Circulating insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) in PSA-detected prostate cancer: the large case control study ProtecT

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Running title: Insulin-like growth factors in PSA-detected prostate cancer

Keywords: case-control study; insulin-like growth factors; insulin-like growth factor binding proteins; prostate cancer

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Word count text: 4,555. **Abstract:** 230.

Figures and tables: 5

ABSTRACT

Circulating insulin-like growth factor-I (IGF-I) has been studied extensively in prostate cancer, but there is still little information about IGFs and IGF binding proteins (IGFBPs) in cancers detected by the prostate-specific antigen (PSA) test. Here we report the findings of a United Kingdom-based case-control study to investigate circulating IGFs and IGFBPs in PSA-detected prostate cancer with regard to their potential associations with different cancer stages or grades. PSA testing was offered to 110,000 men aged 50-69 years from 2002-2009. Participants with an elevated level of PSA (≥ 3.0 ng/ml) underwent prostate biopsy and measurements of blood serum IGF-I, IGF-II, IGFBP-2 and IGFBP-3 obtained at recruitment. We found that serum levels of IGF-II (OR per standard deviation increase: 1.16; 95%CI 1.08,1.24;ptrend<0.001), IGFBP-2 (1.18;1.06,1.31;ptrend<0.01) and IGFBP-3 (1.27;1.19,1.36;ptrend<0.001), but not IGF-I (0.99;0.93,1.04;ptrend=0.62), were associated with PSA-detected prostate cancer. After controlling for IGFBP-3, IGF-II was no longer associated (0.99;0.91,1.08;ptrend=0.62) and IGF-I was inversely associated (0.85;0.79,0.91;ptrend<0.001) with prostate cancer. In addition, no strong associations existed with cancer stage or grade. Overall, these findings suggest potentially important roles for circulating IGF-II, IGFBP-2 and IGFBP-3 in PSA-detected prostate cancer, in support of recent in vitro evidence. While our findings for IGF-I agree with previous results from PSA-screening trials, they contrast with positive associations in routinely-detected disease, suggesting that reducing levels of circulating IGF-I might not prevent the initiation of prostate cancer but might nonetheless prevent its progression.

PRECIS

This large UK-based case control study suggests potentially important associations of circulating IGF-II, IGFBP-2 and IGFBP-3 in prostate cancers that are detected by the PSA test.

INTRODUCTION

Prostate cancer is the commonest male cancer in industrialised countries, but options for primary prevention, particularly by lifestyle interventions, are currently limited.(1) Observations that mortality is several-fold greater in 'western' than Asian countries, and amongst immigrants to high-mortality countries,(2) point to the metabolic consequences of 'westernisation' (high meat, milk and alcohol intake; low exercise; and obesity) as being potentially important in prostate cancer. A common endpoint of westernisation is obesity, insulin resistance and perturbations of the insulin-like growth factor (IGF) system.(3) Nutritionally-regulated IGFs and their binding proteins (IGFBPs), not only play a key role in somatic growth but also activate potentially carcinogenic intracellular signalling networks.(3) The IGF system, therefore, may link westernised diet, obesity and metabolic factors with prostate cancer.(4)

Epidemiological studies have observed positive associations of circulating IGF-I with prostate cancer.(5) Our recent systematic review, however, found that most studies were small (mean=180 cancers), contained a heterogenous admixture of cases (screen-detected, clinically-detected, metastatic and fatal) and the results were highly inconsistent,(6) with effect-sizes being larger for retrospective (pooled OR per standard deviation increase in IGF-I=1.26; 95% CI:1.05,1.52) compared with prospective (1.07; 0.97,1.18) studies. The retrospective studies were largely based on clinically-detected disease, and may be more likely to contain advanced cases, which could lead to an overestimation of associations as a result of reverse causality. Furthermore, detection bias is a concern in studies lacking standardised methods to determine case-control status.(7) For example, if prostate cancer in men with benign prostatic hyperplasia (BPH) is more likely to present symptomatically and hence be diagnosed than in men without BPH, studies based on comparing clinically-identified prostate cancer with unscreened controls could show positive associations of IGF-I with prostate cancer if IGF-I causes BPH, not cancer.(7) Few epidemiological studies investigate components of the IGF-system other than IGF-I or IGFBP-3. However, a genome-wide association study has identified a susceptibility locus for prostate cancer in the region of the *IGF-2* gene,(8) and IGFBP-2 is upregulated

in prostate cancer cell lines,(9) regulates the tumour suppressor gene PTEN,(10) increases in progressing prostate cancer,(11, 12) and falls after prostatectomy.(13)

We investigated associations of circulating IGF-I, IGF-II, IGFBP-2 and IGFBP-3 with prostate cancer prevalence, stage and grade, in men who had PSA-detected disease, allowing inference to focus on the development of early stage cancers as opposed to progression of cancer. Our study involved nearly 3,000 case-control pairs identified from among over 110,000 men who attended a PSA testing clinic in primary care, attenuating detection bias, and making it the largest project to date that we are aware of to investigate the IGF-system in prostate cancer. Based on previous research, albeit with predominantly clinically-detected cases,(5, 6, 14) our primary hypothesis was that IGF-I and IGF-II would be positively associated and IGFBP-3 would be inversely associated with risk of PSA-detected prostate cancer and that the magnitude of associations would be stronger for advanced versus localized disease, suggesting a role for IGFs in the progression rather than initiation, of prostate cancer . Our secondary hypothesis was that IGFBP-2 would be positively associated with prostate cancer, given previous suggestions that this peptide may be a tumour marker.(9-13)

MATERIALS AND METHODS

Study Population

We carried out a cross-sectional case-control analysis in which cases and controls were identified from (nested within) the Prostate Testing for Cancer and Treatment (ProtecT) study.(15) In the ProtecT study, all (approximately 227,000) men aged 50-69 years registered at 337 general practices from nine UK cities (centers) were invited, between 2002-2009, to have a PSA test at a prostate check clinic. Over 110,000 of these men attended the clinic. Participants with a raised PSA level (≥ 3.0 ng/ml, approximately 11% of men tested) underwent digital rectal examination (DRE) and 10-core prostate biopsy, and those with confirmed localised prostate cancer were invited to take part in a randomised trial comparing radical prostatectomy, radical radiotherapy and active monitoring. Histologic material obtained at biopsy was assigned a Gleason score by specialist uro-pathologists following a standard proforma, and then categorized as low- (score<7), mid- (score=7) and high- (score>7) grade. There was no central review of histology but the uropathologists participated in a blinded audit scheme. Cancers were staged clinically (physical exam, DRE, PSA, biopsy, isotope bone scan where indicated), using the TNM staging system, as either localized (T1/T2, NX, M0) or advanced (T3/T4, N0-3, M0-1). Trent Multicentre Research Ethics Committee approved the ProtecT study and allied prostate cancer research under the auspices of the Prostate Mechanisms of Prostate cancer and Treatment (ProMPT) study. There is no overlap in study period with a previous study based on the feasibility phase of ProtecT, conducted between 1999 and early 2001.(16)

Selection of Cases and Controls

The sample size (3,000 cases, 3,000 controls) was determined *a priori* to detect odds ratios of 1.22 comparing the highest *vs.* lowest three quartiles of IGFs or IGFBPs at 5% significance, 90% power. Cases were selected from among all men diagnosed with localized or advanced cancer who had provided a blood sample for research. Men who had no evidence of prostate cancer (PSA below the 3ng/ml threshold, or above the threshold but with one or more sets of negative prostate biopsies) and who had provided a blood sample for research, were eligible for random selection as controls (≈80,000 potentially eligible controls). We stratum matched cases to controls (1:1) by 5-year age group and recruiting general practice.

Laboratory Assays

PSA was measured using standard methods in hospital laboratories at each of the study centers. Blood samples for IGF measurement were drawn at the time of the PSA test, frozen at -80°C within 36 hours, then transferred for assay on dry ice. Concentrations of IGF-I, IGF-II and IGFBP-3 were measured in the laboratory of Professor Holly by in-house radioimmunoassay (RIA) which measures total IGF-I,(17) IGF-II(18) or IGFBP-3(19) levels, including all forms of IGFBP-3 that have undergone minor fragmentation. Serum IGFBP-2 was measured using a one-step sandwich enzyme-linked immunosorbent assay (ELISA) (DSL-10-7100; Diagnostic Systems Laboratories, Webster, TX). The molar ratio of IGF-I: IGFBP-3, which may reflect bioavailability of IGF-I, was calculated as (0.13xIGF-I concentration in ng/ml)/(0.025xIGFBP-3 concentration in ng/ml).

All assays were performed blind to knowledge of case or control status. Each stratum-matched case/control pair were thawed and assayed together wherever possible (this occurred 79% of the time; occasionally case-control pairs were separated at the assay stage, for example if not all samples in the individual boxes used for transport were assayed together, thus separating the pairs without the knowledge of the laboratory staff who were strictly blinded to case-control status). Serum pools were collected with high and low levels of each analyte; these were aliquoted and assayed in every assay to assess between-assay variability and replicates of the same pool were analysed within each assay to assess within-assay variability. Assays were undertaken between 2007 and 2010 and each assay typically contained 40 samples for IGF-I and IGF-II, 32 samples for IGFBP-2 and 80 samples for IGFBP-3. The intra-class correlations (ICCs) for within-assay variability for IGF-I, IGF-II, IGFBP-2 and IGFBP-3 were 0.86, 0.91, 0.95 and 0.88, respectively; the ICCs for between-assay variability were 0.66. 0.84, 0.81 and 0.71.

Other Covariables

Self-reported data on ethnicity, smoking, alcohol, exercise, occupation, urinary symptoms, history of benign prostatic hypertrophy (BPH), family history of prostate cancer (father and brother), height and weight (to calculate body mass index, BMI kg/m^2) were collected from questionnaires completed before receipt of the initial PSA test result.

Statistical Analysis

IGFBP-2 was log transformed before analysis as its distribution was positively skewed. IGF-I, IGF-II and IGFBP-3 distributions were approximately normally distributed. To investigate associations of IGFs and IGFBPs with prostate cancer risk, serum IGFs and IGFBPs were categorized into quintiles, based on the control distribution. Odds ratios (ORs) and 95% CIs for associations of each quintile of IGF or IGFBP with risk of prostate cancer, using the lowest quintile as the reference group, were estimated using conditional logistic regression to account for the matching variables (five-year age group and recruiting general practice), further adjusted for exact age at recruitment as a continuous variable to prevent residual age-confounding. In models assuming a linear dose-response relationship, we computed ORs for associations of prostate cancer per SD increase in IGF or IGFBP using conditional logistic regression adjusted for exact age.

We compared our basic conditional logistic regression model, with models also controlling for the following potential confounding factors (each entered separately): BMI (continuous), height (continuous), smoking (current smoker, ex-smoker, never smoked or unknown), genetic susceptibility (indexed by family history of prostate cancer in father or brother, categorized as yes or no), occupational social class (managerial/professional class, intermediate class, working class or unknown, based on the social categorization of Rose and O'Reilly(20), physical exercise (low, moderate, high or unknown intensity(21)), and current alcohol consumption (never, special occasions, lowest third, middle third, highest third and never). Where these variables were not observed to confound the associations of IGFs and IGFBPs with prostate cancer (by altering the regression coefficient by ≥10%), they were not subsequently included in fully adjusted models. Duration of serum sample storage and assay kit number were observed to cause some variation in IGFs or IGFBPs and were controlled for in all models; although not associated with case-control status, inclusion of these variables in the models reduced the degree of unexplained variation in effect-estimates (narrowing the confidence intervals).

In several previous studies,(6) models including IGF-I and IGF-II were additionally adjusted for IGFBP-3, because levels of IGFBP-3 regulate the bioactivity of IGFs.(22) We therefore also present such models. Likewise, we present models of the association of IGFBP-3 with prostate cancer that are additionally adjusted for IGF-I or IGF-I plus IGF-II, because IGFBP-3 is partly regulated by these factors.(23) All components of the IGF system can affect the concentrations of others, either through changes in equilibria, and consequently clearance, or via feedback at the pituitary. We therefore also developed a model that was mutually adjusted for all measured IGFs and IGFBPs, for completeness. However, we made an *a priori* decision to interpret these mutually adjusted models with caution because of the complexity of the inter-relationships between IGF-I, IGF-II, IGFBP-2 and IGFBP-3; for example, because IGFBP-3 is itself partly regulated by IGF-I and IGF-II,(23) controlling IGF-I or IGF-II associations for IGFBP-3 may represent statistical over-adjustment, attenuating or biasing real causal associations.(24) We have previously reported on associations of IGFs and IGFBPs with BMI in *ProtecT*(25) and shown associations of these peptides with BMI, particularly for IGFBP-2. However, further controlling for BMI in the models made little difference to the effect estimates and we present parsimonious models not controlling for BMI.

In a case-only analysis, we assessed associations of IGFs and IGFBPs with cancer stage (advanced *vs.* localized) and grade (high- *vs.* mid- *vs.* low-grade). Stage analysis was performed using unconditional logistic regression, and grade analysis was performed using ordinal logistic regression, both models adjusting for exact age and the study centre where the recruiting general practice was based (9-level variable). We investigated whether there was any evidence that associations of IGFs and IGFBPs with prostate cancer differed by age (≤60 years, >60 years) using likelihood ratio tests for interaction.

We conducted a sensitivity analysis in which we compared cases to those controls who had also undergone a biopsy because of a raised PSA but who had no histological evidence of prostate cancer ("biopsy-negative" controls). We also investigated by linear regression whether IGF or IGFBP concentrations were associated with PSA level among controls, to determine if IGFs could cause a rise in PSA levels that may be unrelated to prostate cancer.

Finally, we undertook a meta-analysis of the published literature, stratified by cancer detection method (PSA- or routinely-detected), to obtain pooled estimates of the world-wide literature on associations of IGF-I and IGFBP-3 with prostate cancer. We classified "PSA-detected studies" as those that were nested within PSA-based screening trials for prostate cancer, in order to capture studies with case-control pairs that would be very similar to our own. All other studies were classified as "routinely-detected", although we accept that these will likely contain an admixture of screen- and clinically-detected cases. We identified relevant studies from a published systematic review(6) and searched for newly-published articles up to September 2011. To compare across studies, we calculated the log OR or risk ratio per SD increase in IGF or IGFBP level using the method of Chene and Thompson(26) or Greenland and Longnecker(27) (detailed methods presented in Rowlands (2009)(6)), comparing cases with controls. We then used these estimates to perform random and fixed effects meta-analysis using the metan command in Stata.(28) Our inference is based on random-effects models, because we identified substantial heterogeneity; however, we present fixed-effect models for completeness. The I^2 statistic was calculated as a measure of the degree of heterogeneity across studies. The meta-analysis was further stratified by study design (retrospective *vs.* prospective), because of our previous finding that study design is associated with effect-size,(6) and by whether or not effect-estimates were mutually adjusted for other components of the IGF system, because this may have been a source of between-study heterogeneity. All statistical analyses were carried out using Stata 11.0 (Stata Corp., 2009).

RESULTS

Baseline characteristics

We identified 2890 men with prostate cancer, of whom 2699 (93.4%) provided a blood sample for research and were eligible for our study. We obtained serum samples for 2686 (99.5%) eligible cases and 2766 stratum-matched controls, randomly selected from the sample of all potential controls (≈80,000) who provided sufficient blood sample for research purposes (87.8% of all potential controls provided a blood sample). The 13 eligible cases that we could not include in our analysis had an insufficient volume of blood to perform the assays. Most men self-classified themselves as white ethnicity (99%). Cases were more likely to report a family history of prostate cancer, but BMI, height and other lifestyle characteristics did not differ between cases and controls (**Table 1**). IGF-I concentrations were on average lower, and IGF-II, IGFBP-2 and IGFBP-3 higher, in cases compared with controls (**Table 1**).

Of the 2,686 cases, 2,355 (88%) were localized, 311 (11%) advanced and stage was not available for 20 (1%). Of the advanced cases, most were T3a (67%), only 7 (2%) had metastases. Most men had low-grade (Gleason < 7) cancer (1,808; 67%), with 720 (27%) mid-grade (Gleason = 7) and 152 (6%) high-grade (Gleason > 7) cancers (not available for 6 men). 176 controls had PSA levels ≥ 3 ng/ml and subsequent negative prostate biopsies. IGF-I and IGF-II concentrations were correlated with each other (r=0.21) and with IGFBP-3 (r=0.37 and r=0.58, respectively); IGFBP-2 was inversely correlated with IGF-I (r=-0.16), IGF-II (r=-0.11) and IGFBP-3 (r=-0.19).

Serum Concentrations of IGF and IGFBP, and Prostate Cancer Risk

In analyses controlling for age, assay kit and storage duration (model 2), there was no evidence of an association of IGF-I with prostate cancer (OR: 0.99 per SD increase in IGF-I; 95% CI: 0.93, 1.04) (**Table 2**). IGF-II was positively associated with prostate cancer (OR: 1.16; 1.08, 1.24), although there was evidence against the assumption of linearity (p<0.01), likely due to the second quintile having a similar OR (1.45) to the fourth (1.43). The odds of prostate cancer increased with increasing IGFBP-2 (OR: 1.18; 1.06, 1.31) and IGFBP-3 (OR: 1.27; 1.19, 1.36).

When we additionally controlled for IGFBP-3 (model 3), the previously observed null association between IGF-I and prostate cancer became inverse (OR: 0.85; 0.79, 0.91); further adjustment for all other measured components of the

IGF system (IGF-II, IGFBP-2 and IGFBP-3) made little difference to the IGFBP-3 adjusted model (OR: 0.81; 0.75, 0.87; P<0.001).

The positive association of IGF-II disappeared after controlling for IGFBP-3 (OR: 0.99; 0.91, 1.08) and remained null after controlling for all other measured components of the IGF system, IGF-I, IGFBP-2 and IGFBP-3 (OR: 1.04; 0.95, 1.13; p=0.4). IGFBP-3 remained positively associated with prostate cancer after controlling for IGF-I (OR: 1.42; 1.32, 1.53) and additionally controlling for IGF-II (OR: 1.40; 1.28, 1.52) or IGF-II and IGFBP-2 (OR: 1.42; 1.30, 1.55). The observed positive association of IGFBP-2 with prostate cancer remained after controlling for all other measured components of the IGF system, IGF-I, IGF-II and IGFBP-3 (OR: 1.10; 1.028, 1.17; p=0.005).

There was no evidence of an interaction of age with IGFs or IGFBPs on prostate cancer risk (all $p_{interaction}$>0.39). There was no strong evidence that associations of any of the IGFs or IGFBPs with prostate cancer differed according to stage (**Table 3**) or grade.

IGF-I was weakly positively related to PSA level in controls, but IGF-II, IGFBP-2 and IGFBP-3 were not related to PSA level (**Appendix Table 1**). In sensitivity analyses, associations of IGF-I with prostate cancer did not change appreciably when biopsy negative controls were used, although confidence intervals were wide, reflecting imprecision around point estimates with a small sample size (**Appendix Table 2**). The magnitude of the positive associations of IGF-II and IGFBP-3 became stronger (OR = 1.43 per SD increase in IGF-II; 95% CI: 1.15, 1.77; OR = 1.36 per SD increase in IGFBP-3; 95% CI: 1.13, 1.63) but the direction of the previously observed positive association with IGFBP-2 was reversed (OR 0.88 per SD increase in IGFBP-2; 95% CI: 0.75, 1.03).

Meta-Analysis of PSA-Detected Prostate Cancer Studies

We identified four publications based on PSA-screened populations (2 retrospective/2 prospective), and 51 studies of routinely-detected cancers (34 retrospective/17 prospective) (see **Appendix** for reference list). The forest plots and pooled OR for these studies combined with the current study are shown in **Figures 1** (IGF-I) and **2** (IGFBP-3). The pooled IGF-I ORs per SD increase in IGF-I for PSA-detected studies, from random effects models, were 1.02 (95% CI:

0.79,1.33;I^2: 81%) for retrospective studies, and 1.04 (95% CI: 0.92,1.17;I^2:0%) for prospective studies. For routinely-detected cancers, the pooled IGF-I ORs were 1.19 (95% CI: 1.00,1.41;I^2:92%) for retrospective studies, and 1.08 (95% CI: 0.99,1.17;I^2: 57%) for prospective studies. For IGFBP-3, the pooled ORs were 1.06 (95% CI: 0.85,1.34;I^2:84%) for retrospective, PSA-detected cancers; 0.97 (95% CI: 0.86,1.09;I^2:1%) for prospective, PSA-detected cancers. For routinely-detected cancers, the pooled IGFBP-3 ORs were 0.73 (95% CI: 0.57,0.93;I^2:94%) for retrospective studies and 1.03 (95% CI: 0.95,1.12;I^2:40%) for prospective studies.

DISCUSSION

This is the largest study to date investigating associations of the circulating IGF system with prostate cancer. In models that were not mutually adjusted for other components of the IGF system, we found positive associations of IGF-II, IGFBP-2 and IGFBP-3 levels, although no association of IGF-I, with PSA-detected prostate cancer. There was no strong evidence that any of the IGFs or IGFBPs were related to prostate cancer stage or grade, although we were underpowered to detect modest associations. The magnitude of the increased risk associated with the highest versus lowest quintile of IGF-II, IGFBP-2 and IGFBP-3 is similar to that seen for men with a family history of prostate cancer in a first degree relative (approximately 50-100 % increased risk).

Our finding of a positive association of IGF-II with prostate cancer is supported by a genome-wide association study that identified a susceptibility locus for prostate cancer in the *TH-INS-IGF2AS-IGF2* region on chromosome 11p15.5 (rs7127900).(8) *IGF-2* gene over-expression, which can occur via loss of imprinting or heterozygosity,(29) has been observed in prostate,(30) as well as colorectal(31) and more aggressive ovarian(32) cancers. Few epidemiological studies have investigated circulating IGF-II in prostate cancer, but two studies report that raised IGF-II was associated with increased prostate cancer risk, the association being stronger in advanced disease.(16, 33) That the positive association of IGF-II with prostate cancer disappeared after controlling for IGFBP-3, however, indicates that the observed effect may be secondary to the positive association of IGFBP-3 with prostate cancer. A possible secondary effect of the tumour on levels (reverse causality) should be considered, as positive associations of IGF-II with prostate cancer have mostly been observed in small retrospective studies(6) and IGF-II levels were found to fall after removal of colorectal adenomas, precursors of colorectal cancer.(34) Tumor load in early screen-detected prostate cancers (such as were present in our study), however, would be expected to be negligible, making reverse causality an unlikely explanation of our findings.

There have been very few epidemiological studies investigating IGFBP-2 in prostate cancer, but some have observed increased IGFBP-2 levels in prostate cancer,(12, 35, 36) particularly advanced(12) compared with early disease.(37)

IGFBP-2 inversely regulates the tumor suppressor gene PTEN,(10) that when lost is associated with IGFBP-2 over-expression(38) and prostate cancer progression.(39, 40) Thus, serum IGFBP-2 may be an accessible marker of PTEN status and cancer progression. As with IGF-II, reverse causality should be considered as an explanation for our findings, because there is some evidence that IGFBP-2 may be originating from the prostate(13) and small studies indicate that serial measures of IGFBP-2 rise over time in prostate cancer that is progressing,(12) but fall after radical prostatectomy.(13) Further research, based on serial measures of IGF-II and IGFBP-2 after diagnosis of prostate cancer, is required to determine whether these peptides are tumour markers of potential clinical importance.

We observed a strong, linear positive association of IGFBP-3 with screen-detected prostate cancer, that gets stronger with adjustment for IGF-I and IGF-II, in line with a recent large study(41) and a previous meta-analysis of prospective studies.(5) IGFBP-3 has antiproliferative and pro-apoptotic effects, as well as stimulatory effects on cell growth, for example by increasing the concentration of IGFs near the IGF-IR(42) and, independent of IGF-binding, activating MAPK signaling.(43, 44) Our meta-analysis (**Figure 2**) reveals highly heterogeneous results, however (I^2=92%). A possible reason for inconsistent IGFBP-3-prostate cancer associations could be assay differences: IGFBP-3 exists as both intact and fragmented forms in the blood, each of which may be present with multiple additional post-translational modifications. Which of these forms is measured in any assay is poorly defined, and it is possible that different IGFBP-3 assays used in different studies, are measuring different forms of the peptide, leading to heterogeneity in effect estimates.

Our findings for IGF-I do not appear to be in line with previously published meta-analyses.(5, 6, 14) Previous meta-analyses, however, have combined PSA-detected and clinically-detected prostate cancers and, therefore, consist of a mixed group of cancer types. Cases detected by PSA testing, such as those in *ProtecT*, represent a very different phenotype (low volume, well-differentiated, localised cancers,(45) a substantial proportion of which will never become clinically identifiable(46)), compared to routinely-detected cases, which have progressed and have either become large enough, or have metastasized, to be diagnosed routinely. As demonstrated by others,(47) it is likely that at least some risk factors for the initiation of prostate cancer differ from those that cause a sub-group of such cancers to eventually progress into clinically manifest disease. The meta-analysis presented here indicates no or

weak associations of IGF-I with PSA-detected prostate cancer. Although the pooled estimate from the stratified meta-analysis of cross-sectional studies was largely driven by our *ProtecT* data, importantly we also observed no association of IGF-I with PSA-detected prostate cancer in prospective studies, an analysis which did not include ProtecT. In contrast, there was a positive association of IGF-I with routinely-detected cancer, which was particularly marked in retrospective studies. Positive associations seen in previous reports involving clinically detected disease could, therefore, indicate that IGF-I is not associated with the development of early stage cancers but with progression to clinically identifiable disease, in line with some studies (but not all(5)) that observed strong positive associations of IGF-I with clinically advanced or aggressive, but not localized, prostate cancer.(6) An effect of IGF-I on progression, but not initiation, would be consistent with IGF-I mediating effects of a western diet on the prevalence of clinically-detected and fatal prostate cancers (which varies considerably across the world)(48) but not on the prevalence of autopsy-detected sub-clinical cancers (which does not vary to the same extent).(49) We observed inverse associations of IGF-I with prostate cancer after controlling for IGFBP-3 or all other measured components of the IGF system. Inverse associations of IGF-I with localized prostate cancer have previously been observed,(50-52) but have not previously elicited much attention because of imprecision in effect-estimates. As previously stated we made an *a priori* decision to interpret associations observed in mutually adjusted models with caution, due to the complexity of the IGF-IGFBP-3 relationship and the risk of statistical over-adjustment and bias.(24) Nevertheless, the findings are of interest given that the correlation of IGFPB-3 with either IGF-I or IGF-II is only moderate (probably because IGFBP-3 levels are primarily regulated by growth hormone) and IGFBP-3 has important IGF-independent effects.(43, 44)

The limitations of the study include the fact that very few men had metastatic or high-grade disease, limiting our ability to identify differences in associations by stage or grade; residual confounding due to unmeasured variables may be present; and it comprised mainly white British men, so the results may not be generalizable to other ethnic groups. The IGFs and IGFBPs were measured around the time of diagnosis, so levels may be influenced by the presence of disease; however, it is difficult to understand why the presence of cancer should lower levels of IGF-I and thus explain the null/inverse association we observed for IGF-I with prostate cancer. Our cancers were screen-

detected, so were very early in their pathogenesis, and a large proportion of them are likely to remain indolent;(46) however, we feel that this is a strength of the study as it allows focused inference about associations with the development of early stage cancers rather than the progression or spread of cancer to those that are clinically apparent. Measurement of circulating IGF and IGFBP levels may not represent biologically active levels in the tissues; therefore we cannot exclude that IGF-I at the tissue level may play a role in the initiation of early prostate cancer. This is an important consideration given our findings for IGFBP-3, which has been shown to increase the concentration of IGFs near the IGF-IR of cells.(42) Assays measuring free IGF-I are available but were not used in this study, as there is some debate that they only measure a subset of the more bioavailable IGF-I in the circulation(53, 54) and free IGF-I in the circulation may be unrelated to free IGF-I in the tissues where the proportion of IGFBPs is very different. An investigation of free-IGF-I levels in 545 prostate cancer cases from the Physicians' Health Study found no association between free IGF-I and prostate cancer risk.(54) **Figures 1** and **2** show wide variation between studies in whether or not associations were mutually adjusted for other components of the IGF system. There were too few studies to undertake a formal meta-regression analysis of the impact of mutual adjustment. However, stratifying the results by whether or not mutual adjustment was performed did not reduce the observed heterogeneity and visual inspection does not indicate that effect estimates were markedly different when unadjusted versus mutually adjusted estimates were computed.

This study provides the largest assessment of the role of the IGF system (IGF-I, IGF-II, IGFBP-2 and IGFBP-3) in the development of PSA-detected prostate cancer. The evidence suggests potentially important roles for IGFBP-2 and IGFBP-3 (and possibly IGF-II), but no association of IGF-I. We speculate that circulating IGF-I has a limited role in the development of early prostate cancer but may remain an important risk factor for disease progression. Thus reduction of circulating IGF-I is unlikely to be a useful strategy for the primary prevention of prostate cancer. Future research should focus on large studies that can confirm or refute a role of IGF-II, IGFBP-2 and IGFBP-3 in PSA-detected prostate cancer and investigate the association of IGF-I with progression of PSA-detected prostate cancer; such analyses would determine whether IGFs have a potential clinical translational role in predicting which men with localized disease might progress and hence require immediate radical treatment.

Acknowledgements

The authors would like to acknowledge the tremendous contribution of all members of the ProtecT study research group, and especially the following who were involved in this research: Prasad Bollina, Sue Bonnington, Lynne Bradshaw, James Catto, Debbie Cooper, Michael Davis, Liz Down, Andrew Doble, Alan Doherty, Garrett Durkan, Emma Elliott, David Gillatt, Pippa Herbert, Peter Holding, Joanne Howson, Mandy Jones, Roger Kockelbergh, Howard Kynaston, Athene Lane, Teresa Lennon, Norma Lyons, Hing Leung, Malcolm Mason, Hilary Moody, Philip Powell, Alan Paul, Stephen Prescott, Derek Rosario, Patricia O'Sullivan, Pauline Thompson, Lynne Bradshaw, Sarah Tidball. They would also like to thank the men who participated in this study and the NIHR Cambridge Biomedical Research Centre. Ross Harris performed the dose-response calculations for the meta-analysis. Val Laundy, Semih Dogan, Li Zeng, Ola Wojtowicz and Kalina Zdunek performed the IGF assays. The authors declare no conflict of interest. M Rowlands had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Grant support

This work was supported by Cancer Research UK project grant C18281/A7062. The ProtecT study is funded by the UK Health Technology Assessment (HTA) Programme of the National Institute for Health Research, HTA 96/20/99; ISRCTN20141297. The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Department of Health. The authors would like to acknowledge the support of the National Cancer Research Institute (NCRI) formed by the Department of Health, the Medical Research Council (MRC) and Cancer Research UK. The NCRI provided funding through ProMPT (Prostate Mechanisms of Progression and Treatment) and this support is gratefully acknowledged. GDS and RMM work within the CAITE centre, which is supported by the MRC (G0600705) and the University of Bristol. DG, FH, JD and DN are NIHR Senior Investigators. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Reference List

(1) Frankel S, Davey Smith G, Donovan J, Neal D. Screening for prostate cancer. Lancet 2003;361:1122-8.

(2) Peto J. Cancer epidemiology in the last century and the next decade. Nature 2001;411:390-5.

(3) Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915-28.

(4) World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective. Washington DC: AICR; 2007.

(5) Roddam AW, Allen NE, Appleby P, Key TJ, Ferrucci L, Carter HB, et al. Insulin-like Growth Factors, Their Binding Proteins, and Prostate Cancer Risk: Analysis of Individual Patient Data from 12 Prospective Studies. Ann Intern Med 2008;149:461-71.

(6) Rowlands M, Gunnell D, Harris R, Vatten LJ, Holly JMP, Martin RM. Circulating insulin-like growth factor peptides and prostate cancer risk: A systematic review and meta-analysis. Int J Cancer 2009;124:2416-29.

(7) Cohen P. Serum insulin-like growth factor-I levels and prostate cancer risk-interpreting the evidence. J Natl Cancer Inst 1998;90:876-9.

(8) Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G, et al. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nat Genet 2009;41:1116-21.

(9) 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.

(10) Perks CM, Vernon EG, Rosendahl AH, Tonge D, Holly JMP. IGF-II and IGFBP-2 differentially regulate PTEN in human breast cancer cells. Oncogene 2007;26:5966-72.

(11) Woodson K, Tangrea JA, Pollak M, Copeland TD, Taylor PR, Virtamo J, 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.

(12) Ho PJ, Baxter RC. Insulin-like growth factor-binding protein-2 in patients with prostate carcinoma and benign prostatic hyperplasia. Clin Endocrinol (Oxf) 1997;46:333-42.

(13) Shariat SF, Lamb DJ, Kattan MW, Nguyen C, Kim J, Beck J, et al. Association of preoperative plasma levels of insulin-like growth factor I and insulin-like growth factor binding proteins-2 and -3 with prostate cancer invasion, progression, and metastasis. J Clin Oncol 2002;20:833-41.

(14) 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.

(15) Lane JA, Hamdy FC, Martin RM, Turner EL, Neal DE, Donovan JL. Latest results from the UK trials evaluating prostate cancer screening and treatment: The CAP and ProtecT studies. Eur J Cancer 2010;46:3095-101.

(16) Oliver SE, Gunnell D, Donovan J, Peters TJ, Persad R, Gillatt D, 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.

(17) Holly JM, Biddlecombe RA, Dunger DB, Edge JA, Amiel SA, Howell R, et al. Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin?.[Erratum appears in Clin Endocrinol (Oxf) 1989 Feb;30(2):211]. Clin Endocrinol (Oxf) 1988;29:667-75.

(18) Davies SC, Wass JA, Ross RJ, Cotterill AM, Buchanan CR, Coulson VJ, et al. The induction of a specific protease for insulin-like growth factor binding protein-3 in the circulation during severe illness. J Endocrinol 1991;130:469-73.

(19) Cheetham TD, Holly JM, Baxter RC, Meadows K, Jones J, Taylor AM, et al. The effects of recombinant human IGF-I administration on concentrations of acid labile subunit, IGF binding protein-3, IGF-I, IGF-II and proteolysis of IGF binding protein-3 in adolescents with insulin-dependent diabetes mellitus. J Endocrinol 1998;157:81-7.

(20) Rose D, O'Reilly K. The ESRC Review of Government Social Classifications. London: Office for National Statistics & Economic and Social Research Council; 1998.

(21) Godin G, Shephard RJ. A simple method to assess exercise behavior in the community. Can J Appl Sport Sci 1985;10:141-6.

(22) Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebaek NE. The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. Clin Endocrinol 1994;41:85-93.

(23) Holly J, Perks C. The role of insulin-like growth factor binding proteins. Neuroendocrinology 2006;83:154-60.

(24) Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, et al. Illustrating bias due to conditioning on a collider. Int J Epidemiol 2009;dyp334.

(25) Rowlands M-A, Holly J, Gunnell D, Gilbert R, Donovan J, Lane J, et al. The relation between adiposity throughout the life course and variation in IGFs and IGFBPs: evidence from the ProtecT (Prostate testing for cancer and Treatment) study. Cancer Causes and Control 2010;21:1842.

(26) Chene G, Thompson SG. Methods for summarizing the risk associations of quantitative variables in epidemiologic studies in a consistent form. Am J Epidemiol 1996;144:610-21.

(27) Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135:1301-9.

(28) Harris RJ, Bradburn MJ, Deeks JJ, Harbord RM, Altman DG, Sterne JAC. metan: fixed– and random–effects meta–analysis. The Stata Journal 2008;8:3-28.

(29) Toretsky JA, Helman LJ. Involvement of IGF-II in human cancer. J Endocrinol 1996;149:367-72.

(30) Liu ZW, Zhou FJ, Luo YM, Qin ZK, Wang DJ, Luo JH, et al. Genomic imprinting of insulin-like growth factor II in prostate cancer and its clinical significance. Chinese Journal of Cancer 2006;25:765-70.

(31) Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, et al. Loss of IGF2 Imprinting: A Potential Marker of Colorectal Cancer Risk. Science 2003;299:1753-5.

(32) Lu LG, Katsaros D, Wiley A, de la Longrais IAR, Risch HA, Puopolo M, et al. The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-alpha expression to disease progression in epithelial ovarian cancer. Clin Cancer Res 2006;12:1208-14.

(33) 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.

(34) Renehan AG, Painter JE, O'Halloran D, Atkin WS, Potten CS, O'Dwyer ST, et al. Circulating insulin-like growth factor II and colorectal adenomas. J Clin Endocrinol Metab 2000;85:3402-8.

(35) Cohen P, Peehl DM, Stamey TA, Wilson K, Clemmons D, Rosenfeld R. Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients. J Clin Endocrinol Metab 1993;76:1031-5.

(36) Kanety H, Madjar Y, Dagan Y, Levi J, Papa MZ, Pariente C, et al. Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen. J Clin Endocrinol Metab 1993;77:229-33.

(37) 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.

(38) Mehrian-Shai R, Chen CD, Shi T, Horvath S, Nelson SF, Reichardt JKV, et al. Insulin growth factor-binding protein 2 is a candidate biomarker for PTEN status and PI3K/Akt pathway activation in glioblastoma and prostate cancer. PNAS 2007;104:5563-8.

(39) McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 1999;59:4291-6.

(40) Koksal IT, Dirice E, Yasar D, Sanlioglu AD, Ciftcioglu A, Gulkesen KH, et al. The assessment of PTEN tumor suppressor gene in combination with Gleason scoring and serum PSA to evaluate progression of prostate carcinoma. Urologic Oncology 2004;22:307-12.

(41) Nimptsch K, Platz EA, Pollak M, Kenfield SA, Stampfer MJ, Willett WC, et al. Plasma insulin-like growth factor 1 is positively associated with low-grade prostate cancer in the Health Professionals Follow-up Study 1993-2004. Int J Cancer 2010;Epub:NA.

(42) Firth SM, Baxter RC. Cellular Actions of the Insulin-Like Growth Factor Binding Proteins. Endocr Rev 2002;23:824-54.

(43) Martin JL, Weenink SM, Baxter RC. Insulin-like Growth Factor-binding Protein-3 Potentiates Epidermal Growth Factor Action in MCF-10A Mammary Epithelial Cells. J Biol Chem 2003;278:2969-76.

(44) Butt AJ, Martin JL, Dickson KA, McDougall F, Firth SM, Baxter RC. Insulin-Like Growth Factor Binding Protein-3 Expression Is Associated with Growth Stimulation of T47D Human Breast Cancer Cells: The

Role of Altered Epidermal Growth Factor Signaling. Journal of Clinical Endocrinology Metabolism 2004;89:1950-6.

(45) Stamey TA, Caldwell M, McNeal JE, Nolley R, Hemenez M, Downs J. The prostate specific antigen era in the United States is over for prostate cancer: What happend in the last 20 years? The Journal of Urology 2004;172:1297-301.

(46) Draisma G, Etzioni R, Tsodikov A, Mariotto A, Wever E, Gulati R, et al. Lead Time and Overdiagnosis in Prostate-Specific Antigen Screening: Importance of Methods and Context. J Natl Cancer Inst 2009;101:374-83.

(47) Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC. Risk factors for prostate cancer incidence and progression in the health professionals follow-up study. Int J Cancer 2007;121:1571-8.

(48) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide. Lyon, France: International Agency for Research on Cancer; 2010.

(49) Haas GP, Delongchamps NB, Brawley OW, Wang CY, de la Roza G. The Worldwide Epidemiology of Prostate Cancer: Perspectives from Autopsy Studies. Can J Urol 2008;15:3866-71.

(50) 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.

(51) Ismail HA, Pollak M, Behlouli H, Tanguay S, Begin LR, Aprikian AG. Insulin-like growth factor-1 and insulin-like growth factor binding protein-3 for prostate cancer detection in patients undergoing prostate biopsy. J Urol 2002;168:2426-30.

(52) Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M, 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.

(53) Bang P, Ahlson M, Berg U, Carlsson-Skwirut C. Free Insulin-Like Growth Factor I: Are We Hunting a Ghost? Hormone Research in Paediatrics 2001;55:84-93.

(54) Mucci LA, Stark JR, Pollak MN, Li H, Kurth T, Stampfer MJ, et al. Plasma Levels of Acid-Labile Subunit, Free Insulin-Like Growth Factor-I, and Prostate Cancer Risk: A Prospective Study. Cancer Epidemiology Biomarkers & Prevention 2010;19:484-91.

Table 1. Characteristics of 2686 cases and 2766 controls included in the analysis (UK-wide Prostate testing for cancer and Treatment study (ProtecT), 2002-2009)

	Controls n=2766		Cases n=2686	
Continuous variables [*]	**Mean**	**SD**	**Mean**	**SD**
Age (years)	61.7	5.0	61.9	5.0
PSA baseline (ng/ml) (geometric mean)	1.0		6.2	
BMI (kg/m^2)	27.3	3.9	27.2	3.7
Height (cm)	176.1	6.7	176.3	6.7
IGF-I (ng/ml)	166.4	55.5	163.5	54.9
IGF-II (ng/ml)	769.1	283.2	804.8	295.2
IGFBP-2 (ng/ml) [†]	579.5	395-865	594.5	404-872
IGFBP-3 (ng/ml)	4429	1028	4644	1024
IGF-I:IGFBP-3 molar ratio (%)	20.1	7.0	18.6	6.0
Categorical variables	**n**	**%**	**n**	**%**
Smoking				
Current smoker	248	9.0	259	9.6
Ex-smoker	930	33.6	951	35.4
Never smoker	603	21.8	704	26.2
Unknown	985	35.6	772	28.7
Alcohol				
Never	89	3.2	87	3.2
On special occasions only	143	5.2	151	5.6
Lowest third (<9.8 units/week)	465	16.8	536	20.0
Middle third (9.9 to 20.2 units/week)	521	18.8	539	20.1
Upper third (>20.3 units/week)	475	17.2	496	18.5
Unknown	1073	38.8	877	32.7
Weekly exercise §				
Low (0-14 units)	339	12.3	389	14.5
Moderate (15-43 units)	360	13.0	380	14.2
High (44+ units)	361	13.1	351	13.1
Unknown	1706	61.7	1566	58.3
Family history of prostate cancer [‖]				
Yes	128	4.6	198	7.4
No	2607	94.3	2442	90.9
Unknown	31	1.1	46	1.7
Social class [¶]				
Managerial/professional	1108	40.1	1110	41.3
Intermediate	427	15.4	383	14.3
Working	940	34.0	965	35.9
Unknown	291	10.5	228	8.5
Lower urinary tract symptom score [**]				
No LUTS	169	6.1	140	5.2
1 to 3	1947	70.4	1899	70.7
4 to 10	432	15.6	464	17.3
Unknown	218	7.9	183	6.8
History of BPH				
No	2451	88.6	2408	89.7
Possible	134	4.8	117	4.4
Yes	116	4.2	89	3.3
Unknown	65	2.4	72	2.7

Subjects in this table are those included in at least one analysis. [*]Mean levels and standard deviation (SD) of continuous variables calculated by linear regression. IGFs and IGFBPs additionally adjusted for age. [†]Median and

inter-quartile range presented for IGFBP-2. ‡p heterogeneity calculated by Chi square test. §Weekly exercise calculated from the frequency of participation in exercise of mild, moderate and strenuous intensity.[21] ||Family history of prostate cancer is coded yes if father or brother were diagnosed with prostate cancer. ¶Three-class social categorization from Rose and O'Reilly (1998)[20]. **Lower urinary tract symptom score is a sum of the occurrence of hesistancy, urgency, frequency, nocturia and leakage.

Table 2. Association of IGFs and IGFBPs with prostate cancer risk (UK-wide Prostate testing for cancer and Treatment study (ProtecT), 2002-2009)

	Quintile range	n controls	n cases	Model 1* OR (95% CI)	Model 2† OR (95% CI)	Model 3‡ OR (95% CI)
IGF-I (ng/ml)		**2764**	**2686**			
Q1	<=120	558	584	1.0	1.0	1.0
Q2	121-148	566	542	0.89 (0.75 , 1.06)	0.91 (0.76 , 1.08)	0.83 (0.69 , 0.99)
Q3	149-175	537	557	0.97 (0.82 , 1.15)	1.03 (0.86 , 1.22)	0.81 (0.67 , 0.98)
Q4	176-209	553	516	0.88 (0.74 , 1.05)	0.96 (0.80 , 1.14)	0.73 (0.60 , 0.88)
Q5	>=210	550	487	0.83 (0.70 , 0.99)	0.96 (0.80 , 1.16)	0.64 (0.52 , 0.79)
per SD increase in IGF-I				0.94 (0.89 , 1.00)	0.99 (0.93 , 1.04)	0.85 (0.79 , 0.91)
p linear trend §				*0.04*	*0.62*	*<0.001*
(standard deviation IGF-I= 55 ng/ml)						
IGF-II (ng/ml)		**2714**	**2642**			
Q1	<=539	544	413	1.0	1.0	1.0
Q2	540-663	542	575	1.41 (1.18 , 1.69)	1.45 (1.21 , 1.74)	1.28 (1.06 , 1.54)
Q3	664-785	544	480	1.19 (0.99 , 1.43)	1.25 (1.03 , 1.51)	1.02 (0.84 , 1.25)
Q4	786-959	544	533	1.34 (1.12 , 1.62)	1.43 (1.18 , 1.73)	1.10 (0.89 , 1.36)
Q5	>=960	540	641	1.77 (1.45 , 2.15)	1.76 (1.43 , 2.17)	1.21 (0.94 , 1.55)
per SD increase in IGF-II				1.18 (1.11 , 1.26)	1.16 (1.08 , 1.24)	0.99 (0.91 , 1.08)
p linear trend §				*<0.001*	*<0.001*	*0.85*
(standard deviation IGF-II=290 ng/ml)						
IGFBP-2 (ng/ml)		**2732**	**2664**			
Q1	<=356	550	459	1.0	1.0	
Q2	357-496	543	539	1.18 (0.99 , 1.41)	1.12 (0.77 , 1.63)	
Q3	497-661	544	548	1.19 (1.00 , 1.42)	1.37 (0.95 , 1.99)	
Q4	662-943	548	557	1.23 (1.03 , 1.46)	1.65 (1.15 , 2.37)	N/A
Q5	>=944	547	561	1.24 (1.04 , 1.48)	1.72 (1.21 , 2.45)	
per SD increase in IGFBP-2				1.05 (1.00 , 1.11)	1.18 (1.06 , 1.31)	
p linear trend §				*0.06*	*<0.01*	
(geometric standard deviation IGFBP-2=1.75)						
IGFBP-3 (ng/ml)		**2679**	**2637**			
Q1	<=3540	539	386	1.0	1.0	1.0
Q2	3541-4210	535	463	1.25 (1.04 , 1.50)	1.31 (1.07 , 1.60)	1.42 (1.16 , 1.73)
Q3	4121-4650	539	521	1.39 (1.15 , 1.67)	1.45 (1.19 , 1.77)	1.67 (1.36 , 2.04)
Q4	5651-5300	531	588	1.68 (1.39 , 2.02)	1.67 (1.37 , 2.04)	2.00 (1.62 , 2.47)
Q5	>=5301	535	679	1.97 (1.64 , 2.38)	1.99 (1.62 , 2.44)	2.57 (2.05 , 3.22)
per SD increase in IGFBP-3				1.28 (1.21 , 1.36)	1.27 (1.19 , 1.36)	1.42 (1.32 , 1.53)
p linear trend §				*<0.001*	*<0.001*	*<0.001*
(standard deviation IGFBP-3= 1031 ng/ml)						
IGF-I:IGFBP-3 molar ratio (%)		**2678**	**2637**			
Q1	<=14.6	536	678	1.0	1.0	
Q2	14.6-17.5	535	552	0.80 (0.67 , 0.94)	0.75 (0.63 , 0.91)	
Q3	17.6-20.7	535	573	0.80 (0.68 , 0.95)	0.85 (0.70 , 1.02)	
Q4	20.8-25.0	537	500	0.69 (0.58 , 0.82)	0.74 (0.61 , 0.90)	N/A
Q5	>=25.1	535	334	0.45 (0.37 , 0.54)	0.49 (0.39 , 0.61)	
per SD increase in IGF-I: IGFBP-3 molar ratio				0.75 (0.71 , 0.80)	0.78 (0.72 , 0.84)	
p linear trend §				*<0.001*	*<0.001*	

Quintiles of IGFs/IGFBPs based on the distribution in control subjects. All models are conditional logistic regression, stratum matched on 5 year age band and GP surgery, and additionally adjusted as follows: *Model 1: adjusted for age. †Model 2: adjusted for age, assay kit number, sample storage duration. ‡Model 3: adjusted for age, assay kit number, sample storage duration, plus mutual adjustment for IGF-I (for IGFBP-3 association) or IGFBP-3 (for IGF-I and IGF-II association).§p linear trend calculated from the dose-response (per standard deviation increase in IGF or IGFBP) analysis

Table 3. Associations of IGFs and IGFBPs with risk of advanced (T3-T4) versus localized (T1-T2, N0, M0) prostate cancer (UK-wide Prostate testing for cancer and Treatment study (ProtecT), 2002-2009)

	Quartile range	n localized cases	n advanced cases	Model 1* OR (95% CI)	Model 2† OR (95% CI)	Model 3‡ OR (95% CI)
IGF-I (ng/ml)		**2354**	**311**			
Q1	<=128	627	97	1.0	1.0	1.0
Q2	129-161	586	73	0.78 (0.56 , 1.08)	0.79 (0.56 , 1.10)	0.79 (0.56 , 1.12)
Q3	162-199	614	73	0.80 (0.57 , 1.11)	0.80 (0.57 , 1.12)	0.81 (0.56 , 1.15)
Q4	>=200	527	68	0.89 (0.64 , 1.26)	0.85 (0.59 , 1.21)	0.82 (0.55 , 1.23)
per SD increase in IGF-I				0.93 (0.82 , 1.06)	0.91 (0.79 , 1.04)	0.89 (0.76 , 1.04)
p linear trend §				*0.26*	*0.15*	*0.13*
IGF-II (ng/ml)		**2311**	**310**			
Q1	<=573	482	63	1.0	1.0	1.0
Q2	574-721	574	83	1.11 (0.78 , 1.59)	1.11 (0.77 , 1.59)	1.14 (0.79 , 1.64)
Q3	722-909	581	85	1.20 (0.84 , 1.71)	1.14 (0.80 , 1.65)	1.18 (0.80 , 1.73)
Q4	>=910	674	79	0.92 (0.64 , 1.32)	0.83 (0.54 , 1.26)	0.89 (0.55 , 1.44)
per SD increase in IGF-II				0.99 (0.88 , 1.12)	0.97 (0.83 , 1.12)	1.01 (0.84 , 1.21)
p linear trend §				*0.91*	*0.66*	*0.92*
IGFBP-2 (ng/ml)		**2334**	**309**			
Q1	<=395	558	68	1.0	1.0	
Q2	396-578	563	63	0.87 (0.60 , 1.26)	0.90 (0.62 , 1.31)	
Q3	579-865	630	91	1.05 (0.75 , 1.49)	1.10 (0.78 , 1.56)	N/A
Q4	>=866	583	87	1.10 (0.78 , 1.56)	1.12 (0.79 , 1.60)	
per SD increase in IGFBP-2				1.10 (0.96 , 1.25)	1.10 (0.97 , 1.26)	
p linear trend §				*0.16*	*0.13*	
IGFBP-3 (ng/ml)		**2307**	**309**			
Q1	<=3700	430	67	1.0	1.0	1.0
Q2	3701-4380	517	67	0.91 (0.63 , 1.32)	0.94 (0.65 , 1.37)	0.98 (0.67 , 1.43)
Q3	4381-5120	625	88	1.00 (0.71 , 1.42)	1.01 (0.70 , 1.45)	1.09 (0.74 , 1.58)
Q4	>=5121	735	87	0.94 (0.66 , 1.34)	0.95 (0.66 , 1.38)	1.06 (0.71 , 1.59)
per SD increase in IGFBP-3				0.94 (0.82 , 1.06)	0.94 (0.82 , 1.08)	0.97 (0.83 , 1.13)
p linear trend §				*0.30*	*0.37*	*0.70*
IGF-I:IGFBP-3 molar ratio (%)		**2307**	**309**			
Q1	<=15.4	701	96	1.0	1.0	
Q2	15.5-19.0	646	78	0.96 (0.69 , 1.32)	0.97 (0.69 , 1.36)	
Q3	19.1-23.5	538	89	1.16 (0.85 , 1.60)	1.19 (0.84 , 1.68)	N/A
Q4	>=23.6	422	46	0.75 (0.51 , 1.10)	0.64 (0.41 , 0.98)	
per SD increase in IGF-I: IGFBP-3 molar ratio				0.94 (0.82 , 1.08)	0.89 (0.76 , 1.04)	
p linear trend §				*0.41*	*0.14*	

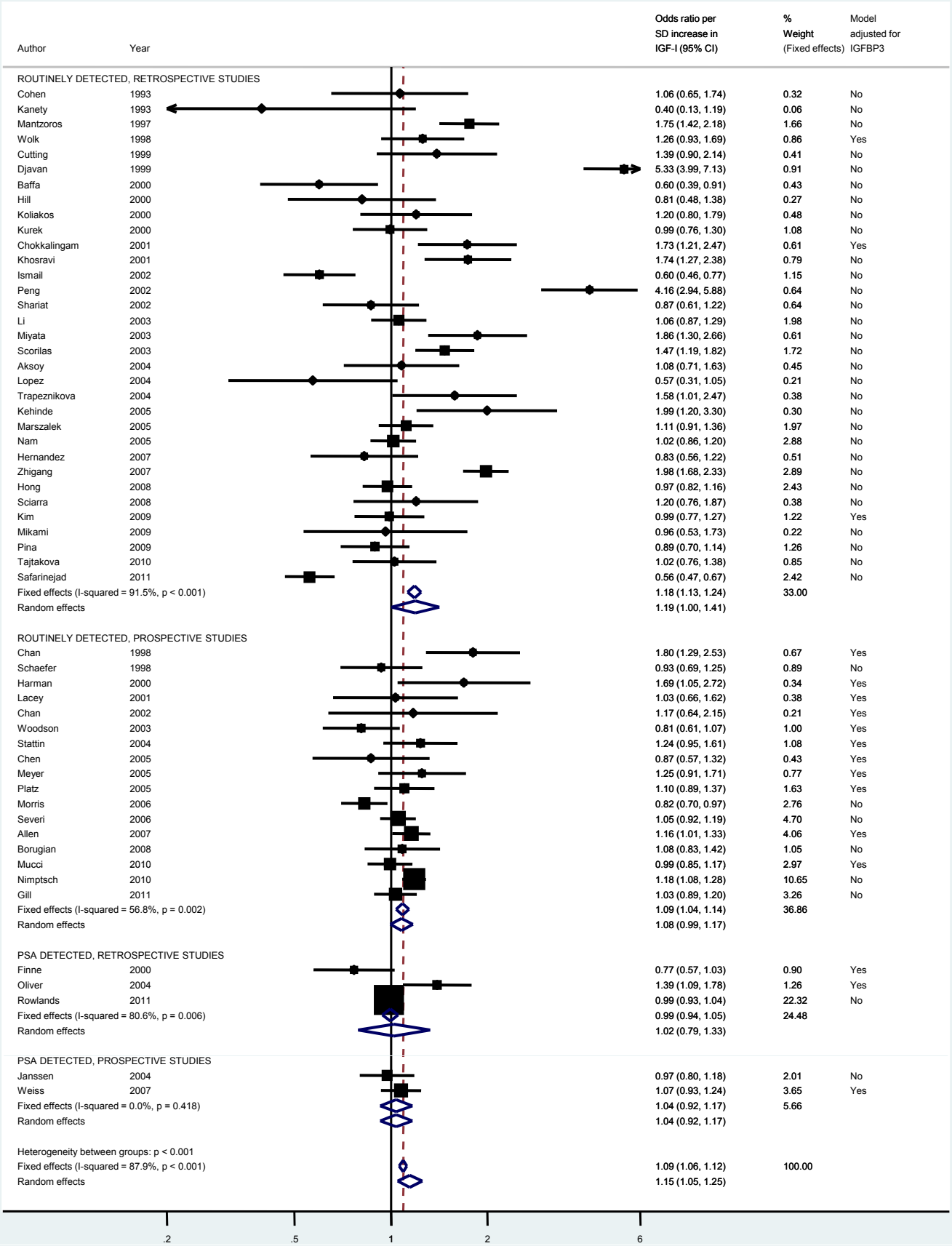
Quintiles of IGFs/IGFBPs based on the distribution in control subjects. OR<1 indicates that higher IGF / IGFBP is associated with decreased risk of advanced compared to localized prostate cancer. OR>1 indicates that higher IGF / IGFBP is associated with increased risk of advanced compared to localized prostate cancer. All models are logistic regression, adjusted as follows: *Model 1: adjusted for age and center. †Model 2: adjusted for age, center,

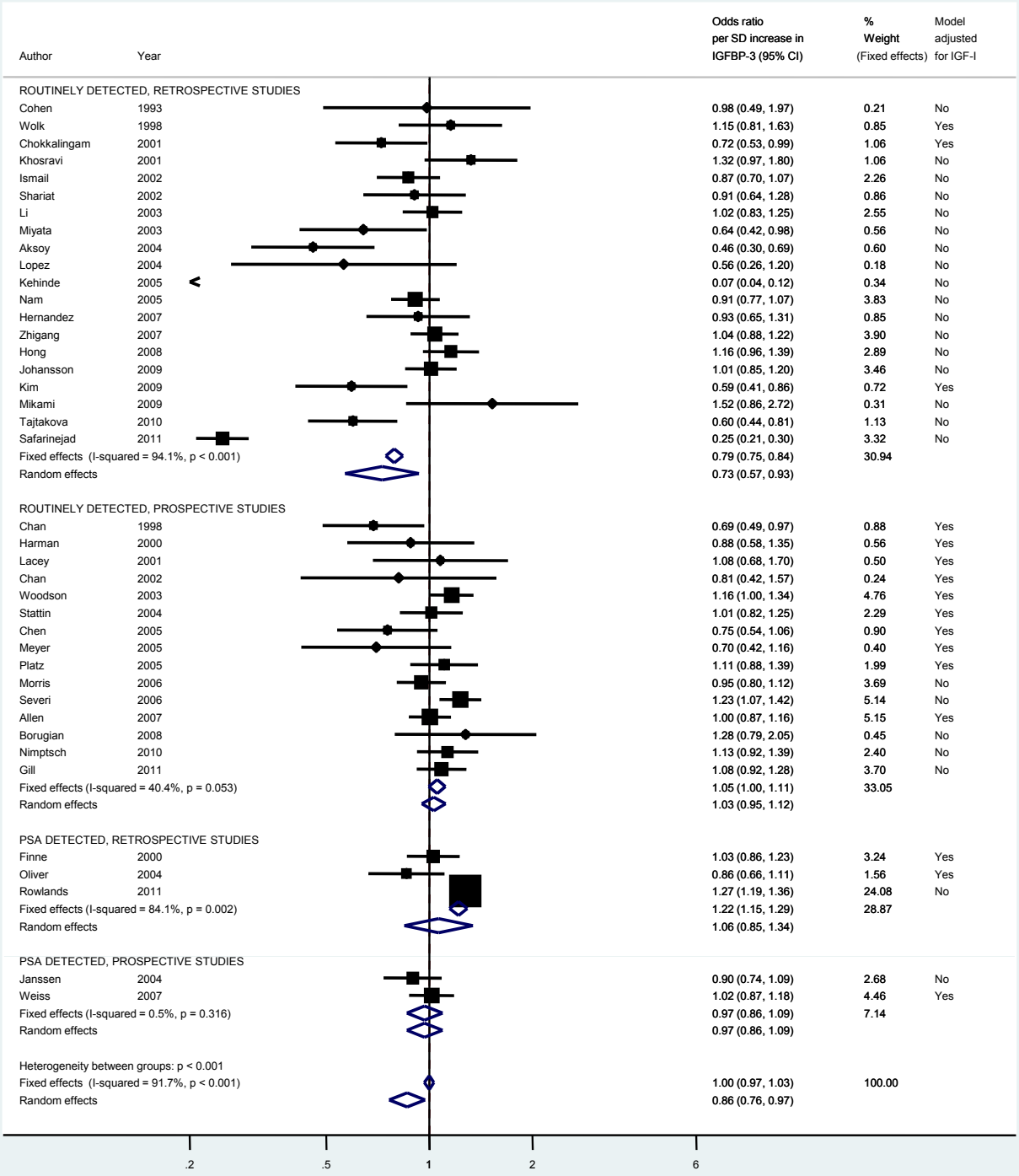
assay kit number, sample storage duration. ‡Model 3: adjusted for age, center, assay kit number, sample storage duration, plus mutual adjustment for IGF-I (for IGFBP-3 association or IGFBP-3 for IGF-I and IGF-II association). §p linear trend calculated from the dose-response (per SD increase in IGF or IGFBP) analysis.

FIGURE LEGENDS

Figure 1. Forest plot of the association of IGF-I with prostate cancer, stratified by studies conducted in PSA-screened populations and those that involved routinely–detected cases. I-squared: the degree of heterogeneity across studies.

Figure 2. Forest plot of the association of IGFBP-3 with prostate cancer, stratified by studies conducted in PSA-screened populations and those that involved routinely–detected cases. I-squared: the degree of heterogeneity across studies.





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The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

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Cancer Res Published OnlineFirst November 21, 2011.