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Article

Circulating Insulin-Like Growth Factor 1–Related Biomarkers and Risk of Lethal Prostate Cancer

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Abstract

Background: Experimental and epidemiologic evidence supports the role of circulating insulin-like growth factor-1 (IGF-1) levels with the risk of prostate cancer. Most circulating IGF-1 is bound to specific binding proteins, and only about 5% circulates in a free form. We explored the relation of free IGF-1 and other components of the IGF system with lethal prostate cancer. **Methods:** Using prospectively collected samples, we undertook a nested case-only analysis among 434 men with lethal prostate cancer and 524 men with indolent, nonlethal prostate cancer in the Physicians' Health Study and the Health Professionals Follow-up Study. Prediagnostic plasma samples were assayed for free IGF-1 and total IGF-1, acid labile subunit, pregnancy-associated plasma protein A (PAPP-A), and intact and total IGF binding protein 4. We estimated odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the associations between IGF-1–related biomarkers and lethal prostate cancer using unconditional logistic regression models adjusted for age, height, and body mass index. **Results:** Men in the highest quartile of PAPP-A levels had 42% higher odds of lethal prostate cancer (pooled adjusted OR = 1.42, 95% CI = 1.04 to 1.92) compared with men in the lowest 3 quartiles. There were no statistically significant differences in the other plasma analytes. The positive association between PAPP-A and lethal prostate cancer was present among men with intact *PTEN* but not among those with tumor *PTEN* loss (2-sided $P_{interaction} = .001$). **Conclusions:** Our study provides suggestive evidence that among men who later develop prostate cancer, higher plasma PAPP-A levels measured prior to diagnosis are associated with increased risk of lethal compared with indolent disease.

Prostate cancer is the second leading cause of cancer mortality among men in the United States (1). In vitro studies show that insulin-like growth factor-1 (IGF-1) stimulates growth of IGF-1 receptor-positive prostate cancer cells by increasing proliferation, enhancing survival, and decreasing apoptosis (2-4). Prostate tumors from patients reveal statistically significant IGF-1 receptor expression compared with adjacent normal tissue, and high tumor expression is associated with lethal disease (5,6). Epidemiological evidence also supports the relevance of the IGF-1 system to prostate cancer. We previously found that healthy men with high levels of total IGF-1 had a higher risk of total prostate cancer in the Physicians' Health Study (PHS) and Health Professionals Follow-up Study (HPFS) (7,8). Results from meta-analysis of prospective studies have confirmed a positive association between total circulating IGF-1 levels and prostate cancer risk (9-11). Additionally, total IGF-1 was strongly associated with advanced prostate cancer but not for early prostate cancer (12), suggesting that IGF-1 may potentially play a role specifically in aggressive and lethal disease. A recent Mendelian randomization study also suggests a role for IGF-1 in determining prostate cancer risk (13).

Of total circulating IGF-1, approximately 1%-5% exists in a bioactive or free form and can bind to the IGF receptor, whereas the balance is complexed with IGF-binding proteins (14,15). Previous ultracentrifugation-based measurements of free IGF-1 were not practical for processing large numbers of archived samples (15,16), but recently a novel enzyme-linked immunosorbent assay (ELISA) for free IGF-1 was developed. Several other IGF-1–related biomarkers are also of potential interest. Human pregnancy-associated plasma protein-A (PAPP-A) was

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originally described in plasma of pregnant women (17), but now it is known to be widely expressed and to be present in plasma of both men and women. PAPP-A, a protease capable of cleaving insulin-like growth factor binding protein–4 (IGFBP-4), thereby releases free IGF-1 (18). Compared with more abundant IGFBPs in the circulation, such as IGFBP-3, IGFBP-4 is less studied as a candidate prognostic cancer biomarker (14). Prostate-specific antigen (PSA) may serve as a relevant example for the potential importance of PAPP-A, as a protease that cleaves IGFBP-3 (19). Given the well-established link between circulating IGF-1 and prostate cancer, and potential roles of this pathway in lethal disease, we sought to determine whether circulating levels of IGF-1–related biomarkers measured prior to diagnosis could distinguish men who later developed lethal prostate cancer from those later diagnosed with indolent disease.

Methods

Study Population

The men in the current study were participants in the prospective PHS and HPFS with incident prostate cancer. The PHS was a randomized placebo-controlled trial of aspirin and β-carotene including 22 071 US male physicians ages 40-84 years in 1982. Participants were followed up annually through questionnaires about diet, health and lifestyle behaviors, and medical history (20). The HPFS is an ongoing prospective cohort study of risk factors for disease including 51 529 male health professionals (dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians) ages 40-75 years in 1986. Dietary intake was assessed by a validated self-administered semiquantitative food frequency questionnaire every 4 years (21). Nutrient intakes were then calculated by multiplying the frequency of consuming each food or beverage item by the nutrient content of that serving and then summing contributions from all food and beverage items using composition values from the US Department of Agriculture sources, supplemented with other data. Participants were followed up biennially using questionnaires to update information on health-related exposures and health endpoints, including prostate cancer. In the PHS, fasting blood samples collected prior to randomization during 1982-1984 are available from 68% of participants (n = 14 916). In the HPFS, fasting blood samples were collected from 18 018 participants free from prostate cancer during 1993-1995. In both cohorts, blood samples were shipped overnight with cold packs, processed, and stored at −80°C or colder.

The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required.

Ascertainment and Confirmation of Prostate Cancer Endpoints

Men with prostate cancer were initially identified using self-reported questionnaires and then confirmed by medical records and pathology reports. Stage was recorded according to the tumor, node, and metastasis staging system. Men with prostate cancer were followed to ascertain further treatments, recurrence, and development of metastases. Deaths were ascertained through repeated mailings, telephone calls to nonrespondents, and searches of the National Death Index, and specific cause of death was determined by an endpoint committee of physicians. Assignment of prostate cancer–specific death was based on review of medical records, death certificates, and information from the participants' families.

Lethal prostate cancer was defined as metastatic or fatal prostate cancer (primary cause of death) during follow-up. Nonlethal prostate cancer was defined as prostate cancer patients in which the men remained free of known metastases with at least 8 years of follow-up after cancer diagnosis. The follow-up for lethal prostate cancer in the PHS was complete through 2015; for the HPFS, data are complete through 2018. We included 434 men with lethal prostate cancer and 524 men with indolent, nonlethal prostate cancer in the current study.

Measurement of the IGF-1–Related Biomarkers and Validation of the Free IGF-1 ELISA

Plasma levels of IGF-1–related biomarkers, including total and free IGF-1, acid-labile subunit, PAPP-A, and intact and total IGFBP-4, were measured in the prospectively collected plasma in the laboratory of Dr Michael Pollak at McGill University in Montreal, Canada, using reagents from AnshLabs (Webster, TX) and Crystal Chem (Elk Grove Village, IL). All assays were conducted using ELISAs from the same production lot. Because traditional ultracentrifugation-based methods for measuring free IGF-1 was not suitable for processing large numbers of clinically obtained samples, a new ELISA format assay for free IGF-1 was used in the current study. This assay used a sensitive 2-site antibody sandwich method, which allowed detection of unbound (free) IGF-1 because the epitope detected was concealed when IGF-1 was complexed with a binding protein. To validate the performance of this ELISA, we added increasing concentrations of recombinant human IGFBP-3 to a pooled human serum sample. As shown in Figure 1, this reduced the detectable free IGF-1 level, as would be expected.

Seven sets of blinded quality control samples (aliquots of pooled plasma from volunteers) were interspersed randomly among the samples. We evaluated the coefficient of variation (CV) for those quality control samples, and all CVs were below 8.8% (range = 3.2%-8.8%). All biomarkers were measured for twice, and the mean value was used in the current study. If the value was below the limit of detection (eg, <0.55 ng/mL for free IGF-1), we used the value halfway between the lower limit and 0.

Tissue Biomarkers

Prostate tumor tissue was collected from a subset of PHS and HPFS participants who had undergone radical prostatectomy (95%) or transurethral resection of the prostate (5%). Tumor tissue microarrays were constructed by sampling 3 or more 0.6-mm paraffin-embedded tumor tissue cores from the primary tumor nodule or nodule with the highest Gleason grade and embedding the cores on a recipient array block. We assessed 3 IGF-related tissue biomarkers: presence or absence of *TMPRSS2: ERG* gene fusion, phosphatase and tensin homolog (*PTEN*) loss, and staining intensity of IGF-1 receptor (IGF-1R) in tumor tissue by immunohistochemistry of protein expression, as previously described (5,6,22).

Statistical Analysis

We used the paired *t* test and Wilcoxon signed-rank test to compare the distributions of baseline characteristics between men with lethal and nonlethal prostate cancer, including age at blood collection and diagnosis, Gleason grade at diagnosis, height, and body mass index (BMI). Spearman correlation

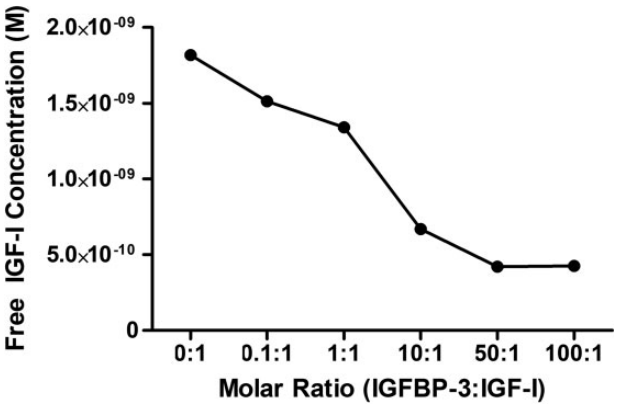

Figure 1. Validation of the free IGF-1 ELISA in plasma. IGF-1 = insulin-like growth factor-1; IGFBP-3 = insulin-like growth factor binding protein-3.

coefficients were calculated to examine the correlation between the IGF-1–related biomarkers.

We estimated cohort-specific odds ratios (ORs), as estimates of the relative risk, and corresponding 95% confidence intervals (CIs) for the associations between IGF-1–related biomarkers and lethal prostate cancer using unconditional logistic regression models adjusted for age at blood collection (in years), height (in quartiles), and BMI ($<25\,kg/m^2$, 25 to $<30\,kg/m^2$, $\geq 30\,kg/m^2$). We categorized men into quartiles of each biomarker, based on the distribution among men with nonlethal prostate cancer, with the lowest quartile as the reference category. For free IGF-1, we used men with levels below the limit of detection as the reference (22.4% for the PHS, 65.9% for the HPFS) and divided the remaining into 2 groups at the median. We then used fixed effects models to calculate the pooled odds ratios, as *P* values for heterogeneity were more than .05 for all. Subgroup analyses were conducted stratified by time from blood collection to cancer diagnosis and Gleason grade. Because of limited sample size, we used linear regression models to compare the adjusted mean circulating levels of IGF-1–related biomarkers across tumor biomarkers: *ERG* fusion, *PTEN* loss, and IGF-1R, adjusting for age (in years), height (in inches), BMI (in kg/m^2), and cohort by adding these variables into the multivariable model. To test whether the association between IGF-1–related biomarkers and lethal prostate cancer was modified by tumor biomarker status, we included the multiplicative interaction term of lethal status and tumor biomarker variable into the multivariable model.

Finally, as exploratory analyses, we used linear regression models to compare the distributions of IGF-1–related biomarkers by tumor protein expression status, including *ERG* fusion, *PTEN* loss, and IGF-1R adjusting for age (in years), height (in inches), BMI (in kg/m^2), and cohort. In addition, Spearman correlation was employed to study the associations between nutrient intakes and IGF-1–related biomarkers in the HPFS, using cumulative average values of nutrient intakes (1986-1990) before blood collection.

All statistical analyses were 2-sided, and a *P* value of less than .05 was considered statistically significant. We conducted all analyses using the SAS software (SAS Institute, Inc, Version 9.4, Cary, NC).

Results

To validate the free IGF-1 ELISA, we measured total and free IGF-1 levels in plasma samples to which we added various concentrations of recombinant IGFBP-3. As shown in Figure 1, addition of the recombinant binding protein decreased the free IGF-1 concentration, as expected, without influencing the total IGF concentration. Although this does not provide a full characterization of the assay, it does indicate that the assay discriminates between total and free IGF-1. Compared with men with nonlethal prostate cancer, men with lethal prostate cancer were more likely to be older at blood collection and diagnosis, and more men with lethal prostate cancer had later stage tumors and a Gleason grade of 7 or higher at diagnosis (Table 1). Free IGF-1 was positively correlated with PAPP-A ($r = 0.14$, $P < .001$) but not with total IGF-1 ($r = 0.02$, $P = .59$) (Table 2).

We observed no statistically significant association between free IGF-1 and lethal prostate cancer (pooled adjusted OR for the group with the highest levels [n = 112 men with lethal prostate cancer] vs those with the lowest levels [n = 192 men with lethal prostate cancer] = 0.93, 95% CI = 0.64 to 1.35). However, men in the highest quartile of PAPP-A levels (n = 147 men with lethal prostate cancer) had 42% higher odds of lethal prostate cancer (pooled adjusted OR = 1.42, 95% CI = 1.04 to 1.92) compared with men in the lowest 3 quartiles (n = 272 men with lethal prostate cancer). We observed no statistically significant associations for the other IGF-1–related biomarkers with lethal prostate cancer (Table 3). There were no statistically significant differences across the 2 cohorts ($P_{heterogeneity}$ ranged from .21 to .96), and no statistically significant interaction was detected by time from blood collection to diagnosis (Supplementary Table 1, available online) or Gleason grade (Supplementary Table 2, available online).

In examining the tissue markers, we found a statistically significant interaction between *PTEN* status and PAPP-A levels and lethal prostate cancer ($P_{interaction} = .001$). Among men with intact *PTEN*, those with lethal prostate cancer had higher PAPP-A levels (adjusted difference = 0.17 ng/mL, 95% CI = 0.01 to 0.33; n = 15) compared with men with nonlethal prostate cancer (n = 178). However, among men with tumor *PTEN* loss, those with lethal prostate cancer had lower PAPP-A levels (adjusted difference = −0.38 ng/mL, 95% CI = −0.66 to −0.09; n = 9) compared with men with nonlethal prostate cancer (n = 34) (Table 4). We observed no statistically significant interactions for the other 2 tissue markers or for any of the other IGF-1 blood biomarkers.

We took advantage of the comprehensive nutrient intake information in the HPFS to explore associations with IGF biomarkers. We found that intakes of dairy-related nutrients (dairy calcium, dairy protein, and lactose) were positively associated with free IGF-1 levels, with Spearman correlation coefficients ranging from 0.11 to 0.14 (Supplementary Table 3, available online).

Discussion

In the current study, we found suggestive evidence that higher plasma PAPP-A levels prior to diagnosis in men who later develop prostate cancer are associated with an increased risk of developing lethal as compared with indolent disease. We observed no statistically significant association of free IGF-1 and other IGF-1 biomarkers with lethal as compared with nonlethal prostate cancer.

Many epidemiological studies support the associations between total IGF-1 levels and prostate cancer risk. In an earlier analysis within the PHS including 152 men who subsequently developed prostate cancer and 152 men who did not develop

Table 1. Participant characteristics in the Physicians' Health Study and the Health Professionals Follow-up Study[a]

Characteristic	Physicians' Health Study		Health Professionals Follow-up Study	
	Nonlethal prostate cancer (n = 209)	Lethal prostate cancer (n = 215)	Nonlethal prostate cancer (n = 315)	Lethal prostate cancer (n = 219)
Age at blood collection, y				
Median (IQR)	53.8 (9.4)	58.4 (10.9)	60.2 (10.5)	68.5 (12.1)
Age at diagnosis, y				
Median (IQR)	68.7 (10.6)	72.1 (13.2)	65.9 (8.0)	73.3 (12.8)
Time from blood collection to diagnosis, y				
Median (IQR)	14.8 (6.9)	13.8 (10.5)	5.9 (4.8)	5.8 (8.0)
Follow-up time from blood collection, y				
Median (IQR)	33.2 (3.0)	23.3 (10.1)	24.8 (1.5)	15.1 (9.5)
Clinical stage, No. (%)				
T1/T2	196 (96.6)	111 (68.9)	290 (95.1)	127 (71.0)
T3	2 (1.0)	10 (6.2)	11 (3.6)	7 (3.9)
T4/N1/M1	5 (2.5)	40 (24.8)	4 (1.3)	45 (25.1)
Gleason score, No. (%)				
2-6	110 (55.3)	48 (31.0)	194 (67.8)	54 (34.6)
3 + 4	31 (15.6)	31 (20.0)	44 (15.4)	27 (17.3)
4 + 3	12 (6.0)	19 (12.3)	31 (10.8)	20 (12.8)
8-10	46 (23.1)	57 (36.8)	17 (5.9)	55 (35.3)
Height, inches				
Median (IQR)	71.0 (3.0)	71.0 (3.0)	70.0 (3.0)	70.0 (4.0)
Body mass index, kg/m^2				
Median (IQR)	24.4 (2.8)	24.8 (3.0)	25.0 (3.9)	25.5 (4.2)

[a]IQR = interquartile range.

Table 2. Spearman's correlations of IGF-1–related biomarkers and age, height, and body mass index in the Physicians' Health Study and the Health Professionals Follow-up Study[a]

Biomarkers	Free IGF-1		Total IGF-1		ALS		PAPP-A		Intact IGFBP-4		Total IGFBP-4	
	r	P[b]	r	P[b]	r	P[b]	r	P[b]	r	P[b]	r	P[b]
Free IGF-1	1	–	0.02	.59	−0.12	.22	0.14	<.001	−0.11	.17	−0.02	.64
Total IGF-1	0.02	.59	1	–	0.61	<.001	−0.15	.07	−0.23	<.001	−0.08	.02
ALS	−0.12	.22	0.61	<.001	1	–	−0.27	.07	−0.24	<.001	−0.09	.007
PAPP-A	0.14	<.001	−0.15	.07	−0.27	.07	1	–	−0.12	.10	0.04	.20
Intact IGFBP-4	−0.11	.17	−0.23	<.001	−0.24	<.001	−0.12	.10	1	–	0.41	<.001
Total IGFBP-4	−0.02	.64	−0.08	.02	−0.09	.007	0.04	.20	0.41	<.001	1	–
Age at blood collection, y	−0.01	.73	−0.24	<.001	−0.15	<.001	0.05	.11	0.16	<.001	0.29	<.001
Height	0.003	.93	0.08	.01	0.04	.21	0.02	.47	−0.05	.14	0.03	.35
Body mass index	0.01	.65	−0.05	.16	0.002	.98	−0.04	.29	0.13	<.001	0.10	.002

[a]ALS = acid labile subunit; IGF-1 = insulin-like growth factor 1; IGFBP-4 = insulin-like growth factor-binding protein 4; PAPP-A = pregnancy-associated plasma protein A.
[b]Spearman correlations were used, and *P* values were 2-sided.

prostate cancer, total IGF-1 was found to be associated with prostate cancer, with the relative risk of 4.3 (95% CI = 1.8 to 10.6) comparing men in the 2 extreme quartiles of total IGF-1 levels (7). In an updated analysis in PHS, including 530 cases and 534 controls, total IGF-1 was observed to be a stronger predictor of advanced rather than early-stage prostate cancer. Men in the highest quartile of total IGF-1 levels had a relative risk of 5.1 (95% CI = 2.0 to 13.2) for advanced-stage prostate cancer compared with men in the lowest quartile of IGF-1 (12). A meta-analysis including 17 prospective studies showed similar results with a statistically significant odds ratio of 1.29 for men in the highest vs the lowest quartile of IGF-1, and the association did not differ statistically significantly by time-to-diagnosis or tumor stage or grade (11). In the present study, we included only men with prostate cancer (no cancer-free controls), as our aim was to distinguish lethal from indolent disease. This may explain the lack of a statistically significant association for total IGF-1.

Only a small proportion (approximately 1%-5%) of IGF-1 in circulation is free, unbound to binding proteins (14,15). Based on the strong evidence linking IGF-1 to prostate cancer risk, we hypothesized that free IGF-1 may represent the biologically active form and might be more strongly linked to lethal prostate cancer. This hypothesis was explored in the past, including by our group indirectly (23), through examination of various ratios of total IGF-1 to its binding proteins, rather than by direct measurement.

Table 3. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) of lethal prostate cancer according to total IGF-1, free IGF-1, ratio of total IGF-1 to free IGF-1, ALS, PAPP-A, intact IGFBP-4, and total IGFBP-4 levels in the Physicians' Health Study and the Health Professionals Follow-up Study[a,b]

Biomarkers	Category 1	Category 2	Category 3	Category 4	P_{trend}[c]
Total IGF-1[d]	1 (Referent)	0.81 (0.56 to 1.18)	0.93 (0.64 to 1.36)	0.71 (0.47 to 1.07)	.18
Free IGF-1[e]	1 (Referent)	1.28 (0.89 to 1.85)	0.93 (0.64 to 1.35)		.36
Ratio of total IGF-1 to free IGF-1[d]	1 (Referent)	1.23 (0.84 to 1.81)	1.10 (0.74 to 1.63)	0.90 (0.62 to 1.33)	.32
ALS[d]	1 (Referent)	0.73 (0.50 to 1.07)	0.98 (0.67 to 1.43)	0.69 (0.47 to 1.02)	.19
PAPP-A[f]					
Comparing quartiles	1 (Referent)	1.02 (0.68 to 1.53)	1.05 (0.69 to 1.58)	1.44 (0.98 to 2.12)	.04
Comparing PAPP-A quartile 4 vs all others	1 (Referent)	1.42 (1.04 to 1.92)			.03
Intact IGFBP-4[d]	1 (Referent)	0.75 (0.49 to 1.13)	0.73 (0.50 to 1.09)	0.81 (0.55 to 1.21)	.50
Total IGFBP-4[d]	1 (Referent)	1.23 (0.80 to 1.89)	0.99 (0.64 to 1.52)	1.49 (0.98 to 2.25)	.07

[a]Adjusted for age at blood collection (in years), height (quartiles), and body mass index (<25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2). ALS = acid labile subunit; IGF-1 = insulin-like growth factor 1; IGFBP-4 = insulin-like growth factor-binding protein 4; PAPP-A = pregnancy-associated plasma protein A.
[b]Fix-effects models were used, *P* for heterogeneity > .05 for all.
[c]Unconditional logistic regression models were used, and *P* values were 2-sided.
[d]Categories were based on quartiles.
[e]The reference group included men with undetectable free IGF-1 values, and the remaining was divided into 2 at its median.
[f]The 2 groups were men at the lowest 3 quartiles (the reference) and men at the highest quartile.

Despite the strong evidence linking IGF-1 with prostate cancer, and the use of a validated assay, we found no statistically significant association between free IGF-1 and lethal prostate cancer compared with indolent prostate cancer. Unlike many prior studies, ours was confined to men who subsequently developed prostate cancer, without the use of cancer-free controls, as we examined the hypothesis that the analytes measured prior to diagnosis might discriminate between those who develop lethal as compared with indolent disease. Perhaps free IGF-1 acts early in prostate cancer development, before tumors are distinguished as potentially lethal. This hypothesis should be explored in nested case-control studies with controls without prostate cancer. Alternatively, free IGF in the circulation could be a poor surrogate for free IGF in the prostate, where IGF bioactivity may influence prostate carcinogenesis. Although free IGF-1 has a circulating half-life of only a few minutes, this does not imply that its levels in circulation are not meaningful. By analogy, the half-life of glucose is about 16 minutes, yet circulating levels are predictive of metabolic disorder. Moreover, taking other related biomarkers in the IGF signaling pathway into account may capture more information to understand the effect of IGF-1 on lethal progression.

We found that intakes of dairy-related nutrients were positively associated with free IGF-1 levels. However, we had no specific a priori hypotheses for the nutrient analyses, and theses analyses were preliminary and exploratory. The findings are suggestive, as a meta-analysis of 5 prospective studies showed dairy products were associated with risk of advanced prostate cancer (24) and may guide future research directions.

Our data identified circulating PAPP-A as a candidate for further study as a possible correlate or determinant of risk of lethal prostate cancer. PAPP-A, originally isolated in 1974, is a protein of placental origin present abundantly in plasma of pregnant women (17). PAPP-A is a protease that cleaves IGFBPs -2, -4, and -5 (25). Whereas levels of this analyte are known to increase with pregnancy in women because of increased placental expression, little is known about the source of circulating PAPP-A in men, as well as determinants of within-person variation over time and between-person variation in men. Circulating PSA levels, an IGFBP-3 protease, is largely determined by its expression in prostate, but this has not been established for PAPP-A. In men without prostate cancer, the circulating concentration of PAPP-A is of the same order of magnitude as PSA. Recent studies have addressed the possible role of PAPP-A in cancer development, including in ovarian, renal, breast, lung, gastric cancers, pleural mesothelioma, melanoma, Ewing sarcoma, and testicular and prostate cancers (26-37). A prior study profiled prostate cancer progression from benign epithelium to metastatic disease and found PAPP-A mRNA levels were statistically significantly increased in prostate cancer (38). Mechanisms by which PAPP-A influences neoplasia are unknown, but it is plausible that it acts by increasing the concentration of free IGFs in the tumor microenvironment, leading to increased IGF-1 receptor activation and downstream signaling (4). It should be noted that the positive association between PAPP-A and lethal prostate cancer was present among men with intact *PTEN* but not among those with tumor *PTEN* loss ($P_{interaction}$ = .001). One may speculate that perhaps the strong effect of *PTEN* loss on lethal progression in prostate cancer might outweigh any effect of PAPP-A (5,39,40).

This is the first population study of the associations of plasma free IGF-1, PAPP-A, and IGFBP-4 levels with lethal prostate cancer. The biomarkers were measured in prospectively collected samples, which reduce the potential for reverse causality. Additionally, we included several potential confounders in the analyses. Although we observed no statistically significant heterogeneity across the 2 cohorts, the proportion of men with free IGF-1 levels below the limit of detection was 66% in HPFS and 22% in PHS. Because the assay was closely standardized, and all CVs below 10%, this may be because of differences in the collection and storage of blood samples. Because the analyses were conducted within each cohort and then results were pooled, we believe this difference did not materially affect our findings. Only a subset of participants had both biomarkers and tumor-related protein expression data available; the statistically significant interaction between PAPP-A and *PTEN* loss on lethal prostate cancer merits further investigation.

In conclusion, we found that free IGF-1 level measured prior to diagnosis among men who later develop prostate cancer does not discriminate between those who develop indolent as

Table 4. Adjusted means, standard errors, differences of circulating IGF biomarkers according to *TMPRSS2: ERG* fusion, phosphatase and tensin homolog (*PTEN*), and IGF-1 receptor (IGF-1R) protein expression status in the Physicians' Health Study and the Health Professionals Follow-up Study[a]

Biomarkers	Men with nonlethal prostate cancer (n = 524)	Men with lethal prostate cancer (n = 434)	Difference (95% CI)	$P_{interaction}$[b]
Total IGF-1 (ng/mL)	221 (3)	213 (3)	−7 (−16 to 1)	
ERG status				.93
ERG− (n = 174)	229 (6)	223 (15)	−6 (−39 to 27)	
ERG+ (n = 160)	227 (6)	221 (15)	−6 (−38 to 27)	
PTEN status				.64
PTEN− (n = 46)	212 (11)	228 (21)	16 (−32 to 64)	
PTEN+ (n = 198)	221 (5)	223 (15)	2.2 (−28 to 32)	
IGF-1R status				.21
Weak to none (0-1, n = 37)	225 (11)	257 (34)	32 (−41 to 105)	
Moderate (>1-2, n = 88)	229 (8)	194 (23)	−35 (−84 to 14)	
Strong (>2, n = 44)	219 (9)	220 (22)	1 (−48 to 50)	
Free IGF-1 (ng/mL)	2.20 (0.21)	2.01 (0.22)	−0.19 (−0.80 to 0.42)	
ERG status				.24
ERG− (n = 174)	1.58 (0.21)	2.52 (0.52)	0.94 (−0.17 to 2.05)	
ERG+ (n = 160)	2.63 (0.57)	1.34 (1.53)	−1.29 (−4.54 to 1.96)	
PTEN status				.20
PTEN− (n = 46)	3.81 (1.56)	1.39 (3.14)	−2.42 (−9.53 to 4.69)	
PTEN+ (n = 198)	2.04 (0.32)	3.18 (0.96)	1.15 (−0.80 to 3.09)	
IGF-1R status				.36
Weak to none (0-1, n = 37)	1.98 (0.69)	5.69 (2.09)	3.70 (−0.74 to 8.15)	
Moderate (>1-2, n = 88)	1.95 (0.39)	1.67 (1.18)	−0.28 (−2.76 to 2.20)	
Strong (>2, n = 44)	2.66 (1.03)	1.15 (2.45)	−1.51 (−6.93 to 3.91)	
Ratio of total IGF-1 to free IGF-1	417 (14)	398 (15)	−19 (−61 to 23)	
ERG status				.64
ERG− (n = 174)	419 (26)	320 (65)	−99 (−238 to 40)	
ERG+ (n = 160)	456 (31)	413 (82)	−43 (−216 to 130)	
PTEN status				.61
PTEN− (n = 46)	357 (49)	415 (97)	58 (−163 to 278)	
PTEN+ (n = 198)	431 (28)	362 (84)	−69 (−239 to 101)	
IGF-1R status				.99
Weak to none (0-1, n = 37)	421 (58)	300 (175)	−121 (−492 to 250)	
Moderate (>1-2, n = 88)	445 (36)	299 (109)	−146 (−376 to 85)	
Strong (>2, n = 44)	419 (50)	392 (117)	−26 (−286 to 233)	
ALS (mU/mL)	1609 (16)	1564 (17)	−45 (−91 to 1)	
ERG status				.31
ERG− (n = 174)	1634 (30)	1538 (74)	−96 (−255 to 62)	
ERG+ (n = 160)	1646 (31)	1648 (82)	2 (−172 to 175)	
PTEN status				.14
PTEN− (n = 46)	1511 (62)	1709 (123)	199 (−81 to 478)	
PTEN+ (n = 198)	1653 (30)	1585 (89)	−69 (−249 to 111)	
IGF-1R status				.18
Weak to none (0-1, n = 37)	1696 (60)	1874 (182)	179 (−208 to 565)	
Moderate (>1-2, n = 88)	1611 (36)	1448 (110)	−162 (−393 to 68)	
Strong (>2, n = 44)	1664 (54)	1550 (129)	−114 (−398 to 171)	
PAPP-A (ng/mL)	1.18 (0.02)	1.23 (0.02)	0.05 (0.01 to 0.10)	
ERG status				.37
ERG− (n = 168)	1.18 (0.03)	1.12 (0.07)	−0.06 (−0.22 to 0.09)	
ERG+ (n = 153)	1.20 (0.03)	1.22 (0.08)	0.03 (−0.15 to 0.20)	
PTEN status				.001
PTEN− (n = 43)	1.25 (0.07)	0.87 (0.12)	−0.38 (−0.66 to −0.09)	
PTEN+ (n = 193)	1.21 (0.03)	1.38 (0.08)	0.17 (0.01 to 0.33)	
IGF-1R status				.66
Weak to none (0-1, n = 34)	1.20 (0.06)	1.10 (0.18)	−0.09 (−0.48 to 0.30)	
Moderate (>1-2, n = 84)	1.21 (0.04)	1.08 (0.12)	−0.13 (−0.39 to 0.12)	
Strong (>2, n = 43)	1.15 (0.07)	1.21 (0.17)	0.06 (−0.31 to 0.43)	
Intact IGFBP-4 (ng/mL)	62.8 (1.0)	61.9 (1.1)	−0.9 (−4.0 to 2.2)	
ERG status				.55
ERG− (n = 169)	62.4 (2.1)	62.8 (5.1)	0.3 (−10.6 to 11.2)	
ERG+ (n = 153)	64.5 (2.1)	59.5 (5.5)	−5.0 (−16.7. 6.7)	

(continued)

Table 4. (continued)

Biomarkers	Men with nonlethal prostate cancer (n = 524)	Men with lethal prostate cancer (n = 434)	Difference (95% CI)	$P_{interaction}$[b]
PTEN status				.42
PTEN− (n = 43)	68.8 (4.1)	55.9 (7.7)	−12.9 (−30.7 to 4.8)	
PTEN+ (n = 193)	64.5 (2.1)	60.8 (6.2)	−3.6 (−16.2 to 8.9)	
IGF-1R status				.11
Weak to none (0-1, n = 34)	62.4 (3.6)	65.7 (10.6)	3.3 (−19.6. 26.2)	
Moderate (>1-2, n = 85)	61.8 (2.6)	65.5 (8.3)	3.7 (−13.7 to 21.2)	
Strong (>2, n = 43)	62.6 (2.8)	41.2 (6.4)	−21.4 (−35.7 to 7.1)	
Total IGFBP-4 (ng/mL)	128 (2)	131 (2)	3 (−2 to 8)	
ERG status				.98
ERG− (n = 168)	133 (4)	140 (11)	7 (−17 to 31)	
ERG+ (n = 153)	125 (2)	135 (6)	10 (−2 to 23)	
PTEN status				.87
PTEN− (n = 43)	122 (4)	128 (7)	5 (−11 to 22)	
PTEN+ (n = 192)	134 (4)	143 (13)	9 (−17 to 35)	
IGF-1R status				.87
Weak to none (0-1, n = 33)	127 (5)	143 (15)	15 (−17 to 47)	
Moderate (>1-2, n = 85)	129 (8)	126 (24)	−3 (−53 to 47)	
Strong (>2, n = 43)	129 (6)	137 (13)	8 (−21 to 38)	

[a]Adjusted for age at blood collection (in years), height (in inches), body mass index (in kg/m^2), and cohort. ALS = acid labile subunit; IGF-1 = insulin-like growth factor 1; IGFBP-4 = insulin-like growth factor-binding protein 4; PAPP-A = pregnancy-associated plasma protein A.
[b]Linear regression models were used, and *P* values were 2-sided.

compared with lethal disease but does provide suggestive evidence that higher PAPP-A levels are associated with an increased risk of developing lethal prostate cancer. This observation should be evaluated in other cohorts, and it also will be of interest to determine if PAPP-A level prior to diagnosis is predictive of aggressive prostate cancer, where the control group is cancer-free.

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Data Availability

The datasets analyzed in the current study are not publicly available because of restricted access, but further information about the datasets is available from the corresponding author on reasonable request.

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