 concentration with risk of low-grade and high-grade prostate cancer in EPIC

	Low grade*		High grade†		$P_{heterogeneity}$
	No. cases/controls	OR (95% CI)	No. cases/controls	OR (95% CI)	
IGF-I					
Low	79/101	1.00	47/43	1.00	
Medium	113/97	1.58 (1.03-2.42)	56/53	0.96 (0.56-1.65)	
High	117/111	1.45 (0.94-2.22)	43/50	0.77 (0.42-1.41)	
P_{trend} ‡		0.06		0.34	0.06
Residuals of IGF-I adjusted for IGFBP-3					
Low	79/101	1.00	47/43	1.00	
Medium	113/97	1.27 (0.84-1.93)	56/53	1.11 (0.59-2.08)	
High	117/111	1.27 (0.82-1.97)	43/50	1.31 (0.71-2.41)	
P_{trend} ‡		0.14		0.69	0.25
IGFBP-3					
Low	86/101	1.00	54/47	1.00	
Medium	117/111	1.27 (0.85-1.89)	40/46	0.79 (0.46-1.34)	
High	106/97	1.33 (0.87-2.03)	52/53	0.86 (0.49-1.53)	
P_{trend} ‡		0.24		0.29	0.12
Residuals of IGBP-3 adjusted for IGF-I					
Low	106/111	1.00	50/52	1.00	
Medium	111/103	1.14 (0.76-1.69)	47/47	1.05 (0.58-1.92)	
High	92/95	1.02 (0.66-1.57)	49/47	1.11 (0.59-2.07)	
P_{trend} ‡		0.95		0.55	0.65

*Based on 309 case-control pairs where the case was diagnosed with low-grade disease.
† Based on 146 case-control pairs where the case was diagnosed with high-grade disease.
‡ P_{trend} based on the continuous (log transformed) variable.

Table 4 shows the associations between IGF-I and IGFBP-3 concentrations and risk of prostate cancer for low-grade and high-grade disease, separately. Increasing IGF-I concentration was weakly positively associated with low-grade disease (OR for the highest versus the lowest third, 1.45; 95% CI, 0.94-2.22; P_{trend} = 0.06) and was inversely related with high-grade disease (OR for the highest versus the lowest third, 0.77; 95% CI, 0.42-1.41; P_{trend} = 0.34), although there was no significant heterogeneity in the linear trends between the two groups ($P_{heterogeneity}$ = 0.06). Neither the IGF-I residuals nor IGFBP-3 was associated with low- or high-grade disease, although the pattern for IGFBP-3 was similar to that seen for IGF-I, being weakly positively associated with low-grade disease and weakly inversely associated with high-grade disease ($P_{heterogeneity}$ = 0.12; Table 4).

To investigate whether the presence of imminent clinical disease could have had an effect on the association between IGF-I concentrations and prostate cancer risk, the analyses were conducted separately in men diagnosed less than 2 years (164 case-control pairs) and 2 or more years since recruitment (466 case-control pairs). The association of serum IGF-I with cancer risk was stronger among men diagnosed soon after recruitment (the OR for the highest versus the lowest third was 2.14; 95% CI, 1.17-3.91; P_{trend} = 0.14) compared with men diagnosed at least 2 years after recruitment (the OR for the highest versus the lowest third was 1.14; 95% CI, 0.80-1.62; P_{trend} = 0.25). A similar association was observed for the IGF-I residuals, but neither of the tests of heterogeneity of risk was statistically significant ($P_{heterogeneity}$ = 0.49 for IGF-I and 0.49 for the IGF-I residuals). There was no difference in the association of IGFBP-3 or the IGFBP-3 residuals and prostate cancer risk according to time between recruitment and diagnosis (data not shown).

We next examined whether the associations of IGF-I and IGFBP-3 concentrations with prostate cancer risk differed by age and BMI and between the participating countries. There was no evidence of heterogeneity of the association of IGF-I or IGFBP-3 with prostate cancer risk between men aged less than or greater than 60 years at blood collection ($P_{heterogeneity}$ = 0.79 and 0.79 for IGF-I and IGFBP-3, respectively) or between men aged less than or greater than 60 years at diagnosis ($P_{heterogeneity}$ = 0.75 and 0.29 for IGF-I and IGFBP-3, respectively). Similarly, there was no significant heterogeneity in the association of IGF-I or IGFBP-3 with prostate cancer risk between men with a BMI less than or greater than the median of 26.6 kg/m^2 ($P_{heterogeneity}$ = 0.69 and 0.57 for IGF-I and IGFBP-3, respectively) or between the seven participating countries ($P_{heterogeneity}$ = 0.35 and 0.39 for IGF-I and IGFBP-3, respectively).

Discussion

This case-control study nested within EPIC is the largest of its kind to examine the association between serum IGF-I and IGFBP-3 concentrations and subsequent prostate cancer risk. Serum IGF-I was weakly positively associated with overall prostate cancer risk, and which was more evident for advanced-stage disease, but these associations were not statistically significant and might therefore be chance findings. However, our results are consistent with data from most of the larger previous prospective studies that have found weak-to-moderate positive associations (12, 13, 16, 17, 21, 22). A few prospective studies have reported null associations between serum IGF-I concentration and total prostate cancer risk, but with the exception of the Melbourne Collaborative Cohort Study (15), these were based on small numbers of cases (i.e., <200 cases; refs. 10, 11, 14, 19, 20).

In our study, serum IGFBP-3 concentration was not strongly associated with overall prostate cancer risk. Some previous prospective studies have found elevated IGFBP-3 concentrations to be associated with an increase in total prostate cancer risk (13, 15, 16, 21), whereas other studies have reported null (19, 22) or nonsignificant reductions in risk (10, 12, 14, 17, 23). The reason for such heterogeneity between studies of relationships between serum IGFBP-3 levels and prostate cancer risk remains unclear, although it could be partly due to different specificities of the immunoassays used for IGFBP-3 measurement, as IGFBP-3 circulates in a variety of intact and proteolytically cleaved forms (26, 27). Indeed, it has been speculated that, whereas the total concentration of intact and cleaved forms combined could be higher among subjects

developing cancer (e.g., because of higher total IGFBP-3 synthesis in the liver), levels of intact IGFBP-3 could be reduced due to more proteolytic cleavage in these same subjects. Further studies are needed on the effect of different assays on absolute levels of IGFBP-3 subforms and their corresponding effects on subsequent cancer risk.

It has been suggested that some of the inconsistencies found between studies in the relationship of IGF-I concentration with prostate cancer risk may, in part, be due to differences in the case-mix of patients, with recent studies having a higher proportion of early-stage disease due to more widespread prostate-specific antigen testing. In particular, the association of IGF-I concentration and prostate cancer risk found after a relatively short follow-up period in the Physicians Health Study (22) was not confirmed after longer follow-up, although there remained a strong positive association between IGF-I concentration and advanced-stage disease (18). Similarly, other studies conducted among men who have undergone prostate-specific antigen testing have found weak-to-moderate positive associations between IGF-I concentrations and total prostate cancer risk (12, 13, 17), and most have found stronger associations for advanced-stage disease (12, 17), suggesting that IGF-I may be associated with tumor aggressiveness. Although nonsignificant, our results (the risk in the highest versus the lowest third of IGF-I was 1.35 for overall prostate cancer and 1.65 for advanced-stage disease) are nevertheless compatible with the hypothesis that serum IGF-I concentration may be associated with a small increase in prostate cancer risk, and which may be stronger for advanced-stage disease. However, it is difficult to determine whether elevated circulating IGF-I levels are a cause of clinically relevant tumors or whether they are a consequence of the presence of prostate cancer at a preclinical stage. In this study, the association of prostate cancer risk with IGF-I concentration was slightly stronger (but not statistically significantly so) among men diagnosed less than 2 years after recruitment compared with men diagnosed after 2 years. Overall, the average time between blood collection and diagnosis in EPIC is still relatively short (mean of 3.4 years), and whether circulating IGF-I concentration is a true precursor of disease remains to be determined with longer follow-up.

We found little evidence that the association of serum IGF-I and IGFBP-3 concentrations with risk differed according to the grade of disease, which is consistent with previous data (17, 18). However, we are aware that there is considerable measurement error in the determination of Gleason scores, with a high proportion of cases likely to be undergraded (28, 29). Indeed, in the present study, 43% of advanced cases were classified as low grade, which is consistent with other data from Europe[27] and most likely reflects some degree of undergrading, which will serve to attenuate any association toward the null. Nonetheless, taken together with the results from previous studies, there is no strong evidence to suggest that circulating IGF-I or IGFBP-3 concentrations are specifically related to the degree of differentiation of prostate cancer.

We found no evidence that the association of serum IGF-I concentrations and prostate cancer risk is modified by age at blood collection, which is consistent with one previous study (14), although others have found IGF-I concentrations to be more strongly related to risk among younger men (<60 years at blood collection; ref. 21) or older men (60+ at blood collection; ref. 22). However, the youngest man in our study was aged 51 at recruitment, and so we cannot exclude the possibility that circulating IGF-I levels during young adulthood or adolescence, rather than during middle age, are more important predictors of subsequent prostate cancer risk.

A limitation of the study is that blood samples were collected at one time point and may not reflect long-term circulating levels. However, other studies have shown that the within-subject reproducibility of serum IGF-I and IGFBP-3 concentrations is relatively high over a period of up to 5 years (30-32), with Spearman rank correlations of 0.87 and 0.73 for IGF-I and IGFBP-3, respectively, over an average of 1.3 years (31). Thus, single measures seem to be representative of usual levels for at least the medium term. Although some intra-individual variation and laboratory measurement errors may have led to attenuation of our relative risk estimates, the high quality control of the assay measurements and the strong association observed between serum IGF-I and age suggest that these overall null results are unlikely to be related to a lack of precision in the measurement of IGF-I and IGFBP-3. Another limitation is that serum IGF-I concentrations reflect the total circulating pool of IGF-I and may not reflect intraprostatic paracrine and autocrine production of IGF-I, whose bioactivity is determined by the local expression of IGFBPs and proteases (4); future work is therefore needed to determine the correlation between serum and tissue concentrations of IGFs.

In conclusion, the findings from this large case-control study nested within EPIC show that serum IGF-I concentration is not strongly associated with prostate cancer risk, although the results are compatible with a small increase in risk, whereas no association for IGFBP-3 was observed.

Acknowledgments

We thank the participants in the EPIC study and Carine Beissy and Bertrand Hemon at IARC for their expertise in data handling.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide. IARC CancerBase No. 5 version 2.0. Lyon: IARC Press; 2004.
2. Wolk A. Diet, lifestyle and risk of prostate cancer. Acta Oncol 2005;44: 277–81.
3. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
4. Pollak M. Insulin-like growth factor physiology and cancer risk. Eur J Cancer 2000;36:1224–8.
5. 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.
6. 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.
7. 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.
8. Harrela M, Koistinen H, Kaprio J, et al. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 1996;98:2612–5.
9. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346–53.
10. Chen C, Lewis SK, Voigt L, Fitzpatrick A, Plymate SR, Weiss NS. Prostate carcinoma incidence in relation to prediagnostic circulating levels of insulin-like growth factor I, insulin-like growth factor binding protein 3, and insulin. Cancer 2005;103:76–84.
11. Janssen JA, Wildhagen MF, Ito K, et al. Circulating free insulin-like growth factor (IGF)-I, total IGF-I, and IGF binding protein-3 levels do not predict the future risk to develop prostate cancer: results of a case-control study involving 201 patients within a population-based screening with a 4-year interval. J Clin Endocrinol Metab 2004;89:4391–6.
12. Meyer F, Galan P, Douville P, et al. A prospective study of the insulin-like growth factor axis in relation with prostate cancer in the SU.VI.MAX trial. Cancer Epidemiol Biomarkers Prev 2005;14:2269–72.
13. Platz EA, Pollak MN, Leitzmann MF, Stampfer MJ, Willett WC, Giovannucci E. Plasma insulin-like growth factor-1 and binding protein-3 and subsequent risk of prostate cancer in the PSA era. Cancer Causes Control 2005;16:255–62.
14. Woodson K, Tangrea JA, Pollak M, et al. Serum insulin-like growth factor I: tumor marker or etiologic factor? A prospective study of prostate cancer among Finnish men. Cancer Res 2003;63:3991–4.

[27] F. Berrino et al., personal communication.

15. Severi G, Morris HA, Macinnis RJ, et al. Circulating insulin-like growth factor-I and binding protein-3 and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:1137–41.
16. Morris JK, George LM, Wu T, Wald NJ. Insulin-like growth factors and cancer: no role in screening. Evidence from the BUPA study and meta-analysis of prospective epidemiological studies. Br J Cancer 2006;95:112–7.
17. Oliver SE, Gunnell D, Donovan J, et al. Screen-detected prostate cancer and the insulin-like growth factor axis: results of a population-based case-control study. Int J Cancer 2004;108:887–92.
18. Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst 2002;94:1099–106.
19. Lacey JVJ, 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.
20. Schaefer C, riedman GD, Quesenberry CP. IGF-I and prostate cancer. Science 1998;282:199.
21. Stattin P, Bylund A, Rinaldi S, 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.
22. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563–6.
23. 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.
24. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
25. Hunter DJ, Riboli E, Haiman CA, et al. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. Nat Rev Cancer 2005;5:977–85.
26. Kaaks R, Rinaldi S, Lukanova A, Akhmedkhanov A, Zeleniuch-Jacquotte A, Toniolo P. Correspondence re: A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 2001;10:1103–4.
27. Rinaldi S, Kaaks R, Zeleniuch-Jacquotte A, et al. Insulin-like growth factor-I, IGF binding protein-3, and breast cancer in young women: a comparison of risk estimates using different peptide assays. Cancer Epidemiol Biomarkers Prev 2005;14:48–52.
28. Montironi R, Mazzuccheli R, Scarpelli M, Lopez-Beltran A, Fellegara G, Algaba F. Gleason grading of prostate cancer in needle biopsies or radical prostatectomy specimens: contemporary approach, current clinical significance and sources of pathology discrepancies. BJU Int 2005;95:1146–52.
29. Bostwick DG. Gleason grading of prostatic needle biopsies. Correlation with grade in 316 matched prostatectomies. Am J Surg Pathol 1994;18:796–803.
30. Decensi A, Johansson H, Miceli R, et al. Long-term effects of fenretinide, a retinoic acid derivative, on the insulin-like growth factor system in women with early breast cancer. Cancer Epidemiol Biomarkers Prev 2001;10:1047–53.
31. Kaaks R, Toniolo P, Akhmedkhanov A, et al. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst 2000;92:1592–600.
32. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, et al. Prediagnostic levels of C-peptide, IGF-I, IGFBP-1, -2 and -3 and risk of endometrial cancer. Int J Cancer 2004;108:262–8.

Serum Insulin-like Growth Factor (IGF)-I and IGF-Binding Protein-3 Concentrations and Prostate Cancer Risk: Results from the European Prospective Investigation into Cancer and Nutrition

Naomi E. Allen, Timothy J. Key, Paul N. Appleby, et al.

Cancer Epidemiol Biomarkers Prev 2007;16:1121-1127.