Insulin-Like Growth Factor-I, IGF Binding Protein-3, and Breast Cancer in Young Women: A Comparison of Risk Estimates Using Different Peptide Assays

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Abstract

Circulating insulin-like growth factor-I (IGF-I) and its major binding protein IGF binding protein-3 (IGFBP-3) have been associated with increased risk of premenopausal breast cancer, although risk estimates varied broadly. An extension of a case-control study (138 cases, 259 matched controls) on IGF-I and breast cancer in premenopausal women nested in the New York University Women's Health Study cohort offered the opportunity to address the hypothesis that such variability may have been the result of variations in the ability of different IGFBP-3 assays to specifically measure intact/functional forms of the protein. IGF-I and IGFBP-3 had originally been measured using in-house RIAs. These measurements were repeated using commercially available ELISAs [Diagnostic System Laboratories (DSL), Webster, Texas], and a third ELISA with greater specificity for active forms for IGFBP-3. Pearson's correlations between IGF-I concentrations in the original study and DSL ELISA were very high [r = 0.92; 95% CI, 0.90-0.94]. Correlations with DSL ELISA were much lower for IGFBP-3 (r = 0.58; 0.49-0.66) and even lower still with the assay for functional IGFBP-3 (r = 0.33; 0.20-0.44). IGF-I and IGFBP-3 measurements by the DSL ELISA methods showed statistically significant relationships with risk. The odds ratios (OR) for top versus bottom quartiles were 1.93 (1.00-3.72; P = 0.02) and 2.03 (1.09-3.76; P = 0.02), respectively, in agreement with the original observations. In contrast, measurements of functional IGFBP-3 tended to be unrelated to risk [ORs for the top versus bottom quartile, 0.97 (0.44-2.11)]. The association with IGF-I became substantially weaker and lost statistical significance after adjustment for IGFBP-3 using DSL ELISA, but became considerably stronger when adjusting for the functional IGFBP-3 measurements [OR = 2.43 (1.21-4.90); P = 0.005], or when considering the molar ratio of IGF-I to IGFBP-3 [OR = 2.37 (1.13-5.00); P = 0.02]. These results are consistent with an association of breast cancer risk in young women with elevated IGF-I and IGFBP-3, and show that for IGFBP-3, the strength of such an association could vary substantially depending on the assay used. (Cancer Epidemiol Biomarkers Prev 2005;14(1):48–52)

Introduction

Recently, several prospective cohort studies (1-4) and case-control studies (5-8) have shown an increased risk of breast cancer in women with elevated blood concentrations of insulin-like growth factor-I (IGF-I), a mitogenic and antiapoptotic protein. The increase in risk seemed to be most pronounced in premenopausal women who developed breast cancer before the age of 50 (1, 2). Because of the small sample size, the risk estimates observed in these studies had wide CI, and were also noticeably heterogeneous (1-3), with one prospective study (9) showing no association.

Heterogeneity of risk estimates was more pronounced for IGF binding protein-3 (IGFBP-3), the major binding protein of IGF-I in blood, than for IGF-I itself. Some studies showed a direct relationship of risk with IGFBP-3 concentrations (2-4), whereas others showed either a protective effect (1), or no association at all (9). Such heterogeneity, and the positive correlations between IGFBP-3 and IGF-I, resulted in a broadly variable effect of adjustments for IGFBP-3 on the reported associations between IGF-I and risk of breast cancer. In one study (1), IGFBP-3 adjustment resulted in a considerable increase in risk estimates, although in others (2-4), the same adjustment did not strengthen, but weakened the associations. A similar discrepancy has been noted in studies of cancers of the large intestine (10, 11) and prostate (12, 13).

We previously speculated that the observed between-study heterogeneity in relation to IGFBP-3 might be the result of differences in assay specificity. IGFBP-3 in blood undergoes specific proteolytic cleavage, and therefore, different analytic methods may measure, more or less intact, biologically active forms of IGFBP-3 (14). A case-control study of serum IGF-I, IGFBP-3, and risk of breast cancer in young women, nested within the New York University Women's Health Study cohort, offered the opportunity to address the hypothesis that the observed heterogeneity in IGF-I, and especially in IGFBP-3, may have been the result, in part, of variations in the ability of different assays used in different studies to measure these proteins with a comparable degree of specificity.

Materials and Methods

Subjects. This case-control study was nested within the New York University Women's Health Study and has been described in detail previously (2). About 86% of the women were non-Hispanic Whites, 6% Black, 6% Hispanic, and 2% from other ethnic backgrounds.

Case subjects were women who were premenopausal at the time of the baseline blood drawing (at cohort recruitment), who

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were diagnosed with invasive breast cancer before age 51, at least 6 months after the baseline blood collection, and had no previous diagnosis of cancer except possibly nonmelanoma skin cancer. Women who reported the use of oral contraceptives or hormone replacement therapy in the 6 months prior to enrollment were not included in the cohort. Control subjects were chosen from all cohort subjects who were alive and free of cancer at the date of diagnosis of the index case subject, using an incidence density sampling method. The control subjects were matched individually to the cases according to age at recruitment (±3 months), menopausal status and date of baseline blood sampling (±3 months). In the original case-control study, four controls per case were selected (2). Because of the high cost of the laboratory analyses, in the present study, we randomly selected two controls out of the original four, and selected two controls for each newly diagnosed case.

A total of 138 eligible premenopausal cases and 259 controls were identified and included in this study (Table 1). The Ethical Review Boards of the New York University School of Medicine and the IARC, in Lyon, France, reviewed and approved the present study.

IGF-I and IGFBP-3 Assays. All laboratory measurements were done on serum aliquots continuously stored at −80°C. The IGF-I and IGFBP-3 assays of the original study had been done in the laboratory of Dr. Johannes Bonfrer at the Netherlands Cancer Institute in Amsterdam, as previously described (2). IGF-I was measured after acid Sep-Pack C18 chromatography (15), whereas IGFBP-3 was measured by an in-house RIA after a dilution step (16). The new assays were done at the laboratory of the Hormones and Cancer Group at the IARC, Lyon, France using common commercially available ELISA kits (Diagnostic System Laboratories, Webster, Texas) for the measurements of IGF-I and IGFBP-3 in blood. IGF-I assays included an acid-ethanol precipitation of IGF-I binding proteins to avoid interferences of IGFBPs in the measurements. IGFBP-3 was also measured with an additional ELISA kit commercialized for the measurement of functional IGFBP-3 concentrations (IGFBP-3 FLIA, Functional Ligand Labelling Immunoassay; Immunological and Biochemical Test Systems, Reutlingen, Germany). In this assay, samples are acidified to release IGFs, and are then neutralized in the presence of excess biotinylated IGF-II, which binds only to functional IGFBP-3 (fragments of the protein with lost affinity for IGFs are therefore not labeled). Samples are then transferred to a plate coated with an antibody that recognizes IGFBP-3 and fragments that are bound to the plate. Only IGF-labeled, functional IGFBP-3 are then detected by addition of a streptavidin-peroxidase complex which is necessary for detection in the ELISA procedure.

For quality control, three serum samples were measured in duplicate in each batch. For each peptide, the overall intrabatch coefficient of variation (CV) was calculated as the average CV of the duplicate measurements in each batch for the three different serum samples. The overall inter-batch CV was calculated for the three different quality control samples as the average interbatch CV for the means of each of the duplicate measurements. For the DSL ELISA, mean intrabatch and interbatch CV were 6.3% and 8.3%, and 7.2% and 13.9% for IGF-I and IGFBP-3, respectively. For the functional IGFBP-3 assay, the mean intrabatch and interbatch CV were 10.1% and 21.4%, respectively.

As in the original study, samples from all case subjects and their matched controls were analyzed on the same day for each assay kit. Laboratory personnel were not able to distinguish between case and control samples.

Statistical Analysis. Peptide hormone data were ln-transformed to reduce departures from the normal distribution. A pairwise t test was used to test for mean differences between subjects' characteristics and hormone levels for cases and matched controls. Pearson partial correlations between IGF-I and IGFBP-3 adjusted for batch and case-control status were calculated. Intraclass correlations between repeated hormone measurements were calculated from variance components estimated by the SAS MIXED procedure (17) and their 95% CI were calculated as previously described (18).

Odds ratios (OR) for disease by quartile levels of the hormone variables, and on a continuous scale, were estimated by conditional logistic regression models using the SAS PHREG procedure. The quartile cutoff points were based on the distribution of analyte values using the cases and controls combined. For the calculation on a continuous scale, hormones were $\log_2$-transformed, so that a unit increase on the scale would represent a doubling of hormone concentrations. Likelihood ratio tests were used to assess linear trends in ORs over the quartiles, scoring the categories according to the median of the ln-transformed value for each quartile. Adjustments for benign breast disease, body mass index (BMI), family history of breast cancer and age at menarche in logistic regression models altered the OR estimates only trivially, therefore, unadjusted estimates are reported. All statistical tests and corresponding P values were two-sided, and $P < 0.05$ were considered statistically significant.

Results

In the extended study, cases reported a family history of breast cancer [OR: 2.2 (95% CI, 1.3-3.5)] and benign breast disease [OR: 3.1 (1.7-5.6)] more frequently than the controls. No case-control differences were observed for age at menarche (12.3 for cases versus 12.4 for controls), age at first full-term pregnancy (26.9 years versus 25.6), number of children (2.1 for cases and controls), and for having been pregnant (52.9% versus 58.3%). Patients with breast cancer were slightly leaner than controls (body mass index, 22.9 versus 23.8; $P = 0.01$).

We first assessed the agreement of the various assay techniques with each other. The DSL ELISA assays for IGF-I and IGFBP-3 yielded higher absolute values than those in the original study ($P < 0.001$; ref. 2). The Pearson correlation between DSL ELISA and original assays was high for IGF-I [r = 0.92 (0.90-0.94)], but much lower for IGFBP-3 [r = 0. 58 (0.49-0.66)]. The assay for functional IGFBP-3 resulted in lower mean values than those obtained by DSL ELISA or the original study,

Table 1. Mean and range (5th and 95th percentiles) of IGF-I and IGFBP-3 concentrations for cases and controls

	Original study			Extended study		
	Cases (n = 87)	Controls (136)	P	Cases (n = 138)	Controls (n = 259)	P
IGF-I (ng/mL)						
Original method	229.7 (129.0-323.0)	217.5 (110.0-347.0)	0.02			
DSL ELISA	294.1 (172.3-410.8)	279.0 (147.1-428.4)	0.04	303.1 (174.1-428.9)	287.5 (165.7-434.5)	0.01
IGFBP-3 (ng/mL)						
Original method	3,507 (2,200-4,800)	3,332 (2,000-4,625)	0.16			
DSL ELISA	3,807 (2,620-4,844)	3,635 (2,571-4,836)	0.10	3,907 (2,634-5,339)	3,723 (2,532-5,028)	0.04
Functional IGFBP-3	2,440 (905-4,560)	2,562 (1,290-4,040)	0.11	2,848 (1,070-5,025)	2,926 (1,410-4,890)	0.19

and the corresponding correlations were both weak [0.45 (0.34-0.55) and 0.33 (0.20-0.44), respectively]. For a subgroup of 111 study subjects (36 cases and 75 controls) serum samples were available in addition to those at baseline, and were collected between 9 and 65 months (average, 26 months) after the baseline blood collection. The relevant intraclass correlations for DSL ELISA measurements were relatively high for both IGF-I [0.81 (0.75-0.88)] and IGFBP-3 [0.87 (0.83-0.92)], thus suggesting that intraindividual variations in analyte concentrations were not a major factor.

As shown in Table 1, in the extended study, mean IGF-I and IGFBP-3 concentrations obtained with the DSL ELISA were 5.1% and 4.7% higher, respectively, among cases than among controls. In logistic regression analyses (Table 2), the ORs of breast cancer for elevated levels of both peptides indicated an average doubling in breast cancer risk: for IGF-I, the top versus bottom quartile OR = 1.93 (1.00-3.72, $P = 0.02$), and a doubling of IGF-I levels yielded an OR = 1.98 (1.12-3.50); and for IGFBP-3, the top versus bottom quartile OR = 2.03 (1.09-3.76, $P = 0.02$), and a doubling of IGFBP-3 levels gave an OR = 2.22 (1.08-4.53). The DSL ELISA assays of IGF-I and IGFBP-3 were highly correlated [$r = 0.66$ (0.59-0.71)] so that adjustment of one hormone for the other did not modify their associations with risk. The molar ratio of IGF-I/IGFBP-3 when using DSL ELISA measurements also showed no relationship with breast cancer risk.

With the assay for functional IGFBP-3, the controls had 2.7% higher, nonsignificant IGFBP-3 concentrations than the cases (Table 1). Logistic regression analysis showed no association with breast cancer risk [top versus lowest quartile, OR = 0.97 (0.44-2.11), or OR = 0.75 (0.46-1.23) for a doubling in levels; Table 2], but adjustment of the DSL ELISA IGF-I model for the functional IGFBP-3 concentrations substantially strengthened the ORs from 1.93 to 2.43 (1.21-4.90, $P = 0.005$). Likewise, the molar ratio of IGF-I (DSL ELISA) and functional IGFBP-3 showed a significant and direct association with breast cancer risk [OR, 2.37 (1.13-5.00); $P = 0.02$].

For comparison with the original study results (Table 3), we re-calculated the breast cancer ORs for the subgroup of cases and controls that were part of it. IGF-I was associated with increased breast cancer risk only when the original measurements were used [top versus lowest quartile OR = 2.22 (0.94-5.25); $P = 0.03$]. With the DSL ELISA, the OR was substantially lower [1.65 (0.69-3.99)] and was of borderline significance. IGFBP-3 was associated with an increase in risk when either the original measurements or the DSL ELISA method were used. With the assay for functional IGFBP-3, there was no longer any evidence of a positive association, as the ORs for the two upper quartiles fell below unity. Adjustment of IGF-I for IGFBP-3 showed a significant increase in risk only when IGF-I measurements (both original method and DSL ELISA) were adjusted with the functional IGFBP-3 measurements [top versus lowest quartile OR = 2.99 (1.19-7.51), $P = 0.01$ when IGF-I was measured by the original method, and OR =2.37 (0.92-6.07) p=0.02 when IGF-I was measured by DSL ELISA]. The IGF-I/IGFBP-3 molar ratios were statistically associated with an increase in breast cancer risk only when the functional IGFBP-3 concentrations were considered [top versus lowest quartile OR 2.34 (0. 92-5.96), $P = 0.03$, when IGF-I was measured by original method, and OR 3.72 (1.44-9.63), $P = 0.01$, when using DSL ELISA IGF-I measurements]. With any other combination of measurements, the ORs were appreciably lower and the trends not significant.

Discussion

We present the results of an extension of a previous study on the association between circulating IGF-I, IGFBP-3, and breast cancer occurring at age 51 or younger. The two principal aims of this study were to increase the precision of relative risk estimates with a larger study size, and to assess the effects that the use of different IGF-I and/or IGFBP-3 assays might have on relative risk estimates. The final numbers of cases and controls were 138 and 259, respectively, for whom IGF-I and IGFBP-3 were re-measured by a common assay (DSL ELISA), as well as by an ELISA assay that determines the concentrations of functional IGFBP-3 (i.e., total IGFBP-3 or fragments which can bind IGF).

In the extended data set, the risks observed for IGF-I concentrations confirmed our previous findings (2), where a significant increase in breast cancer risk with increasing IGF-I serum concentrations was found (OR, 2.30; top versus bottom quartile). These data suggest a role for IGF-I in breast cancer etiology, which seems to be associated with a relatively early onset of the disease.

IGFBP-3 has been found to reduce cancer risk in several previous studies where this binding protein was measured by DSL ELISA. This was the case for studies on breast cancer in young women (1), as well as on prostate (12, 19) and colorectal cancers (10). In each of these studies, the association of IGF-I with cancer risk became stronger after adjustment for IGFBP-3 levels. A physiologic explanation for the stronger association of cancer risk with IGF-I adjusted for binding capacity to IGFBP-3 could be that levels of IGF-I relative to this IGFBP-3 may reflect more accurately a fraction of IGF-I that is more directly available to target tissues, where IGF-I acts as a

Table 2. ORs of breast cancer (95% CI) by quartiles of IGF-I (measured by ELISA) and IGFBP-3, and IGF-I/IGFBP-3 molar ratio in the extended study (138 cases of 259 controls)

	Quartiles				P trend median*	Continuous ($\log^2$ scale)
	1	2	3	4		
IGF-I DSL ELISA	1.00	1.38 (0.74-2.59)	2.23 (1.19-4.16)	1.93 (1.00-3.72)	0.02	1.98 (1.12-3.50)
Adjusted for IGFBP-3 ELISA DSL	1.00	1.28 (0.67-2.47)	1.90 (0.91-3.95)	1.56 (0.68-3.59)	0.23	1.61 (0.75-3.47)
Adjusted for Functional IGFBP-3	1.00	1.48 (0.78-2.80)	2.69 (1.38-5.22)	2.43 (1.21-4.90)	0.005	2.54 (1.37-4.73)
Number of cases and controls	27/73	32/67	42/57	37/62		
IGFBP-3 DSL ELISA	1.00	1.53 (0.85-2.77)	1.63 (0.91-2.94)	2.03 (1.09-3.76)	0.02	2.22 (1.08-4.53)
Adjusted for IGF-I ELISA DSL	1.00	1.36 (0.73-2.52)	1.31 (0.67-2.59)	1.49 (0.69-3.23)	0.33	1.47 (0.56-3.87)
Number of cases and controls	26/74	35/63	36/63	41/59		
Functional IGFBP-3	1.00	0.87 (0.47-1.63)	0.59 (0.29-1.18)	0.97 (0.44-2.11)	0.68	0.75 (0.46-1.23)
Adjusted IGF-I ELISA DSL	1.00	0.78 (0.41-1.49)	0.43 (0.20-0.93)	0.59 (0.24-1.43)	0.15	0.54 (0.31-0.95)
Number of cases and controls	39/60	36/64	26/73	37/62		
Molar ratios						
IGF-I ELISA/IGFBP-3 DSL ELISA	1.00	1.07 (0.58-1.96)	1.07 (0.58-1.99)	1.17 (0.59-2.33)	0.68	1.42 (0.68-2.96)
Number of cases and controls	34/66	35/63	34/66	35/64		
IGF-I ELISA/Functional IGFBP-3	1.00	1.00 (0.51-1.96)	1.32 (0.65-2.66)	2.37 (1.13-5.00)	0.02	2.11 (1.30-3.43)
Number of cases and controls	30/69	29/71	34/64	45/55		

*Linear trends in ORs over the quartiles by scoring the categories according to the median (ln) hormone value for each quartile.

mitogenic and antiapoptotic agent. In several other studies where IGFBP-3 was measured by assays different from DSL ELISA, elevated IGFBP-3 tended to be associated with an increase rather than a decrease in cancer risk (2, 13). We had suggested that such inconsistencies in the relationship between IGFBP-3 and cancer risk may reflect differences in assay specificity (14). IGFBP-3 exists in a variety of phosphorylated and glycosylated forms (20, 21) and undergoes proteolytic cleavage by specific proteases. More or less intact forms of IGFBP-3 and IGFBP-3 fragments can be found in blood circulation (22). IGFBP-3 proteolysis is increased during pregnancy, diabetes, severe illness (20, 23, 24), and also in patients with cancers of the breast and prostate (8, 25). These observations previously led us to speculate (14) that subjects at increased cancer risk might have increased hepatic synthesis and blood levels of IGFBP-3, possibly as a reflection of altered circulating growth hormone, although they may have lower levels of intact (uncleaved) forms of IGFBP-3, and higher proteolysis (26). Thus, it was possible that the DSL ELISA method would measure mostly specific intact forms of IGFBP-3, whereas other assays, commercialized for measurements of total IGFBP-3, might detect more or different forms combined.

In contrast to our expectations and to several previous studies, however, breast cancer risk was not inversely related to IGFBP-3 measured by DSL ELISA, on the contrary, an increase in risk was observed with increasing IGFBP-3 concentrations measured by DSL ELISA. Given this direct association with risk and the positive association of DSL-IGFBP-3 with DSL IGF-I, a multivariate adjustment for DSL-IGFBP-3 tended to weaken the observed association of breast cancer risk with IGF-I. We do not have a clear explanation as to why the relationship of cancer risk with DSL ELISA measurements of IGFBP-3 should be different from those observed in previous studies. There are, however, indications that the performance of the assay has changed over time. The IGFBP-3 standard used for the ELISA assay has been changed from a purified glycosylated to a recombinant nonglycosylated standard. It is possible that the binding to antibodies differs between the pure recombinant IGFBP-3 protein currently used to prepare the standards, and the mixture of the glycosylated, phosphorylated, and proteolysed IGFBP-3 forms found in biological (blood) samples (22, 24, 27, 28).

Measurements of functional IGFBP-3 showed modest and statistically insignificant inverse relationships with risk. Adjustments for Immunological and Biochemical Test systems' measurements of IGFBP-3, and the IGF-I/functional IGFBP-3 molar ratios, resulted in a much stronger and statistically significant trend of breast cancer risk with IGF-I levels, and risk was also directly related to the IGF-I/IGFBP-3 molar ratio. Although the assay commercialized by Immunological and Biochemical Test systems indeed seems very promising, in our hands, this assay showed relatively high intrabatch and interbatch CVs. Its applicability to large-scale epidemiologic study therefore needs further confirmation in other studies. Moreover, it is not entirely clear what the functional assay measures that is distinct from other assays because the current evidence indicates that, other than in renal failure, all of the IGFBP-3 forms that are present in the circulation are functional in terms of binding to IGFs, and any forms of IGFBP-3 which do not bind to IGFs are very rapidly cleared from the circulation.

In conclusion, our study is compatible with previous observations of an increased risk of breast cancer among young women with elevated blood levels of IGF-I, although it also indicates that the biochemical assays used for the measurement of IGFBP-3 may lead to rather different conclusions. Taken together, our results stress the need for further, larger prospective studies on IGF-I and breast cancer risk among young women, and underline the need for careful studies on the specificity particularly of IGFBP-3 assays used in epidemiologic studies.

Table 3. ORs of breast cancer (95% CI) by quartiles of IGF-I and IGFBP-3 for the subset of subjects who were part of the original study (87 cases of 136 controls)

	Quartiles				*P* trend median*	Continuous (log^2 scale)
	1	2	3	4		
IGF-I						
Original method	1.00	1.23 (0.55-2.73)	2.24 (0.95-5.28)	2.22 (0.94-5.25)	0.03	1.98 (1.00-3.92)
Number of cases and controls	18/38	18/37	25/31	26/30		
DSL ELISA method	1.00	0.99 (0.41-2.38)	2.41 (1.02-5.66)	1.65 (0.69-3.99)	0.09	1.95 (0.93-4.07)
Number of cases and controls	18/37	18/39	28/28	23/32		
IGFBP-3						
Original method	1.00	1.92 (0.81-4.52)	1.65 (0.73-3.72)	2.22 (0.99-4.98)	0.06	1.69 (0.79-3.60)
Number of cases and controls	17/43	21/30	22/32	27/31		
DSL ELISA method	1.00	1.45 (0.66-3.20)	1.73 (0.81-3.72)	2.05 (0.94-4.47)	0.05	2.03 (0.81-5.06)
Number of cases and controls	16/40	22/34	23/32	26/30		
Functional IGFBP-3	1.00	1.22 (0.53-2.83)	0.55 (0.24-1.26)	0.76 (0.28-2.06)	0.24	0.58 (0.31-1.07)
Number of cases and controls	24/31	27/30	16/39	20/36		
IGF-I adjusted for IGFBP-3						
IGF-I, original method						
Adjusted for IGFBP-3 original method	1.00	1.21 (0.54-2.73)	2.17 (0.86-5.51)	2.13 (0.79-5.73)	0.10	1.89 (0.82-4.33)
Adjusted for IGFBP-3 DSL ELISA	1.00	1.21 (0.53-2.75)	2.16 (0.82-5.71)	2.12 (0.73-6.11)	0.13	1.82 (0.76-4.35)
Adjusted for functional IGFBP-3	1.00	1.34 (0.60-3.01)	2.70 (1.09-6.69)	2.99 (1.19-7.51)	0.01	2.63 (1.26-5.48)
IGF-I ELISA Method						
Adjusted for IGFBP-3 original method	1.00	0.94 (0.38-2.29)	2.19 (0.87-5.47)	1.43 (0.52-3.95)	0.24	1.77 (0.73-4.32)
Adjusted for IGFBP-3 DSL ELISA	1.00	0.93 (0.38-2.29)	2.13 (0.83-5.51)	1.37 (0.46-4.09)	0.32	1.71 (0.64-4.52)
Adjusted for functional IGFBP-3	1.00	1.06 (0.42-2.66)	3.27 (1.29-8.30)	2.37 (0.92-6.07)	0.02	2.72 (1.22-6.08)
Molar ratios						
IGF-I original method						
IGFBP-3 original method	1.00	2.02 (0.85-4.81)	1.62 (0.65-4.06)	1.86 (0.70-4.97)	0.29	1.46 (0.69-3.10)
IGFBP-3 DSL ELISA	1.00	2.18 (0.88-5.43)	1.56 (0.62-3.95)	1.73 (0.71-4.21)	0.35	1.67 (0.72-3.86)
Functional IGFBP-3	1.00	1.54 (0.65-3.64)	3.17 (1.27-7.92)	2.34 (0.92-5.96)	0.03	2.48 (1.39-4.42)
IGF-I ELISA method						
IGFBP-3 original method	1.00	1.27 (0.58-2.81)	1.79 (0.78-4.10)	0.83 (0.32-2.16)	0.85	1.28 (0.59-2.78)
IGFBP-3 ELISA	1.00	1.39 (0.60-3.21)	2.06 (0.91-4.69)	1.32 (0.51-3.40)	0.28	1.49 (0.59-3.75)
Functional IGFBP-3	1.00	1.46 (0.61-3.49)	1.84 (0.71-4.74)	3.72 (1.44-9.63)	0.01	2.51 (1.36-4.62)

*Linear trends in ORs over the quartiles by scoring the categories according to the median (ln) hormone value for each quartile.

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