CLINICAL STUDY

Growth hormone-binding protein is directly and IGFBP-3 is inversely associated with risk of female breast cancer

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Abstract

Objective: Several components of the GH and IGF systems have been implicated in the development of malignancies. All components of these hormonal systems have never been jointly evaluated in female breast cancer, and previous studies have not examined the role of IGF-binding proteins (IGFBP-4, IGFBP-6) or GH-binding protein (GHBP).

Design: Hospital-based case-control study.

Methods: In this sample of primarily postmenopausal women, we obtained serum measures of IGF-I, IGF-II, and binding proteins IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-6, as well as GHBP, insulin, and leptin from 74 breast cancer cases and 76 control subjects.

Results: In crude analyses, we found lower age-standardized mean IGF-I, IGFBP-3, IGFBP-4, IGFBP-6, and higher IGFBP-1 and GHBP in breast cancer cases when compared with controls. Multivariate models mutually adjusted for other GH-IGF system components and classical breast cancer risk factors demonstrated an inverse association between IGFBP-3 and risk of breast cancer (odds ratio (OR) = 0.2, $P < 0.01$) and a direct association between GHBP and disease risk (OR = 3.3, $P < 0.01$). No significant associations were detected in multivariate analyses among IGF-I, IGF-II or IGFBP-1, IGFBP-4, IGFBP-6 with risk of breast cancer, indicating that these factors may not have effects independent of and/or comparable with IGFBP-3 and GHBP.

Conclusions: These results support a protective role of IGFBP-3 and demonstrate for the first time an increased risk of breast cancer with higher GHBP, after accounting for variation in IGFs, IGFBPs, and classical breast cancer risk factors.

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Introduction

The insulin-like growth factor (IGF) system is an intricate arrangement of growth factors (peptide hormones IGF-I and IGF-II), high affinity-binding proteins (IGFBP-1 to 6), as well as cell surface receptors, IGFBP proteases, and low affinity IGFBP-related proteins (1). This system plays a substantial role in human metabolism and cell proliferation, differentiation, and apoptosis, and thus has been proposed to be a determinant of risk for carcinoma, including breast cancer. Another closely related hormone that may be involved in the underlying mechanism is human growth hormone (GH), which regulates IGF-I production mainly in the liver (2). GH, like IGF-I, which is usually bound in binary and ternary complexes with IGFBP-3, circulates in complexes with growth hormone-binding protein (GHBP). Since GH is secreted in a pulsatile fashion and displays considerable circadian

variability, obtaining reliable measurements that reflect its integrated mean levels presents a challenge, and as such, the relationship between GH and cancer has not yet been adequately studied in epidemiology studies. However, serum concentrations of GHBP, which is the cleaved extracellular ligand-binding domain of GH receptors, may reflect the GH tissue receptor status (3, 4) and has been inversely associated with GH levels in several clinical conditions (5, 6).

Numerous epidemiologic studies have examined the association between components of the IGF system, in particular IGF-I and IGFBP-3, and female breast cancer (7–17). Most nested case-control studies have demonstrated an increased risk of premenopausal breast cancer with higher concentrations of IGF-I, but results have been mixed with respect to IGFBP-3, with reports of positive, negative, and no association. Studies with postmenopausal populations have tended to report no association between IGF-I or IGFBP-3 and risk of breast

cancer. Strong theoretical bases suggest that the GH and IGF systems are important factors in tumor formation and proliferation (18–20). Specifically, IGF-I is known to induce cell proliferation *in vitro*, and IGFBPs have been shown to exert effects both dependent and independent of IGF function and may induce apoptosis (21). However, no prior study has examined the role of IGFBP-4 or IGFBP-6 in breast cancer, nor has any previous work included GHBP. Since the components of the GH and IGF systems are highly correlated, accurate measurement of each factor is imperative for both crude assessment and in mutually adjusted models to determine the central forces driving potential associations. Thus, we have simultaneously examined the role of circulating IGF-I, IGF-II, IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-6, and GHBP, as well as insulin and leptin, accounting for effects of insulin resistance and fat mass respectively, in predicting risk of breast cancer in the context of a hospital-based case–control study consisting mostly of postmenopausal women.

Materials and methods

Study subjects

Study subjects were recruited during the 16-month period from September 2003 until February 2005, inclusive, from 81 consecutively diagnosed and histologically confirmed incident cases of female breast cancer at the department of surgery of the Theagenio Cancer Hospital in Greece. Of these cases, five women refused participation and two were excluded from the study on the basis of previous cancer at another site. The remaining 74 cases were enrolled in the study. Control subjects were identified among 83 women examined at the same site using identical diagnostic procedures and criteria in the context of a routine breast cancer prevention program but were free of breast cancer but may have been diagnosed with a benign lesion (such as fibroadenoma) from mammogram and/or biopsy tests. Of the potential controls, 76 consented to the study and met eligibility requirements. Study subjects responded to an interviewer-administered questionnaire covering demographic, anthropometric and clinical information, and fasting blood samples were taken by research assistants blinded to the case or control status of the 150 subjects and were subsequently stored on ice in Styrofoam containers at -70°C . These samples were then shipped via overnight courier to the Beth Israel Deaconess Medical Center (Boston, MA, USA) for assay. Estrogen receptor (ER) and progesterone receptor (PR) status were identified immunohistochemically and/or biochemically. The study protocol was approved by the Theagenio Cancer Hospital Scientific Committee. All participants submitted written informed consent prior to enrolment in the study.

Serum analysis

Measurements of serum leptin (Diagnostic Systems Laboratories, Webster, TX, USA), insulin, IGF-I, IGF-II, IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-6, and GHBP (LINCO, St Louis, MO, USA) were obtained for all samples from a single run of commercially available radioimmunoassays and ELISAs in the principal investigator's laboratory at the Beth Israel Deaconess Medical Center as described previously (22, 23). For the newly studied markers IGFBP-4, IGFBP-6, and GHBP, we report the following assay characteristics: IGFBP-4 had a sensitivity of 0.008 ng/ml and intraassay coefficient of variation (CV) values of 3.0–6.3%, IGFBP-6 had a sensitivity of 1.1 ng/ml and intraassay CV values of 6.4–10.7%, and GHBP had a sensitivity of 1.69 pmol/l and intraassay CV values of 3.2–5.6%. Laboratory personnel conducting analyses were blinded to the case or control status of the samples.

Statistical analysis

Case and control groups were compared on demographic, somatometric, reproductive, and biochemical variables using standard *t*-tests or χ^2 -tests as appropriate. Log transformation was used on biochemical variables to achieve normal distribution. Nonparametric Mann–Whitney comparisons were also performed and yielded similar results (data not shown). Linear regression models were used to obtain age-standardized estimates of mean biochemical measures for case and control groups. Spearman unadjusted and partial correlations controlling for age and body mass index (BMI) were calculated between biochemical measures for control subjects. Risk of breast cancer was modeled using unconditional simple and multivariate logistic regression models of hormonal variables to produce crude and adjusted odds ratio (OR) estimates and 95% confidence intervals. Biochemical markers were analyzed in tertile groups based on distributions of the control population. Reported *P* values are two-sided, and a level of $\alpha = 0.05$ was used to determine statistical significance. Analyses were conducted using the SAS statistical package (SAS, Version 8.2 for UNIX, SAS Institute, Cary, NC, USA).

Results

Characteristics of cases and controls are presented in Table 1. Among cases, tumor size ranged from 1 to 5 cm in diameter. At diagnosis, nine (12.2%) of the cases had metastasis. Ductal carcinomas accounted for the majority of cases, with 58 (78%) diagnoses. The remaining cases were 13 (18%) lobular carcinomas and 3 (4%) carcinomas otherwise differentiated (medullary, mucinous, papillary, and tubular carcinomas). When compared with control subjects, breast cancer patients were older, had higher age at first birth

Table 1 Descriptive and biochemical variable measures by case and control status.

Variable	All subjects			Postmenopausal subjects		
	Cases (n=74)	Controls (n=76)	P value	Cases (n=61)	Controls (n=54)	P value
Case characteristics						
Stage, n (%)						
I	19 (25)			14 (23)		
IIA,B	32 (43)			24 (39)		
IIIA,B,C	14 (19)			14 (23)		
IV	9 (12)			9 (15)		
Nodal positive, n (%)	48 (65)			37 (61)		
ER+/PR+, n (%)	40 (54)			33 (57)		
ER+/PR-, n (%)	11 (15)			9 (16)		
ER-/PR+, n (%)	3 (4)			2 (3)		
ER-/PR-, n (%)	17 (23)			14 (24)		
Descriptive						
Age, mean (s.d.), years	62.5 (11.6)	55.6 (11.6)	<0.01	66.2 (8.9)	60.6 (8.3)	<0.01
Height, mean (s.d.), cm	158.2 (5.6)	159.3 (5.9)	0.18	158.0 (5.8)	158.6 (5.4)	0.49
BMI, mean (s.d.), kg/m ²	29.1 (4.6)	29.3 (6.1)	0.45	29.2 (4.1)	29.8 (6.0)	0.58
Age at menarche, mean (s.d.), years	12.9 (1.3)	12.9 (1.6)	0.95	13.0 (1.3)	13.1 (1.6)	0.60
Age at menopause, mean (s.d.), years	49.4 (4.0)	47.7 (4.8)	0.05	49.4 (4.0)	47.7 (4.8)	0.05
Age at first birth, mean (s.d.), years	24.6 (4.0)	23.5 (4.5)	0.11	24.8 (4.1)	23.0 (3.4)	0.02
Nulliparous, n (%)	7 (9)	5 (7)	0.76	5 (8)	4 (7)	1.00
Family history of breast cancer, n (%)	5 (7)	4 (5)	1.00	2 (3)	2 (4)	1.00
Family history of cancer, n (%)	20 (27)	17 (23)	0.70	19 (31)	13 (24)	0.41
Biochemical, age-standardized mean (s.e.)						
Insulin (ng/ml)	12.8 (0.9)	14.7 (0.9)	0.28	13.4 (1.0)	14.6 (1.0)	0.38
Leptin (ng/ml)	10.9 (0.6)	11.4 (0.6)	0.84	11.4 (0.7)	11.5 (0.7)	0.55
IGF-I (ng/ml)	126 (13)	138 (12)	<0.01	105 (13)	115 (14)	0.06
IGF-II (ng/ml)	1035 (32.5)	1013 (33.2)	0.40	1002 (37)	1018 (40)	0.96
IGFBP-1 (ng/ml)	36.7 (2.3)	31.8 (2.4)	<0.01	35.9 (2.4)	33.0 (2.6)	<0.01
IGFBP-3 (ng/ml)	4137 (134)	4279 (135)	0.02	3966 (152)	4226 (162)	0.37
IGFBP-4 (ng/ml)	556 (23)	596 (23)	<0.01	574 (26)	629 (27)	<0.01
IGFBP-6 (ng/ml)	518 (31)	599 (30)	<0.01	547 (35)	642 (36)	<0.01
GHBP (pmol/l)	1731 (89)	1656 (90)	0.57	1805 (94)	1695 (100)	0.02

Abbreviations: s.d., standard deviation; BMI, body mass index; IGF, insulin-related growth factor; BP, binding protein.

(postmenopausal women only) and menopause (all women). Cases also had higher age-standardized IGFBP-1 and GHBP and lower IGF-I, IGFBP-3, IGFBP-4, and IGFBP-6. Subjects did not differ significantly on other measured variables with respect to case or control status (Table 1).

Since substantial interrelationships are expected between the components of the GH and IGF systems, we present Spearman's correlations of age, BMI, hormones insulin and leptin, IGFs, IGFBPs, and GHBP among the 76 control subjects and 54 postmenopausal controls in Table 2. Similar results were obtained both including and excluding premenopausal women. IGF-I had moderate inverse associations with BMI and direct associations with IGF-II and IGFBP-3, and IGF-II had very strong positive correlation with IGFBP-3 and moderate positive correlation with GHBP. IGFBP-1 increased moderated with age, and showed modest but significant negative associations with BMI, leptin, and insulin. IGFBP-1 had positive correlation with GHBP, whereas IGFBP-3 and GHBP had a direct association. Both IGFBP-4 and IGFBP-6 were directly related to age and showed significant positive association with each other. GHBP showed weak negative correlation with age and positive correlations with BMI and IGFBP-4 (Table 2). Adjusting for age and BMI using partial correlations did not appreciably alter

associations between biochemical variables, and similar measures were obtained when including the 74 breast cancer cases in calculating the correlation coefficient (data not shown).

Age- and multivariate-adjusted odds ratios (ORs) for all subjects are presented in Table 3. In age-adjusted models, higher tertile of GHBP was associated with a marginal 54% increased risk of breast cancer ($P=0.06$). After adjusting further for BMI, this association intensified to a 77% increase in risk ($P=0.02$). In multivariate models, including age, BMI, height, reproductive and family history, and mutually adjusting for components of the GH-IGF-IGFBP system, each tertile increase of GHBP conferred a 3.3-fold increase in breast cancer risk ($P<0.01$). In addition, higher tertile of IGFBP-3 was significantly associated with an 81% reduced risk of breast cancer after multivariate adjustment. Results were similar when premenopausal women were excluded from the analysis (Table 4). In bivariate models, higher tertile of IGFBP-3 was marginally associated with a 44% decreased risk of breast cancer ($P=0.10$). This association was somewhat strengthened after adjustment for classical breast cancer risk factors ($P=0.09$). After mutually adjusting for the effects of the other components of the IGF-IGFBP system and hormones insulin and leptin, higher tertile of IGFBP-3 was significantly associated with a 77%

Table 2 Correlation matrix for biochemical factors from Spearman partial correlation coefficients.

	Insulin	Leptin	IGF-I	IGF-II	IGFBP-1	IGFBP-3	IGFBP-4	IGFBP-6	GHBP
All controls (n=76)									
Age	0.06	0.06	-0.32 [†]	-0.19	0.23	-0.25*	0.35 [†]	0.43 [†]	-0.02
BMI	0.35 [†]	0.64 [†]	-0.38 [†]	-0.02	-0.40 [†]	-0.08	0.36 [†]	0.06	0.44 [†]
Insulin		0.265*	-0.19	-0.13	-0.33 [†]	-0.17	0.08	0.05	0.36 [†]
Leptin			-0.22	0.20	-0.42 [†]	0.16	0.32 [†]	-0.06	0.46 [†]
IGF-I				0.53 [†]	-0.11	0.68 [†]	-0.32 [†]	-0.05	0.04
IGF-II					-0.20	0.91 [†]	0.12	-0.24*	0.46 [†]
IGFBP-1						-0.21	-0.04	0.09	-0.58 [†]
IGFBP-3							0.03	-0.24*	0.41 [†]
IGFBP-4								0.34 [†]	0.35 [†]
IGFBP-6									-0.13
Postmenopausal (n=54)									
Age	-0.15	-0.15	-0.17	-0.27	0.52 [†]	-0.22	0.35 [†]	0.52 [†]	-0.32*
BMI	0.27	0.70 [†]	-0.41 [†]	0.16	-0.47 [†]	0.11	0.25	0.03	0.41 [†]
Insulin		0.24	-0.21	-0.12	-0.33*	-0.14	0.01	0.07	0.26
Leptin			-0.18	0.20	-0.48 [†]	0.22	0.21	-0.03	0.39 [†]
IGF-I				0.54 [†]	-0.09	0.65 [†]	-0.16	-0.06	0.14
IGF-II					-0.25	0.91 [†]	0.21	-0.16	0.52 [†]
IGFBP-1						-0.28	-0.10	0.17	-0.56 [†]
IGFBP-3							0.15	-0.14	0.53 [†]
IGFBP-4								0.51 [†]	0.29*
IGFBP-6									-0.12

* $P < 0.05$; [†] $P < 0.01$. Spearman partial correlations based on data from control subjects. Partial correlations adjusting for age and BMI did not substantially alter associations among biochemical variables.

decreased risk and GHBP with a threefold increased risk of breast cancer ($P = 0.02$ for both, Table 4). Excluding metastatic cases ($n = 9$) from the analysis and adjusting for the stage of disease did not substantially alter reported results for either all or postmenopausal subjects (not shown).

Discussion

In this hospital-based case-control study of predominantly postmenopausal women, we did not find a significant association of either IGF-I or IGF-II with risk of breast cancer, but demonstrated a protective role of

Table 3 Crude and multivariate mutually adjusted risk of breast cancer by tertiles of insulin, leptin, IGF-I, IGF-II, IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-6, and GHBP.

	OR ^a (95% CI)	OR ^b (95% CI)	OR ^c (95% CI)	OR ^d (95% CI)	OR ^e (95% CI)
Hormones					
Insulin	0.99 (0.65–1.51)	1.06 (0.68–1.65)	1.09 (0.68–1.74)	0.78 (0.43–1.42)	0.60 (0.30–1.19)
<i>P</i> value	0.97	0.80	0.72	0.42	0.14
Leptin	0.94 (0.62–1.43)	1.00 (0.62–1.61)	1.06 (0.64–1.77)	0.72 (0.40–1.32)	0.85 (0.41–1.76)
<i>P</i> value	0.94	0.99	0.82	0.29	0.67
Growth factors					
IGF-I	1.12 (0.70–1.79)	1.07 (0.66–1.74)	1.08 (0.66–1.78)	1.30 (0.64–2.67)	1.59 (0.68–3.73)
<i>P</i> value	0.65	0.77	0.75	0.47	0.29
IGF-II	1.20 (0.77–1.87)	1.22 (0.78–1.91)	1.36 (0.84–2.19)	1.20 (0.50–2.88)	1.70 (0.63–4.55)
<i>P</i> value	0.43	0.39	0.98	0.69	0.29
Binding proteins					
IGFBP-1	1.36 (0.88–2.10)	1.30 (0.82–2.06)	1.21 (0.73–2.02)	1.51 (0.82–2.78)	0.91 (0.44–1.86)
<i>P</i> value	0.16	0.26	0.46	0.19	0.79
IGFBP-3	0.79 (0.51–1.21)	0.78 (0.51–1.21)	0.81 (0.52–1.26)	0.37 (0.15–0.96)	0.19 (0.06–0.58)
<i>P</i> value	0.27	0.27	0.35	0.04	<0.01
IGFBP-4	0.89 (0.57–1.41)	0.96 (0.58–1.59)	0.89 (0.52–1.51)	1.21 (0.65–2.25)	0.91 (0.43–1.92)
<i>P</i> value	0.63	0.88	0.66	0.55	0.80
IGFBP-6	0.79 (0.57–1.10)	0.79 (0.57–1.10)	0.74 (0.52–1.05)	0.98 (0.66–1.46)	0.80 (0.50–1.28)
<i>P</i> value	0.16	0.17	0.09	0.92	0.36
GHBP	1.54 (0.98–2.41)	1.77 (1.09–2.89)	2.13 (1.23–3.68)	2.52 (1.26–5.02)	3.32 (1.49–7.41)
<i>P</i> value	0.06	0.02	<0.01	<0.01	<0.01

^aAdjusted for age.

^bAdjusted for age and BMI.

^cAdjusted for age, BMI, height, age at menarche, age at menopause, nulliparous status, and family history of breast cancer.

^dMutually adjusted IGF-I, IGF-II, IGFBP-1, IGFBP-3, GHBP, IGFBP-4, IGFBP-6, insulin, and leptin.

^eMutually adjusted IGF-I, IGF-II, IGFBP-1, IGFBP-3, GHBP, IGFBP-4, IGFBP-6, insulin, and leptin; controlling additionally for age, BMI, and height; age at menarche, age at menopause, nulliparous status, and family history of breast cancer.

Table 4 Crude and multivariate mutually adjusted risk of postmenopausal breast cancer by tertiles of insulin, leptin, IGF-I, IGF-II, IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-6, and GHBP.

	OR ^a (95% CI)	OR ^b (95% CI)	OR ^c (95% CI)	OR ^d (95% CI)	OR ^e (95% CI)
Hormones					
Insulin	1.03 (0.65–1.61)	1.06 (0.67–1.69)	1.10 (0.68–1.77)	0.75 (0.41–1.35)	0.64 (0.33–1.22)
<i>P</i> value	0.91	0.81	0.71	0.33	0.17
Leptin	0.99 (0.61–1.62)	1.07 (0.61–1.89)	1.13 (0.61–2.09)	0.74 (0.38–1.43)	0.91 (0.41–2.01)
<i>P</i> value	0.96	0.80	0.69	0.37	0.82
Growth factors					
IGF-I	0.82 (0.50–1.35)	0.80 (0.48–1.32)	0.76 (0.45–1.30)	1.15 (0.55–2.43)	1.20 (0.54–2.69)
<i>P</i> value	0.44	0.78	0.32	0.71	0.65
IGF-II	1.00 (0.61–1.65)	1.03 (0.62–1.70)	1.00 (0.58–1.72)	1.31 (0.46–3.71)	1.68 (0.53–5.34)
<i>P</i> value	0.99	0.92	0.99	0.61	0.38
Binding proteins					
IGFBP-1	1.23 (0.72–2.10)	1.16 (0.66–2.04)	1.19 (0.66–2.13)	1.21 (0.63–2.34)	0.77 (0.35–1.67)
<i>P</i> value	0.44	0.60	0.57	0.57	0.50
IGFBP-3	0.66 (0.41–1.08)	0.67 (0.41–1.09)	0.64 (0.38–1.08)	0.32 (0.11–0.90)	0.23 (0.07–0.75)
<i>P</i> value	0.10	0.11	0.09	0.03	0.02
IGFBP-4	0.92 (0.54–1.58)	0.98 (0.55–1.74)	0.99 (0.54–1.82)	1.32 (0.67–2.63)	1.14 (0.53–2.42)
<i>P</i> value	0.77	0.95	0.98	0.42	0.74
IGFBP-6	0.83 (0.57–1.20)	0.83 (0.57–1.20)	0.80 (0.54–1.18)	1.15 (0.73–1.80)	0.96 (0.58–1.60)
<i>P</i> value	0.32	0.32	0.26	0.53	0.88
GHBP	1.22 (0.91–1.64)	1.75 (0.95–3.24)	1.78 (0.95–3.36)	2.28 (0.98–5.30)	3.08 (1.16–8.21)
<i>P</i> value	0.18	0.07	0.07	0.05	0.02

^aAdjusted for age.^bAdjusted for age and BMI.^cAdjusted for age, BMI, height, age at menarche, age at menopause, nulliparous status, and family history of breast cancer.^dMutually adjusted IGF-I, IGF-II, IGFBP-1, IGFBP-3, GHBP, IGFBP-4, IGFBP-6, insulin, and leptin.^eMutually adjusted IGF-I, IGF-II, IGFBP-1, IGFBP-3, GHBP, IGFBP-4, IGFBP-6, insulin, and leptin; controlling additionally for age, BMI, and height; age at menarche, age at menopause, nulliparous status, and family history of breast cancer.

IGFBP-3. Our results also suggest novel findings of increased risk for breast cancer with higher levels of GHBP and indicate that IGFBP-1, IGFBP-4, and IGFBP-6 are not significantly associated with breast cancer risk. The association with GHBP persisted after mutual adjustment for the components of the IGF–IGFBP axis, implying a potential role of either GHBP *per se* or the status of the GH receptor in breast cancer pathophysiology independent of the IGF system. These analyses also took into account potential effects of hyperinsulinemia, by including insulin and leptin, which we show to have no independent associations with breast cancer of their own accord.

Serum IGF-I appears to be associated with risk of breast cancer among premenopausal women (7, 10, 12, 13, 15, 17, 24, 25), but no similar association has been found among postmenopausal women (10, 26). Several explanations for this dichotomy have been proposed. One mechanism may include the induction IGF-I production by higher estrogen levels in premenopausal women (9). This explains the inverse correlation between serum IGF-I and age ($r = -0.33$ in our study, Table 2), and it is plausible that these increased levels would exert a greater effect on the development of premenopausal breast cancer. Premenopausal breast cancer has previously been shown not to be associated with high BMI, while postmenopausal breast cancer has been linked with obesity (27). In our study, IGF-I was inversely associated with BMI ($r = -0.38$). Thus, since our study population consisted primarily of

postmenopausal women, it is not surprising that we detected no relationship between IGF-I and breast cancer risk. We also did not find any association between IGF-II and risk of breast cancer, as has been reported previously (7). However, evidence from animal and cell line experiments examining the role of IGF-II in carcinogenesis indicates increased rates of malignancies, including breast tumors (28–33), and results from one case–control study found a positive association between IGF-II and ER-positive but not ER-negative breast cancer among postmenopausal women (9). We report a nonsignificant increase in risk by ~70%, and thus a potential role of IGF-II in pre- and postmenopausal breast cancer needs to be studied further.

More than 99% of circulating IGFs are bound to IGFBPs. IGFBPs not only regulate IGF action and bioavailability, but also appear to mediate IGF-independent actions, including inhibition or enhancement of cell growth and induction of apoptosis (21). The most thoroughly studied IGFBP in relation to breast cancer has been IGFBP-3. Several studies have reported a direct relationship between IGFBP-3 and risk of breast cancer in premenopausal women (8, 10–12, 14, 17); however, results from observational studies have been particularly inconsistent among postmenopausal women (9, 11, 12, 14, 26). Our data support an inverse relationship between IGFBP-3 and breast cancer. Beneficial actions of IGFBP-3 may include anti-proliferative effects and induction of apoptosis, both through sequestration of IGF-I and

via IGF-independent effects (34). It is also possible that cleaved IGFBP-3 fragments may either promote or inhibit tumor growth (35). We have used a previously validated and reliable assay (22, 23) to measure IGFBP-3. Other IGFBPs have been studied less extensively. We did not find any significant independent association between risk of breast cancer and IGFBP-1, as reported previously (12, 15, 26), nor IGFBP-4 or IGFBP-6, which have not been evaluated prior in breast cancer patients.

Since GH increases serum IGF-I levels, concern has been raised regarding its possible role in breast cancer. Some studies have identified an association between acromegaly and overall cancer risk (18–20), but others found no such association (36, 37). Moreover, a recent interventional study showed that human GH treatment induced mammary gland hyperplasia in aging animals, and this effect was not mediated through IGF-I (38). It has also been demonstrated that human GH can act in an autocrine/paracrine manner in human mammary carcinoma cells to promote proliferation and transcriptional activation (39, 40). A recent study (16) of premenopausal women failed to find an association between breast cancer and GH but did not examine GHBP. GH itself is a difficult marker to study because of its pulsatile secretion and circadian variability and heterogeneity of available assays, which can increase the complexity of GH quantification especially at the low range of GH concentrations (41–46). Thus, a single serum GH determination is not adequate for assessing GH levels in epidemiology studies. GHBP reflects GH receptor status, and circulating GHBP levels are inversely related to GH levels in healthy subjects or patients with acromegaly or GH deficiency. We used this marker in our study and found a more than threefold increased risk of breast cancer for women in higher tertiles of serum GHBP concentration. This risk estimate remained significant after adjustment for other components of the IGF system. Further studies are needed to confirm an IGF-independent relationship between the GHBP and the risk for breast cancer and to study the potential underlying mechanisms.

In summary, our study is the first to examine simultaneously the role of components of the GH–IGF–IGFBP axis that can be reliably measured using current technology in the pathogenesis of breast cancer. Advantages of the study include the simultaneous measurement of biochemical variables in a standardized and established manner and the blinding of laboratory personnel to case–control status. In addition, we were able to examine the effect of each component adjusting only for classical breast cancer risk factors and also were able to detect associations independent of other GH–IGF components in mutually adjusted models (Table 3), a matter of particular importance given the high collinearity between these factors (Table 2). Of particular importance, negative confounding due to positive correlations between IGFBP-3 and GHBP attenuated

the associations of both variables with breast cancer toward the null in crude models. After mutual adjustment, significant negative association with IGFBP-3 and positive association with GHBP became apparent. The study was relatively heterogeneous with respect to age, but we adjusted for this potential confounder in statistical analyses. The results of our study likely apply most closely to women with postmenopausal breast cancer and may not be as applicable to premenopausal women. Our results do not appear to be affected by severity of breast cancer, as similar associations were observed whether excluding late-stage cancers or taking into account stage of breast cancer in statistical analyses. A separate consideration is the fact that blood samples were not obtained prospectively, which precludes the establishment of causation. In spite of these limitations, our results are largely consistent with previous findings from prospective studies on the role of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 in postmenopausal breast cancer. In addition, we have demonstrated for the first time that GHBP significantly increased breast cancer risk independently of the IGF system. We also show that IGFBP-4 and IGFBP-6, which have not been studied prior in breast cancer, appear to have no significant association with disease risk, although this remains to be verified by larger studies. The cross-sectional nature and wide scope of biochemical factors explored provide a solid hypothesis-generating body of data in this area of research. Further matched case–control, prospective, and interventional studies are needed to confirm and/or extend our results and to provide insight into the biological mechanisms of the GH, GHBP, and the IGF system in breast cancer pathophysiology.

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