Plasma Levels of Acid-Labile Subunit, Free Insulin-Like Growth Factor-I, and Prostate Cancer Risk: A Prospective Study

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Abstract

Background: The acid-labile subunit (ALS) acts in the insulin-like growth (IGF) system by binding circulating IGF-I in a ternary complex with binding protein (IGFBP)-3 to prevent IGF-I from crossing the endothelial barrier. Given the role of the IGF system in prostate cancer, ALS may influence carcinogenesis by modulating IGF-I levels or bioavailability.

Methods: We undertook a prospective study nested in the Physicians' Health Study to examine ALS, free IGF-I, and prostate cancer. We assayed circulating levels of ALS and IGF components among 545 incident cases and 545 matched controls. We calculated relative risks (RR) and 95% confidence intervals (95% CI) adjusted for life-style factors, total IGF-I, and IGFBP3.

Results: ALS was positively correlated with total IGF-I (r = 0.58), IGFBP3 (r = 0.68), and free IGF-I (r = 0.36). Comparing highest versus lowest quartiles, we found no association between free IGF-I and prostate cancer risk (RR, 0.9; 95% CI, 0.6-1.3). In contrast, ALS was positively associated with risk among men in the 2nd (RR, 1.5; 94% CI, 1.0-2.3), 3rd (RR, 1.6; 94% CI, 1.1-2.5), and 4th quartiles (RR, 1.4; 94% CI, 0.9-2.1) compared with lowest quartile. The association was stronger for advanced stage tumors (RR, 2.0; 94% CI, 0.8-4.6). There was a suggestion of an interaction between ALS and total IGF-I, whereby high circulating IGF-I was associated with an increased risk of advanced prostate cancer among men with low but not higher ALS levels.

Discussion: Plasma ALS is positively associated with prostate cancer risk, and may interact biologically with IGF-I to affect carcinogenesis. These data provide further support for the role of the IGF axis in prostate cancer.

Introduction

Growing epidemiologic evidence implicates components of the insulin-like growth factor (IGF) axis in prostate carcinogenesis. In prospective studies, higher circulating levels of total IGF-I have been associated with an increased risk of prostate cancer (1-6), particularly for advanced stage disease (2, 6). By binding with the IGF-receptor, IGF-I imparts mitogenic and antiapoptotic effects on prostate epithelium (7). IGF is bound in circulation with a family of binding proteins, predominantly binding protein-3 (IGFBP3). Circulating levels of IGFBP3 may affect risk by modulating the bioavailability of IGF-I (7-9), by competing for the IGF receptors (10), or by independently promoting apoptosis (8, 9). Some studies have found that higher circulating IGFBP3 are linked with lower risk (11). Still, the epidemiologic data are not completely convergent because some studies have found no association between total IGF-I (12, 13) or IGFBP3 (6, 12) and prostate cancer risk. A recent pooled analysis of individual level data found a relative risk (RR) of 1.4 [95% confidence interval (95% CI), 1.2-1.6] comparing extreme quintiles of IGF-I, and no association for IGFBP3 after accounting for IGF-I levels (11).

Approximately 80% to 85% of circulating IGF-I is bound in a ternary complex with its binding protein and the glycoprotein acid-labile subunit (ALS; refs. 14, 15). Synthesis of ALS occurs primarily in the liver, although local synthesis may occur in some tissues (16) under regulation by growth hormone (17), which also controls circulating IGF-I and IGFBP3 levels. Indeed, reductions in levels of the subunit are observed in conditions categorized as growth hormone deficient (15).

ALS has no affinity for free IGF-I, has very low affinity for uncomplexed IGFBP3, but readily binds to binary complexes of IGF-I bound to IGFBP3. Given its high affinity, ALS in high concentrations leaves little free IGF-I in circulation. In the ternary complex, IGF-I cannot cross

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the endothelial cell barrier (16). As a consequence, its half-life in the circulation is prolonged, from 10 minutes in the free IGF-I form to 12 hours in the ternary complex (16). Levels of ALS seem to directly affect both IGF-I and IGFBP3 levels. In a mouse model with an inactivated *ALS* gene, circulating levels of IGF-I and IGFBP3 were substantially reduced compared with the wild-type genotype (18). This observation is striking given a lack of reduction in IGF-I or IGFBP3 synthesis, as evidenced by retained gene expression. Similarly, in a case report of a young patient with a genetic mutation that completely inactivated the *ALS* gene, there were marked decreases in serum levels of total IGF-I and IGFBP3 (19). Treatment of the patient with growth hormone stimulated levels of IGF-I and IGFBP3, albeit below normal levels, whereas the levels of the subunit remained undetectable. Taken together, these data suggest that ALS is crucial in preventing the degradation of IGF-I and IGFBP3 in circulation. On the other hand, there is evidence that the overexpression of the *ALS* gene is associated with impaired postnatal growth in animal models, which is likely due to alterations in the tissue availability of IGF rather than circulating levels (20).

ALS seems to have direct physiologic effects on the circulating and tissue concentrations of IGF. Given its importance in modulating the availability IGF-I and IGFBP3, ALS may play a role in prostate carcinogenesis. Higher concentrations of ALS may be associated with a lower risk of prostate cancer risk by decreasing the bioavailability of IGF-I and prohibiting IGF-I access to tissue. Alternatively, the subunit may protect against the degradation of IGF-I and IGFBP3 in circulation, and thus may prolong their respective biological effects.

Prior epidemiologic studies of IGF-I and prostate cancer have generally measured total levels in circulation, rather than the free form. Moreover, no published study has assessed the role of ALS within the normal physiologic range on prostate carcinogenesis and progression. To this end, we undertook a nested case-control study within the Physicians' Health Study to examine the relation between ALS, free IGF-I, and prostate cancer risk using prospectively collected plasma samples.

Materials and Methods

Study Population

Initiated in 1982, the Physicians' Health Study was a randomized controlled trial of aspirin and β-carotene supplementation among 22,071 U.S. male physicians ages 40 to 84 y. Participants were excluded from the study if they had (*a*) previous diagnoses of myocardial infarction, stroke, transient ischemic attack, or cancer (except nonmelanoma skin cancer); (*b*) current renal or liver disease, peptic ulcer, or gout; and (*c*) current use of aspirin, vitamin A, or β-carotene supplements. Participants are followed through annual questionnaires to collect data on diet, health and life-style behaviors, and medical history, and biannually through postcards to ascertain compliance and health end points, including prostate cancer. Follow-up of the participants for morbidity and mortality is 99% complete (21).

During 1982 to 1984, physicians were sent blood collection kits to obtain baseline blood samples before randomization. Prospectively collected plasma samples are available from 14,916 (68%) of physicians who represent the study base for the nested case-control study. The Physicians' Health Study specimens were continuously frozen at −82°C (and more recently at −142°C).

Case-Control Ascertainment

At each of the questionnaire and postcard mailings, physicians reported if they have been diagnosed with prostate cancer during the previous 6 months. The study investigators confirmed the report of prostate cancer through the review of medical records and pathology reports. This information was further used to describe the cancers pathologically. We defined advanced disease as those cases with advanced stage at diagnosis ($T_3/T_4/M_1/N_1$) or cases that died of prostate cancer. Localized cases were those with stage T_1 or T_2 cancer confined to the prostate and those who did not die of prostate cancer during the follow-up. Cancers were further classified on histologic grade and were defined as high-grade (Gleason score of ≥7 or poorly differentiated tumors) or low-grade (Gleason score of <7 or well differentiated) tumors. Through December 1995, 786 incident prostate cancer cases arose in the cohort of physicians who initially provided blood samples.

We sought to select controls randomly from participants who had not had a partial or total prostatectomy or prostate cancer by the date of the case's diagnosis, and who had sufficient plasma for biochemical analyses. Controls were matched to cases on age (±1 and ±5 y for elderly participants) and smoking status (current, former, or never).

Measurement of the IGF Axis

Plasma levels of free IGF-I and ALS were measured in the prospectively collected plasma in the laboratory of Dr. Michael Pollak. Data on IGF-I and IGFBP3 were previously measured in Dr. Pollak's laboratory and were included in the previous publication by Chan et al. (2). The mean time between collection of blood and case diagnosis was 8.5 y (range, 0.2-13.2 y). All assays were conducted using ELISAs. Case-control pairs were assayed in adjoining wells, with blinding to case-control status. Assays for total IGF-I and IGFBP3 were conducted in three batches using reagents from the Diagnostic Systems Laboratory, as previously described (2). Analyses of the subunit and free IGF-I were conducted in one batch. Included in the analyses were 545 cases and their matched controls that had sufficient plasma for biomarker assays; of the cases, 161 were advanced stage at diagnosis or died of prostate cancer during follow-up, and 177 had high tumor grade. Free IGF-I data were not available for six controls and one case, and thus, data on free

Table 1. Correlation coefficients between IGF-related factors in plasma among controls (n = 545) at baseline, Physicians' Health Study, 1982 to 1984

	ALS	Free IGF-I	Total IGF-I	IGFBP3	Age
Acid-labile subunit	1.0				
Free IGF-I	0.36	1.0			
Total IGF-I	0.58	0.39	1.0		
IGFBP3	0.68	0.23	0.67	1.0	
Age	−0.28	−0.09	−0.32	−0.22	1.0

NOTE: P values for Pearson correlations between IGF components were <0.001, and P values for Pearson correlations between IGF components and age were <0.05. Data on free IGF-I was available for 538 controls.

IGF-I are based on 538 matched pairs. Intra-assay coefficients of variations were 9.5% for ALS and 5.8% for free IGF-I.

Statistical Analysis

All analyses were undertaken using the SAS statistical software (version 9).

We calculated the Pearson correlation coefficients to examine the correlation between the IGF-related components among the controls. Because IGF-I and IGFBP3 were measured in three different batches, we calculated partial correlation coefficients adjusted for batch differences. We used the least square means procedure to estimate age-adjusted mean levels of ALS and free IGF-I in relation to baseline characteristics among the controls.

Conditional logistic regression models were used to examine the relation between ALS and free-IGF-I and prostate cancer risk, controlling for potential confounders. Conditional analyses also adjust for potential drift in the laboratory assays because case-control pair samples are run in adjoining wells on the same plates, and thus, analyses are essentially stratified on batch. We categorized men into quartiles, based on the distribution of ALS and free IGF-I levels in the control group. Odds ratios, as an estimate of the RR, and 95% CIs were calculated, with the lowest quartile as the reference category. To assess the linearity of ALS and free IGF-I, and prostate cancer risk on the log scale, the median of quartiles were modeled and the results of trend tests are presented.

Conditional logistic regression models were automatically adjusted for the matching factors age and smoking. We further considered as potential confounders the following variables measured at baseline: height (continuous), body mass index (BMI; categorical: <25.0, 25.0-29.9, 30.0+), vigorous physical activity (categorical: 5+ per week, less often/none), alcohol intake (categorical: >1 drinks per day, <1 per day/none), tomato consumption (categorical: >1 servings per day, less often/none), and multivitamin use (categorical: current, former, never). Covariates that changed the main effects for ALS or free IGF-I by >10%, or were significant at $\alpha = 0.20$ were retained in the models. Based on the biological interrelationships of the IGF axis, we also adjusted for total IGF-I and IGFBP3 in subsequent analyses using batch-specific cutpoints.

As previously reported in the literature, total IGF-I and IGFBP3 seem to be more strongly linked to more advanced tumors (2, 6). Thus, we examined the association between ALS and free IGF-I levels stratified by prostate cancer tumor stage and grade. We also addressed whether these associations differed as a function of IGF-I and IGFBP3, by cross-classifying individuals on tertiles of these hormones.

This project was approved by the Institutional Review Board of Partners Healthcare.

Results

Among controls, the mean (SD) plasma level was 15.5 μg/mL (3.6) for ALS and 0.48 ng/mL (0.24) for free IGF-I. The molar concentration of the subunit (177 nmol/L) was 7.2-fold higher than total IGF-I (24.7 nmol/L) and 1.7-fold higher than IGFBP3 (103 nmol/L). Levels of the subunit were positively correlated with total IGF-I (r = 0.58), IGFBP3 (r = 0.68), and to a lesser extent, free IGF-I (r = 0.36; Table 1). There was a significant but more moderate positive correlation between free and total IGF-I levels. Levels of each component of the IGF system decreased with increasing age at baseline.

In Table 2, we examine the differences in mean plasma levels of ALS and free IGF-I by baseline covariates among the controls. Given the strong relation between age and IGF plasma levels, data in this table are age adjusted. Taller men tended to have lower levels of ALS. Greater physical activity and higher intake of tomatoes were modestly associated with lower levels of the subunit, even after adjusting for age. Men who were current smokers had lower levels of free IGF-I, whereas taller men had higher levels.

Table 3 shows the association of ALS and free IGF-I with prostate cancer from three modeling approaches. First, we adjust for the matching factors only. Second,

we present data from the multivariable model additionally adjusted for BMI and height. Finally, in the multivariable model, we further controlled for total IGF-I and IGFBP3 levels. Adjusting for the prostate cancer risk factors, higher levels of ALS were associated with an increased risk of total prostate cancer. The elevation in risk was apparent beginning in the 2nd quartile, with a 50% higher risk compared with the lowest quartile. The data suggest a threshold effect, with no further increase in risk after the second quartile. Combining the 2nd through 4th quartiles, the RR was 1.6 (95% CI, 1.1-2.2). Controlling for total IGF-I and IGFBP3 did not appreciably change the RRs, suggesting that the association with the subunit was independent of the other IGF ternary components in circulation. To address concerns that the positive association with ALS could be due to the influence of subclinical disease on tumor production of the IGF-components, we excluded cases (n = 49) that were diagnosed during the first 3 years of follow-up. The results were the same (data available upon request), suggesting that the association with the subunit was not due to reverse causality. We observed no significant association between plasma levels of free IGF-I and total prostate cancer risk in any of the analyses (Table 3).

Because of prior observations that the associations for total IGF-I and IGFBP3 were stronger for advanced disease (2, 6), we examined the relation between ALS and free-IGF-I stratified by cancer stage and tumor grade (Table 4). In line with the previous study, we found a suggestion that the association of the subunit was stronger for advanced (stage $T_3/T_4/N_1$ or lethal prostate cancer) versus early-stage (stage $T_{1/2}$) cancers. Comparing the highest and lowest quartiles, the subunit was associated with a RR of 2.0 (95% CI, 0.8-4.6) for advanced prostate cancer and 1.5 (95% CI, 0.8-2.8) for early-stage disease. Moreover, the increased risk associated with the subunit was restricted to the lower grade tumors, with no association between higher levels of the subunit and high-grade prostate cancer. The association between free IGF-I and prostate cancer risk was consistently null for advanced and early-stage tumors, and for high versus low-grade cancers (Table 4).

The three components of the IGF system, IGF-I, IGFBP3, and the subunit, interact biologically and this interplay influences the bioavailability and the potential function of these components. Thus, we cross-classified individuals on tertiles of the subunit and separately for total IGF-I (Table 5A), free IGF-I (Table 5B), or IGFBP3 (Table 5C). There was a suggestion that the effect of total

Table 2. Age-adjusted mean (SEM) levels of ALS and free IGF-I according to baseline characteristics among controls, Physicians' Health Study, 1982 to 1984

	*n**	ALS, μg/mL (SEM) *n* = 545	Free IGF-I, ng/mL (SEM) *n* = 538
Smoking status			
Current	47	15.4 (0.6)	0.40 (0.02)
Former	237	15.5 (0.2)	0.48 (0.01)
Never	261	15.4 (0.2)	0.49 (0.02)
Alcohol use			
Daily or more	167	15.6 (0.3)	0.47 (0.03)
Less often	372	15.2 (0.2)	0.47 (0.01)
Vigorous exercise			
5+ times/wk	91	14.9 (0.4)	0.44 (0.02)
1-4 times/wk	294	15.6 (0.2)	0.48 (0.01)
Less often	156	15.4 (0.3)	0.49 (0.03)
Tomato intake			
5 or more per week	167	15.2 (0.3)	0.45 (0.01)
Less/none	373	15.5 (0.2)	0.48 (0.01)
BMI, kg/m^2			
<23.0	138	15.0 (0.3)	0.46 (0.02)
23.0-24.9	184	15.4 (0.3)	0.50 (0.02)
25.0-29.9	209	15.6 (0.3)	0.46 (0.01)
30.0+	14	15.2 (1.0)	0.42 (0.06)
Height, inches			
<68	85	15.9 (0.4)	0.47 (0.02)
68-72	372	15.3 (0.2)	0.46 (0.01)
73+	88	15.2 (0.4)	0.52 (0.05)

*Sample size among *n* = 545 controls with data on ALS; ns may not add up because of missing data on specific covariates.

IGF-I ($P_{interaction}$ = 0.10) differed as a function of levels of ALS. The increase in risk of advanced prostate cancer associated with total IGF-I is confined to those in the lowest levels of ALS, with a RR of 9.3 (95% CI, 1.7-51.3) comparing the highest with lowest tertiles of total IGF-I, although the 95% CIs are wide. There is no evidence of a positive association between total IGF-I and advanced disease among those with higher levels of the subunit. Although we observed no effect of free IGF-I overall, men with low ALS had a 3.3-fold (95% CI, 0.7-15.4) increased risk of advanced disease associated with higher free IGF-I. Among controls, 3% had both high total IGF-I and low ALS and 5% had both high free IGF-I and low ALS. The data suggest that the protective effect of IGFBP3 is consistent across ALS levels (P = 0.84).

Discussion

In this large, prospective study nested within the Physicians' Health Study, we observed that circulating ALS was associated with a small increase in total prostate cancer risk, with an apparent threshold effect after the second quartile of the subunit. The association was consistent even after controlling for circulating levels of other IGF components. Our data further suggest that the association of ALS was stronger for advanced tumors, although the findings were not statistically significant. In an earlier analysis within this cohort, Chan et al. (2) noted that total IGF-I and IGFBP3 levels were more strongly associated with advanced rather than earlier stage disease. In light of these earlier findings, the data suggest that circulating ALS may influence the progression and aggressiveness of prostate cancer.

In our data, we also noted a stronger effect of the subunit on lower grade versus higher grade prostate cancer. Although high tumor grade is an important risk factor for prostate cancer death, (22, 23) this finding could indicate that higher grade cancer represents more autonomous tumors, whereas the more differentiated, lower grade cancer may be more susceptible to regulation and influence by levels of growth factors. A positive finding with low-grade prostate cancer has been shown previously for IGF-I (2).

Circulating levels of ALS varied as a function of life-style factors. Levels of the subunit were lower among men with greater physical activity and increased tomato consumption. These findings are in line with prior research showing that circulating levels of IGF-I may be modulated by life-style factors (24, 25). If confirmed, these findings would add further evidence that diet and life-style affect prostate cancer risk and progression through the IGF system.

We saw suggestive evidence that the effect of ALS on advanced prostate cancer varied as a function of the other IGF components. High ALS was associated with a 3- to 6-fold increased risk among those with lower levels of total IGF-I, free IGF-I, or IGFBP3, whereas at the highest levels, there was no association with ALS. These interactions among the IGF components were evaluated based on biological plausibility. However, some strata are based on small numbers of case-control pairs, and therefore, these findings should be viewed cautiously.

It is unlikely that ALS itself has direct carcinogenic potential. Because expression of ALS is under growth hormone stimulation (17), the positive associations may reflect some other components regulated by growth hormone. For example, sex steroid hormones can influence the secretion of growth hormone, and can affect the production or clearance of IGF-I (26).

More likely, ALS affects prostate cancer risk and progression through its influence on IGF-I and IGFBP3 availability. Indeed, we noted that the associations between total and free IGF-I on advanced prostate cancer were influenced by levels of circulating ALS. The substantial increase in risk associated with total IGF-I was limited to those with the lowest levels of the subunit. This finding

Table 3. RR and 95% CI of total prostate cancer associated with ALS and free IGF-I, Physicians' Health Study, 1982 to 1995

	Quartiles				P_{trend}
	Low (Q1)	Q2	Q3	High (Q4)	
ALS, mean µg/mL (range) among controls	10.7 (2.9-12.9)	14.3 (12.9-15.5)	16.7 (15.5-18.1)	20.4 (18.1-29.0)	
RR (95% CI): adjusted for age/smoking	1.0 (Reference)	1.5 (1.0-2.1)	1.4 (1.0-2.0)	1.2 (0.8-1.7)	0.56
RR (95% CI): multivariate adjusted*	1.0 (Reference)	1.5 (1.0-2.1)	1.4 (1.0-2.0)	1.2 (0.8-1.7)	0.63
RR (95% CI): multivariate adjusted†	1.0 (Reference)	1.5 (1.0-2.3)	1.6 (1.1-2.5)	1.4 (0.9-2.1)	0.28
Free IGF-I, mean ng/mL (range) among controls	0.27 (0.07-0.35)	0.40 (0.35-0.44)	0.50 (0.44-0.57)	0.74 (0.57-4.02)	
RR (95% CI): adjusted for age/smoking	1.0 (Reference)	0.8 (0.6-1.1)	1.1 (0.8-1.5)	1.0 (0.7-1.4)	0.73
RR (95% CI): multivariate adjusted*	1.0 (Reference)	0.7 (0.5-1.0)	1.0 (0.7-1.5)	0.9 (0.7-1.3)	0.90
RR (95% CI): multivariate adjusted†	1.0 (Reference)	0.7 (0.5-1.0)	1.0 (0.7-1.4)	0.9 (0.6-1.3)	0.78

*Adjusted for age, smoking status, height, and BMI in conditional logistic regression model.
†In addition, adjusted for total IGF-I and IGFBP3.

Table 4. RR and 95% CI of prostate cancer associated with quartiles of ALS and free IGF-I, by cancer stage and grade, Physicians' Health Study, 1982 to 1995

	n case-control pairs	Quartiles				P_{trend}
		Low (Q1)	Q2	Q3	High (Q4)	
ALS						
Tumor stage						
Advanced disease*	161	1.0 (Reference)	1.7 (0.8-3.4)	2.2 (1.0-4.7)	2.0 (0.8-4.6)	0.11
Localized disease†	304	1.0 (Reference)	1.5 (0.9-2.7)	1.4 (0.8-2.5)	1.5 (0.8-2.8)	0.32
Tumor grade						
High tumor grade‡	177	1.0 (Reference)	1.0 (0.5-2.0)	1.7 (0.8-3.6)	1.0 (0.4-2.5)	0.62
Low tumor grade§	317	1.0 (Reference)	1.9 (1.1-3.3)	1.7 (1.0-3.0)	1.4 (0.8-2.6)	0.55
Free IGF-I						
Tumor stage						
Advanced disease*	158	1.0 (Reference)	1.2 (0.6-2.5)	1.2 (0.6-2.6)	0.9 (0.4-2.0)	0.83
Localized disease†	302	1.0 (Reference)	0.5 (0.3-0.8)	0.9 (0.6-1.4)	0.7 (0.4-1.1)	0.43
Tumor grade						
High tumor grade‡	176	1.0 (Reference)	1.3 (0.7-2.4)	0.9 (0.5-1.8)	1.2 (0.6-2.4)	0.79
Low tumor grade§	313	1.0 (Reference)	0.5 (0.3-0.8)	1.1 (0.7-1.7)	0.7 (0.4-1.2)	0.64

NOTE: Data adjusted for age, smoking status, height, BMI, total IGF-I, and IGFBP3 using conditional logistic regression.
*Advanced disease: stage $T_3/T_4/N_1/M1$ at cancer diagnosis, or death from prostate cancer.
†Localized disease: stage T_1 or T_2 at cancer diagnosis and did not die from prostate cancer.
‡High grade: tumor graded as Gleason score of 7 or higher or poorly differentiated.
§Low grade: tumor graded as Gleason score of 6 or lower or well differentiated.

is intriguing in light of the observation that ALS circulates in excess compared with total IGF-I and its BP-3, shown in our own data as well as other studies (16, 27). Although based on a smaller number of advanced cancers, these data suggest a complex biological interplay of the IGF components on prostate cancer progression. For example, higher ALS may be more likely to restrict IGF-I to the circulation, and in this way, the local IGF effects on the prostate epithelium are mitigated. Such an effect has been observed in animal models in which overexpression of the *ALS* gene reduces the availability of IGF at the tissue level (20). Among individuals with low levels of ALS, in contrast, the bioavailability of IGF-I seems optimized. A larger, prospective study with sufficient numbers of advanced/lethal prostate cancer cases would help clarify the potential interaction of the IGF components.

We found no overall association between levels of free IGF-I and total prostate cancer risk, nor evidence of an association with advanced stage or lower grade tumors. The coefficients of variation for this assay were relatively good, suggesting that substantial measurement error in the assay is not likely. Although there is some debate that the free IGF-I assay is only measuring a subset of the more bioavailable IGF-I in the circulation, free IGF-I measured is this way does seem to have physiologic correlates (28), suggesting that the assay is capturing important information.

Bound in the ternary complex, the half-life of IGFBP3 is prolonged as well. IGFBP3 has been shown to have independent proapoptotic and cellular growth inhibition effects (14) over and beyond its influence on IGF-I bioavailability. We observed the strong protective effect of IGFBP3 among individuals with either high or low ALS, suggesting that ALS does not affect the independent effects of IGFBP3.

There are some strengths and limitations to consider in assessing the study findings. The IGF components were measured using prospectively collected samples, which reduce the potential for reverse causality. In a subgroup analysis, the exclusion of cases occurring during the first 3 years of follow-up did not influence the study outcome. The blood measurements were based on samples collected at only one point in time and may not reflect long-term changes or variations in levels. In a pilot study in the Physicians' Health Study, we collected blood specimens twice during a 5-year interval among 79 men. We assayed for free and total IGF-I in the specimens and found a high within person correlation (r = 0.70 and 0.75, respectively) between the assays across time points. These findings agree with published data from the Health Professionals Follow-up Study, which used similar blood collection and storage methods as in the Physicians' Health Study. Levels of IGF-I and IGFBP3 were assayed on blood specimens from 149 individuals

Table 5. RR (95% CI) of advanced prostate cancer associated with tertiles of ALS and total IGF-I, free IGF-I, or IGFBP3, cross-classified, Physicians' Health Study, 1982 to 1995

A. RR (95% CI) of advanced prostate cancer associated with tertiles of ALS and total IGF-I;* $P_{interaction}$ = 0.10

		Total IGF-I (tertiles) *n* exposed cases/RR (95% CI)		
		Low	**Medium**	**High**
ALS (tertiles)	Low	26	11	8
		Reference	1.5 (0.5-4.7)	9.3 (1.7-51.3)
	Medium	15	17	23
		4.0 (1.1-14.0)	2.6 (0.9-7.5)	6.0 (1.9-18.9)
	High	10	28	22
		3.1 (0.9-10.8)	6.6 (2.1-20.4)	5.0 (1.5-16.1)

B. RR (95% CI) of advanced prostate cancer associated with tertiles of ALS and free IGF-I;* $P_{interaction}$ = 0.29

		Free IGF-I (tertiles) *n* exposed cases/RR (95% CI)		
		Low	**Medium**	**High**
ALS (tertiles)	Low	21	14	8
		Reference	1.4 (0.5-3.9)	3.3 (0.7-15.4)
	Medium	17	18	19
		4.1 (1.3-13.5)	2.7 (0.9-8.5)	3.3 (1.0-10.5)
	High	11	20	29
		3.8 (1.1-13.3)	7.1 (2.1-24.2)	3.2 (1.1-9.2)

C. RR (95% CI) of advanced prostate cancer associated with tertiles of ALS and IGFBP3;† $P_{interaction}$ = 0.84

		IGFBP3 (tertiles) *n* exposed cases/RR (95% CI)		
		Low	**Medium**	**High**
ALS (tertiles)	Low	32	10	3
		Reference	0.8 (0.2-2.6)	0.4 (0.1-2.9)
	Medium	14	30	11
		1.8 (0.6-5.3)	1.6 (0.6-4.1)	0.5 (0.1-1.5)
	High	8	22	30
		6.1 (1.2-30.1)	2.0 (0.7-5.7)	0.7 (0.3-1.6)

*Data are adjusted for age, smoking, height, BMI, and IGFBP3 using conditional logistic regression.
†Data are adjusted for age, smoking, height, BMI, and total IGF-I using conditional logistic regression.

collected twice at a 3-year interval. For IGF-I and IGFBP3, the correlation coefficients ranged between 0.6 and 0.7. These correlations, which incorporate both measurement error as well as biological variation over time, indicate that a single measure provides a reasonable time-integrated level. Therefore, IGF measured at a single time point provides a reasonable estimate of exposure across time. We did not have pilot data for levels of ALS, however. The blood samples included both fasting and nonfasting samples, which may have a small effect on circulating levels of free IGF-I. The case-control study was nested within a well-defined cohort, and thus, selection bias should not be a concern. In the analysis, we considered several potential confounders that should reduce the likelihood of residual confounding of the association of circulating levels of ALS and prostate cancer risk. It is, however, possible that the possible associations for the subunit may reflect residual confounding by circulating levels of IGF-I or indicate higher levels of other components under growth hormone control that correlated with ALS. A larger, prospective study with additional measured components of the IGF system would help disentangle the issue of potential confounding. Information on family history of prostate cancer was not available from the baseline questionnaire.

In conclusion, higher levels of circulating ALS are associated with a modest increased risk of prostate cancer, particularly for advanced disease. ALS seems to interact biologically with total and free IGF-I in directing their effects on the prostate epithelium. Our data highlight the complexity of the IGF axis in prostate carcinogenesis and progression, and the need to comprehensively assess the IGF pathway to disentangle the

individual and joint effects of the ligands, their binding proteins, as well as additional IGF components.

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References

1. 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.
2. 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.
3. 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.
4. Allen NE, Key TJ, Appleby PN, et al. 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. Cancer Epidemiol Biomarkers Prev 2007;16:1121–7.
5. 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.
6. Stattin P, Rinaldi S, Biessy C, Stenman UH, Hallmans G, Kaaks R. High levels of circulating insulin-like growth factor-I increase prostate cancer risk: a prospective study in a population-based nonscreened cohort. J Clin Oncol 2004;22:3104–12.
7. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. J Natl Cancer Inst 2000;92:1472–89.
8. Furstenberger G, Senn HJ. Insulin-like growth factors and cancer. Lancet Oncol 2002;3:298–302.
9. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505–18.
10. Kostecka Y, Blahovec J. Insulin-like growth factor binding proteins and their functions (minireview). Endocr Regul 1999;33:90–4.
11. Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, 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.
12. 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.
13. Lacey JV, Jr., 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.
14. Baxter RC. Insulin-like growth factor binding proteins in the human circulation: a review. Horm Res 1994;42:140–4.
15. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824–54.
16. Boisclair YR, Rhoads RP, Ueki I, Wang J, Ooi GT. The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system. J Endocrinol 2001;170:63–70.
17. Juul A, Andersson AM, Pedersen SA, et al. Effects of growth hormone replacement therapy on IGF-related parameters and on the pituitary-gonadal axis in GH-deficient males. A double-blind, placebo-controlled crossover study. Horm Res 1998;49:269–78.
18. Ueki I, Ooi GT, Tremblay ML, Hurst KR, Bach LA, Boisclair YR. Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. Proc Natl Acad Sci U S A 2000;97:6868–73.
19. Domene HM, Bengolea SV, Martinez AS, et al. Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. N Engl J Med 2004;350: 570–7.
20. Silha JV, Gui Y, Modric T, et al. Overexpression of the acid-labile subunit of the IGF ternary complex in transgenic mice. Endocrinology 2001;142:4305–13.
21. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with β carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334: 1145–9.
22. Johansson JE, Andren O, Andersson SO, et al. Natural history of early, localized prostate cancer. JAMA 2004;291:2713–9.
23. D'Amico AV, Cote K, Loffredo M, Renshaw AA, Chen MH. Pretreatment predictors of time to cancer specific death after prostate specific antigen failure. J Urol 2003;169:1320–4.
24. Giovannucci E, Pollak M, Liu Y, et al. Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. Cancer Epidemiol Biomarkers Prev 2003;12:84–9.
25. Mucci LA, Tamimi R, Lagiou P, et al. Are dietary influences on the risk of prostate cancer mediated through insulin-like growth factor system? BJU Int 2001;87:814–20.
26. Holly JM, Gunnell DJ, Davey Smith G. Growth hormone, IGF-I and cancer. Less intervention to avoid cancer? More intervention to prevent cancer? J Endocrinol 1999;162:321–30.
27. Baxter RC. Circulating levels and molecular distribution of the acid-labile α subunit of the high molecular weight insulin-like growth factor binding complex. J Clin Endocrinol Metab 1990;70:1347–53.
28. Li HJ, Ji CY, Wang W, Hu YH. A twin study for serum leptin, soluble leptin receptor, and free insulin-like growth factor-I in pubertal females. J Clin Endocrinol Metab 2005;90:3659–64.