Insulin-like Growth Factors and Breast Cancer Risk in Chinese Women[1]

Herbert Yu,[2] Fan Jin, Xiao-Ou Shu, Benjamin D. L. Li, Qi Dai, Jia-Rong Cheng, Hans J. Berkel, and Wei Zheng

Department of Epidemiology and Public Health and Yale Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06520-8034 [H. Y.]; Feist-Weiller Cancer Center and Departments of Medicine and Surgery, Louisiana State University Health Sciences Center, Shreveport, Louisiana 71130 [B. D. L. L., H. J. B.]; Venderbilt-Ingram Cancer Center and Center for Health Services Research, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232 [X. O. S., Q. D., W. Z.]; and Department of Epidemiology, Shanghai Cancer Institute, Shanghai 200032, People's Republic of China [F. J., J-R. C.]

Abstract

Insulin-like growth factor (IGF)-I has mitogenic and antiapoptotic effects on breast cancer cells. High-circulating IGF-I was found to be associated with increased risk of breast cancer in several previous epidemiological studies, mostly conducted in the Caucasian populations. Little is known about the association between IGF and breast cancer in Asian women whose dietary habits differ considerably from their Caucasian counterparts. A population-based case-control study was conducted to assess the associations of IGFs and IGF binding protein-3, a major IGF binding protein in the circulation, with breast cancer risk in Chinese women. The study included 300 incident breast cancer patients diagnosed between August 1996 and March 1998 in Shanghai and 300 age- and menopause-matched controls selected randomly from the general population. Plasma levels of IGF-I, IGF-II, and IGFBP-3 were measured using commercial ELISA kits (Diagnostic Systems Laboratories, Webster, TX). Conditional logistic regression analysis was performed to examine the association between IGF and breast cancer risk after adjusting for potential confounding factors. Breast cancer patients had higher plasma levels of IGF-I and IGFBP-3. A dose-response relationship was observed between breast cancer risk and the level of IGF-I or IGFBP-3. The adjusted odds ratios were 2.01 (95% confidence interval, 1.26–3.19) or 3.01 (95% confidence interval, 1.81–4.99), respectively, for women with the highest tertile of IGF-I or IGFBP-3 compared with those with the lowest tertile of these molecules. These associations were more evident in premenopausal women or women with high body mass index or high waist-to-hip ratio. No significant association was found for IGF-II. The study confirms that high circulating levels of IGF-I are associated with elevated risk of breast cancer. In contrast to the findings from several studies conducted in Caucasian women, we found that IGFBP-3 was positively associated with breast cancer risk in Chinese women.

Introduction

Emerging evidence suggests that IGF-I[3] plays an important role in breast cancer (1, 2). IGF-I is a single-chain polypeptide with 70 amino acids and is involved in the regulation of cell proliferation, differentiation, and apoptosis (3, 4). Laboratory studies demonstrate that IGF-I has strong mitogenic and antiapoptotic effects on breast cancer cells (5–9) and interacts synergistically with estrogen to stimulate breast cancer growth (10–12). Animal studies indicate that IGF-I levels in the circulation are positively correlated with total energy intake and that IGF-I administration can abolish the inhibitory effect of energy restriction on tumor growth (13, 14).

Several epidemiological investigations, including both case-control and cohort studies, found that high circulating levels of IGF-I were associated with increased risk of breast cancer (15–19). High IGF-I was also correlated with the mammagraphic density of breast tissue, a strong indicator of breast cancer risk (20). These findings lend additional support to the role of IGF-I in breast cancer development. Thus far, all of the epidemiological studies reported have been conducted in the Caucasian populations. Little is known about the relationship between IGF-I and breast cancer in Asian women. Given the possible influence of diet and nutrition on circulating IGF-I, and the substantial differences in dietary habits between Asian and Caucasian populations, we conducted a population-based case-control study to examine the associations of IGF-I, IGF-II, and IGFBP-3 with breast cancer risk in Chinese women.

Materials and Methods

Study Subjects. A large population-based case-control study was conducted in the urban area of Shanghai, China, from August 1996 through March 1998. Detailed descriptions of the study have been given elsewhere (21). Briefly, the study enrolled 1459 incident breast cancer patients aged between 25 and 64 years, and 1556 healthy controls with frequency-match on age. The cases represented ~91% of the breast cancer patients newly diagnosed in the region during the study time; the controls represented 90% of eligible women randomly selected, as potential controls, from the general population in Shanghai. The Shanghai Resident Registry, which keeps records for all of the permanent residents in urban Shanghai, was used to select potential controls randomly from female residents, frequency-

Received 4/27/01; revised 4/10/02; accepted 4/22/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by Public Health Service Grants CA83050 and CA64277 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed, at Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, P.O. Box 208034, New Haven, CT 06520-8034. Phone: (203) 785-5688; Fax: (203) 785-6980; E-mail: herbert.yu@yale.edu.

[3] The abbreviations used are: IGF, insulin-like growth factor; CV, coefficient of variation; OR, odds ratio; CI, confidence interval; BMI, body mass index; WHR, waist-to-hip ratio.

matched to cases by age (5-year interval). The number of controls in each age-specific stratum was determined in advance according to the age distributions of the incident breast cancer cases reported to the Shanghai Cancer Registry in recent years. Only women who lived at the address identified during the study period were considered to be eligible for the study. Each potential control was visited by an interviewer according to the home address provided in the resident registry to additionally determine study eligibility and her interest in participating in the study. All of the study participants completed an in-person interview with the use of a structured questionnaire, which elicited information on demographic features, menstrual and reproductive history, hormone use, medical history, physical activity, alcohol and tobacco use, dietary habits, and family history of cancer. Eighty-two percent of the cases (1, 193) and 84% of the controls (1, 310) provided blood samples. The specimens were collected in a 10-ml vacutainer tube with either EDTA or heparin anticoagulant. Plasma was separated immediately after collection and stored at −70°C until the study.

To enhance the comparability between the cases and controls in the current study, we selected 300 cases whose blood samples were collected before any radiotherapy or chemotherapy, and 300 individually matched controls. For each index case, a control was selected randomly from the pool of 1310 healthy women who completed the interview and provided a blood sample. The cases and controls were individually matched on age (±5 years), date of blood collection (±30 days), and menopausal status (except for 14 pairs). The ages of the 300 patients ranged between 28 and 64 years, and the mean was 48.5 years (SD = 8.3), which was comparable with the mean age of the controls, 48.4 years (SD = 8.4; range 29–64). Postmenopausal women accounted for ~43% of the women in the study. Of the 600 women selected, there was only 1 subject whose plasma sample was not available for the study.

Specimen Measurement. Plasma concentrations of IGF-I, IGF-II, and IGFBP-3 were determined with the use of commercially available ELISA kits (DSL, Inc., Webster, TX). These methods were used in most of previous epidemiological studies and had fairly good reproducibility (15, 22, 23). The calibrators used in the assays ranged between 4.5–640 ng/ml for IGF-I, 50–2000 ng/ml for IGF-II, and 2.5–100 ng/ml for IGFBP-3. For IGFBP-3 measurement, plasma samples were diluted at 1:100 in an assay buffer. The intra- and interassay precisions are 1.5–3.4 and 1.5–8.5% of CV, respectively, for IGF-I, 4.2–7.2 and 6.3–10.7% of CV for IGF-II, and 0.5–1.9 and 1.8–3.9% of CV for IGFBP-3 (24). Each assay has no cross-reaction with other members of the IGF family. To improve the precision of case-control comparisons, samples from a matched case-control pair were assayed in the same plate.

Statistical Analysis. Because it was known that the distributions of the IGF variables are positively skewed (24), levels of IGF-I, IGF-II, and IGFBP-3 were compared between the cases and controls using the Wilcoxon signed rank test. The Spearman correlation coefficients were used to evaluate the correlations between the IGF variables or between IGF and other variables. To measure the associations of IGF variables with breast cancer risk, we calculated the adjusted ORs and their 95% CI using the conditional logistic regression model. In the logistic regression analysis, IGFs and IGFBP-3 were analyzed both as continuous and categorical variables. Values of IGFs and IGFBP-3 were log transformed when analyzed as continuous variables and were classified into three categories, low, medium, and high, based on the tertile distributions in the control group, when analyzed as categorical variables. Potential confounding factors adjusted in the logistic regression analysis included BMI (kg/m^2), age at menarche (year), age at first live birth (year), total energy intake (kcal), WHR, history of fibroadenoma (yes *versus* no), and family history of breast cancer (yes *versus* no). The association of IGF with breast cancer was additionally analyzed in women stratified by menopausal status. Stratified analyses were also performed in subgroups of women, stratified by BMI, WHR, and total energy intake, to assess the joint effect of these variables with IGFs and IGFBP-3. Levels of these variables were classified into two groups using median values in the control group as a cutoff. To assess the possible interactions between the IGF variables and menopausal status, BMI, WHR, or total energy intake, we included, in addition to two of the variables of interest, the product of two selected variables, in the conditional logistic regression models, but because menopause was one of the matching conditions, the regression model was developed without including menopausal status. All of the *P*s were derived from two-sided tests.

Results

Comparisons of selected demographic and risk factors between breast cancer patients and their matched controls are shown in Table 1. The ranges of BMI were between 15.9 and 42.2 for cases, and between 17.5 and 36.6 for controls among the premenopausal women, and were between 15.1 and 36.1, and 16.0 and 34.3, respectively, among the postmenopausal women. The WHR ranges were between 0.60 and 0.95 for cases, and 0.64 and 0.97 for controls. There were no statistically significant differences between cases and controls with respect to their age, age at menarche, age at menopause, BMI, total energy intake, education, family income, and number of parity. However, compared with controls, more cases had a later age at first live birth ($P = 0.005$) and a history of fibroadenoma ($P = 0.006$). The results also indicated that cases had slightly higher WHR ($P = 0.048$) and more frequently a family history of breast cancer ($P = 0.067$) than controls.

Table 2 shows the range and median concentrations of IGF-I, IGF-II, and IGFBP-3 in the study subjects. Plasma levels of IGF-I and IGFBP-3 were all substantially higher in cases than in controls ($P < 0.001$). Significant differences were seen in both pre- and postmenopausal women for IGFBP-3 and in premenopausal women for IGF-I. Also, cases had slightly higher levels of IGF-II than controls, and the difference was statistically significant ($P = 0.022$). However, no significant differences were found in IGF-II when the subjects were classified by their menopausal status.

IGF-I levels in plasma were inversely correlated with age, and the correlations were the same in cases and controls ($r = -0.42$; $P < 0.001$). No correlation was found between IGF-II and age in either cases or controls; IGFBP-3 was weakly and positively correlated with age in the control group ($r = 0.14$; $P = 0.019$). IGF-I was positively correlated with IGF-II and IGFBP-3, and the correlation between IGF-I and IGFBP-3 was stronger among controls ($r = 0.26$; $P < 0.001$) than among cases ($r = 0.17$; $P = 0.003$), as was the correlation between IGF-I and IGF-II, $r = 0.33$ ($P < 0.001$) for controls and $r = 0.18$ ($P = 0.002$) for cases. No correlation was found between IGF-II and IGFBP-3. Also, no correlations were observed between IGFs and BMI, total energy intake, or WHR. IGFBP-3 was weakly and positively correlated with BMI both in controls ($r = 0.16$; $P = 0.005$) and cases ($r = 0.12$; $P = 0.046$), but was not correlated with total energy intake and WHR.

The results from conditional logistic regression analyses

Table 1 Comparison of characteristics between breast cancer patients and controls[a]

Variable	Case (n = 300)	Control (n = 300)	P
	Mean (SD)	Mean (SD)	
Age (year)	48.5 (8.3)	48.4 (8.4)	0.139
Age at menarche (year)	14.9 (1.9)	14.7 (1.7)	0.180
Age at first lifebirth (year)[b]	26.6 (4.2)	25.8 (4.1)	0.005
Age at menopause (year)[c]	48.9 (4.4)	48.2 (4.4)	0.185
BMI (kg/m^2)			
Premenopause	22.8 (2.9)	22.4 (3.2)	0.198
Postmenopause	24.5 (3.7)	23.8 (3.7)	0.130
WHR	0.81 (0.05)	0.80 (0.06)	0.048
Total energy intake (calorie)	2277 (507)	2234 (474)	0.275
	Percent	Percent	P^d
Education			
Elementary or less	15.0	17.0	
Middle school	42.7	41.3	
High school	29.3	30.0	
College or higher	13.0	11.7	0.879
Family income			
Low	32.7	29.0	
Middle	29.7	32.7	
High	37.7	38.3	0.576
Menopause			
No	57.2	57.1	
Yes (natural)	39.5	38.6	
Yes (surgical)	3.3	4.3	0.804
Number of parity			
1	61.1	59.0	
2	25.6	27.8	
3+	13.3	13.2	0.839
Fibroadenoma			
No	90.3	96.0	
Yes	9.7	4.0	0.006
Family history			
No	96.3	98.7	
Yes	3.7	1.3	0.067

[a] From paired Student's t test.
[b] Parous women.
[c] Among those with natural menopause.
[d] From χ^2 test.

are shown in Table 3. In the univariate analysis, high IGF-I and IGFBP-3, analyzed either as continuous variables (logarithmically transformed the actual concentrations of these variables, with 1 ng/ml being the minimal increment unit) or categorical variables, were associated with increased risk of breast cancer. Because the change in OR was based on the increase in every unit of $e^{(IGFs)}$ where (IGFs) were the actual values (ng/ml) of these molecules, the associations seemed to be much stronger when IGFs were analyzed as continuous variables as opposed to categorical variables, suggesting that the associations were underestimated in the study when categorical data were used in calculation of the ORs. However, to make our results comparable with the findings of other studies, we presented our results mainly based on the categorical data. The ORs were 2.01 (95% CI, 1.26–3.19) for IGF-I and 3.01 (95% CI, 1.81–4.99) for IGFBP-3 when comparing women in the highest tertile with those in the lowest one. Two multiple logistic regression models were developed to assess the independent effect of IGF-I. The association between IGF-I and breast cancer was not significant after adjusting for IGFBP-3 (OR, 1.59; 95% CI, 0.90–2.80), but remained significant when other variables were adjusted (OR, 1.95; 95% CI, 1.18–3.23), including BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, and family history of breast cancer. Similar analyses were also performed for IGFBP-3. A significant association between IGFBP-3 and breast cancer was found in both multivariate models. For IGF-II, a borderline significant association was seen only when IGF-II was analyzed as a continuous variable without adjusting for other variables, but no association was found when the analysis was adjusted for other risk factors or when it was analyzed as a categorical variable either in univariate or in multivariate models. There was a significant interaction between IGF-II and IGF-I (P = 0.034); a borderline significant interaction between IGF-II and IGFBP-3 was also noted (P = 0.068). However, no interaction was detected between IGF-I and IGFBP-3. Because of the interaction, IGF-II was not adjusted in the multivariate analysis of IGF-I and IGFBP-3, both of which were also not adjusted in the analysis of IGF-II (Table 3).

The associations between breast cancer and IGFs or IGFBP-3 were also examined in subgroups of women stratified by menopausal status (Table 4). For IGF-I, a positive association with breast cancer was seen in premenopausal women but not in postmenopausal women. The significant association in premenopausal women was diminished when IGFBP-3 was adjusted in the analysis. However, it should be noted that the association between IGF-I and breast cancer in premenopausal was detected even after the power was substantially reduced. With or without adjustment, high IGFBP-3 was strongly associated with increased risk of breast cancer, particularly in premenopausal women. Although the ORs for breast cancer were somewhat elevated with IGF-II levels, none of the associations or trends were statistically significant. Because interactions existed between IGF-II and IGF-I and IGFBP-3, mutual adjustment for these variables was not performed. Interactions between menopausal status and IGF variables were not found in the analysis.

To assess the joint effect of IGF-I and IGFBP-3 with BMI, WHR, and total energy intake, we compared the associations of breast cancer with IGF-I, IGF-II, and IGFBP-3 in subgroups of women stratified by BMI (high *versus* low), WHR (high *versus* low), and total energy intake (high *versus* low; Table 5). A weak additive effect appeared to be present between BMI and IGF-I or IGFBP-3 as the ORs between high and low IGF-I or IGFBP-3 increased from 1.71 (95% CI, 1.02–2.88) or 1.97 (95% CI, 1.17–3.33) in women with low BMI to 2.44 (95% CI, 1.50–3.97) or 2.48 (95% CI, 1.51–4.07) in those with high BMI, respectively. Similar joint effects were also suggested between WHR and IGF-I or IGFBP-3, but no joint effects were found between total energy intake and IGF-I or IGFBP-3. For IGF-II, none of the associations were significant. The interactions between the IGF variables and BMI, WHR, or total energy intake were examined, and statistically significant interaction was not found in any of these variables except for IGFBP-3 and total energy intake, which was borderline significant (P = 0.057).

Discussion

In the study, we found high plasma levels of IGF-I to be associated with elevated risk of breast cancer in Chinese women. The association was dose-dependent and was more evident in premenopausal women than in postmenopausal women. These results are consistent with the findings in Caucasian women (15–17), indicating that IGF-I may play a similar role in breast cancer among different ethnic groups. A similar finding was also suggested in a small study of 30 African-

Table 2 Plasma levels of IGF-I, IGF-II, and IGFBP-3 in Chinese women

Variable	Case		Control		P^a
	No.	Median (range)	No.	Median (range)	
All women					
IGF-I (ng/ml)	299	143 (31–334)	300	127 (34–350)	<0.001
IGF-II (ng/ml)	299	860 (326–1,857)	300	856 (407–1,430)	0.022
IGFBP-3 (ng/ml)	299	4340 (2,100–11,810)	300	4030 (1,513–10,740)	<0.001
Premenopausal women					
IGF-I (ng/ml)	171	163 (41–334)	170	146 (69–299)	<0.001
IGF-II (ng/ml)	171	852 (326–1,857)	170	867 (407–1,386)	0.205
IGFBP-3 (ng/ml)	171	4224 (2,100–9,767)	170	3901 (2,263–10,740)	0.002
Postmenopausal women					
IGF-I (ng/ml)	128	114 (31–280)	130	106 (34–350)	0.060
IGF-II (ng/ml)	128	867 (362–1,472)	130	810 (454–1,430)	0.093
IGFBP-3 (ng/ml)	128	4597 (2,209–11,810)	130	4189 (1,513–10,730)	0.008

[a] From Wilcoxon signed rank test.

Table 3 Associations of breast cancer with IGF-I, IGF-II, and IGFBP-3 in all women

Variable	Number	Unadjusted		Number	Adjusted[a]		Adjusted[b]	
	Case/control	OR	95% CI	Case/control	OR	95% CI	OR	95% CI
IGF-I (ng/ml)								
Continuous								
ln (IGF-I)	299/300	2.47 ($P < 0.001$)	1.45–4.22	283/291	2.23 ($P = 0.007$)	1.25–3.99	1.46 ($P = 0.279$)	0.73–2.92
Categorical (by tertile)								
≤107.5	77/98	1.00		72/96	1.00		1.00	
107.5–149.6	89/101	1.18	0.75–1.85	86/98	1.33	0.82–2.14	1.16	0.71–1.92
≥149.6	133/101	2.01	1.26–3.19	125/97	1.95	1.18–3.23	1.49	0.85–2.59
Trend test		$P = 0.003$			$P = 0.009$		$P = 0.199$	
IGF-II (ng/ml)								
Continuous								
ln (IGF-II)	299/300	2.51 ($P = 0.050$)	1.00–6.31	283/291	1.76 ($P = 0.280$)	0.63–4.88		
Categorical (by tertile)								
≤711.6	95/99	1.00		92/94	1.00			
711.6–945.1	91/100	1.06	0.66–1.70	86/98	1.00	0.59–1.69		
≥945.1	113/101	1.54	0.79–2.97	105/99	1.22	0.58–2.57		
Trend test		$P = 0.214$			$P = 0.613$			
IGFBP-3 (ng/ml)								
Continuous								
ln (IGFBP-3)	299/300	5.27 ($P < 0.001$)	2.34–11.88	283/291	4.31 ($P < 0.001$)	1.80–10.31	3.15 ($P = 0.029$)	1.12–8.82
Categorical (by tertile)								
≤3698	65/99	1.00		62/96	1.00		1.00	
3698–4395	92/99	1.46	0.95–2.23	88/96	1.28	0.81–2.04	1.16	0.71–1.88
≥4395	142/102	3.01	1.81–4.99	133/99	3.00	1.70–5.31	2.50	1.37–4.58
Trend test		$P < 0.001$			$P < 0.001$		$P = 0.004$	

[a] Adjusted for BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, and family history of breast cancer.
[b] Adjusted for BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, family history of breast cancer, and either IGFBP-3 or IGF-I.

American women (19). However, for Chinese women, the positive association between IGF-I and breast cancer risk was attenuated when IGFBP-3 was adjusted in the analysis.

In our study, high IGFBP-3 was strongly associated with increased risk of breast cancer, and the association was independent of IGFs and other risk factors. There was also a clear dose-response relationship between plasma IGFBP-3 and breast cancer risk. Our finding of a positive association between IGFBP-3 and breast cancer risk in Chinese women was contradictory to the findings of several studies conducted in the Caucasian populations in which IGFBP-3 was inversely associated with breast cancer risk after adjusting for IGF-I (15–17). The inconsistent finding between Chinese and Caucasians may underscore the complexity of IGF regulation and the possible impacts of lifestyle and diet on this IGF binding protein in the circulation. In addition, most studies reported have shown a strong correlation between IGF-I and IGFBP-3, whereas in our study this correlation was relatively weak. Because no epidemiological studies of IGF have been done in Chinese women, it is difficult to determine whether this difference is because of the study population or the study itself.

However, our study was not the only study that found high IGFBP-3 to be associated with increased risk of breast cancer. Two previous studies also showed a positive association between plasma IGFBP-3 and breast cancer risk (18, 25). Furthermore, high IGFBP-3 levels in the blood have also been reported to be associated with elevated risk for prostate cancer (26) and colorectal cancer (27). The potential adverse, instead of protective, effect of IGFBP-3 on breast cancer was also observed in tumor tissue studies. Clinical studies showed that

Table 4 Associations of breast cancer risk with IGF-I, IGF-II, and IGFBP-3 in pre- and postmenopausal women

Variable	Number	Unadjusted		Number	Adjusted[a]		Adjusted[b]	
	Case/control	OR	95% CI	Case/control	OR	95% CI	OR	95% CI
Premenopausal women								
IGF-I by tertile (ng/ml)								
≤125.8	33/56	1.00		32/56	1.00		1.00	
125.9–170.0	60/58	1.78	0.98–3.22	55/56	1.65	0.85–3.21	1.46	0.70–3.03
>170.0	78/56	2.41	1.35–4.29	74/55	2.29	1.20–4.37	1.92	0.88–4.20
Trend test		$P = 0.003$			$P = 0.012$		$P = 0.236$	
IGF-II by tertile (ng/ml)								
<718.7	52/56	1.00		50/54	1.00			
718.8–936.5	53/58	1.32	0.61–2.89	50/58	1.17	0.50–2.71		
>936.5	66/56	1.96	0.74–5.21	61/55	1.50	0.51–4.44		
Trend test		$P = 0.154$			$P = 0.439$			
IGFBP-3 by tertile (ng/ml)								
≤3593	36/56	1.00		35/56	1.00		1.00	
3594–4258	52/57	1.54	0.86–2.75	48/56	1.35	0.72–2.54	0.98	0.47–2.06
>4258	83/57	3.43	1.72–6.87	78/55	3.71	1.67–8.26	2.69	1.12–6.47
Trend test		$P < 0.001$			$P = 0.002$		$P = 0.022$	
Postmenopausal women								
IGF-I by tertile (ng/ml)								
≤94.47	39/43	1.00		38/43	1.00		1.00	
94.5–121.7	32/44	0.82	0.41–1.67	28/42	0.66	0.27–1.59	0.57	0.22–1.43
>121.7	57/43	1.52	0.80–2.88	56/41	1.97	0.93–4.19	1.56	0.68–3.57
Trend test		$P = 0.155$			$P = 0.042$		$P = 0.166$	
IGF-II by tertile (ng/ml)								
≤703.4	39/43	1.00		38/41	1.00			
703.5–962.2	41/43	1.08	0.59–1.99	39/43	1.08	0.53–2.18		
>962.2 ng/ml	48/44	2.13	0.72–6.33	45/42	2.17	0.60–7.90		
Trend test		$P = 0.281$			$P = 0.367$			
IGFBP-3 by tertile (ng/ml)								
≤3779	26/43	1.00		25/40	1.00		1.00	
3780–4863	47/43	1.65	0.88–3.07	44/42	1.52	0.75–3.10	1.40	0.65–2.98
>4863	55/44	2.37	1.08–5.18	53/44	2.60	1.03–6.56	2.11	0.76–5.87
Trend test		$P = 0.027$			$P = 0.044$		$P = 0.178$	

[a] Adjusted for BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, and family history of breast cancer.
[b] Adjusted for BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, family history of breast cancer, and either IGFBP-3 or IGF-I.

high levels of IGFBP-3 in breast cancer tissue were associated with tumors with poor prognosis, including an inverse association with the status of estrogen and progesterone receptors, and positive correlations with tumor size and S phase fraction (28–31). Interestingly, the association between IGFBP-3 and cancer prognosis varied by cancer site. In ovarian cancer, high IGFBP-3 levels in tumor tissue were associated with favorable prognosis (32). The inconsistent relationship between IGFBP-3, and cancer risk and prognosis seems to support the notion that IGFBP-3 has complex functions and does not act alone on IGF action. It is known that the function of IGFBP-3 is regulated by IGFBP proteases in local tissue (33). Degradation of IGFBP-3 by IGFBP proteases changes the binding affinity of IGFBP-3 to IGFs, which subsequently affects the activity of IGF-I.

IGFBP-3 has been found to exert dual regulatory effects on IGF-I action (34, 35). In most situations, IGFBP-3 inhibits the action of IGF-I on cell proliferation and apoptosis, but sometimes this binding protein enhances the mitogenic effect of IGF-I. Functionally, IGFBP-3 has two distinct impacts on IGF-I as a result of binding to the growth factor. When IGFBP-3 binds to IGF-I, the binding protein suppresses IGF-I action because it blocks the interaction between IGF-I and its receptor, IGF-IR. Binding of IGFBP-3 to IGF-I also protects IGF-I from degradation and transports the molecule to local tissue. If IGFBP proteases are present and active in local tissue, IGFBP-3 is degraded by the proteases and releases free IGF-I, which resumes its activity to interact with the IGF-I receptor. In this case, IGFBP-3 enhances the action of IGF-I by increasing the bioavailability of IGF-I in local tissue. On the basis of the different impact of IGFBP-3 on IGF-I, we speculate that high levels of IGFBP-3 in the circulation may lead to two opposite outcomes: (*a*) less biologically active free IGF-I available for IGF action; and (*b*) more total IGF-I available in the system because of prolonged half-life of IGF-I protected by IGFBP-3. The ultimate effect of IGFBP-3 on IGF-I will be determined by IGFBP proteases and other factors that affect IGFBP-3 function. Therefore, measuring the amount of total IGFBP-3 in the blood without knowing its molecular structure and protease activity may not be adequate in assessing its relationship with disease. This may also be true when we adjust for IGFBP-3 in the analysis of IGF-I without considering other factors.

The association between IGF-II and breast cancer risk has not been investigated in previous epidemiological studies. The lack of interest in IGF-II is probably because of several reasons. First, IGF-II is believed to regulate cell proliferation and differentiation only at an early stage of human development. After birth, IGF-II plays a less important role in growth regulation than IGF-I, and the effect of IGF-II is gradually replaced by IGF-I (36). Second, the action of IGF-II on cell growth may be regulated via paracrine mechanism; endocrine regulation is less important for IGF-II than for IGF-I (1). Third, growth hormone

Table 5 Joint effect of IGFs and IGFBP-3 with BMI, WHR, and total calorie intake on breast cancer risk

Variable	<Median		≥Median	
	Number Case/control	OR (95% CI)	Number Case/control	OR (95% CI)
BMI (median = 22.5)				
IGF-I (ng/ml)				
<127	48/73	1.00 (reference)	62/77	1.11 (0.65–1.88)
≥127	78/78	1.71 (1.02–2.88)	110/72	2.44 (1.50–3.97)
IGF-II (ng/ml)				
<856	65/73	1.00 (reference)	84/77	1.24 (0.78–1.98)
≥856	61/78	0.90 (0.47–1.71)	88/72	1.42 (0.74–2.72)
IGFBP-3 (ng/ml)				
<4019	53/84	1.00 (reference)	59/66	1.48 (0.89–2.47)
≥4019	73/67	1.97 (1.17–3.33)	113/83	2.48 (1.51–4.07)
WHR (median = 0.7979)				
IGF-I (ng/ml)				
<127	40/75	1.00 (reference)	70/75	1.82 (1.06–3.13)
≥127	86/73	2.51 (1.47–4.26)	102/77	3.10 (1.78–5.39)
IGF-II (ng/ml)				
<856	73/86	1.00 (reference)	76/64	1.54 (0.93–2.56)
≥856	53/62	1.04 (0.54–2.00)	96/88	1.38 (0.73–2.59)
IGFBP-3 (ng/ml)				
<4019	51/75	1.00 (reference)	61/75	1.25 (0.75–2.09)
≥4019	75/73	1.62 (0.97–2.72)	111/77	2.56 (1.52–4.32)
Total calorie intake (median = 2234)				
IGF-I (ng/ml)				
<127	58/86	1.00 (reference)	52/63	1.18 (0.71–1.96)
≥127	101/84	2.11 (1.28–3.48)	88/67	2.18 (1.33–3.56)
IGF-II (ng/ml)				
<856	75/81	1.00 (reference)	74/69	1.18 (0.74–1.89)
≥856	84/89	0.99 (0.53–1.85)	66/61	1.16 (0.62–2.16)
IGFBP-3 (ng/ml)				
<4019	57/94	1.00 (reference)	55/56	1.61 (0.96–2.71)
≥4019	102/76	2.57 (1.56–4.23)	85/74	2.06 (1.28–3.32)

regulates the production of IGF-I but not IGF-II. Fourth, energy balance and protein intake have a significant impact on IGF-I levels in the circulation but little on IGF-II. Because our study focused on a special population that has a distinct lifestyle and dietary habit from the Western society, we included IGF-II in the study to assess its impact on breast cancer risk. Although we did not find clear evidence for a positive association of IGF-II with breast cancer risk, there were some indications suggesting a potential relationship, which was more evident in postmenopausal women. However, the reduced power in the subgroup analysis may hamper our ability to detect any significant associations. The possible interactions between IGF-II and IGF-I and IGFBP-3 also indicate that IGF-II may play an interesting role in the relationships of breast cancer with IGF-I and IGFBP-3. Larger studies are warranted to clarify the role of IGF-II in breast cancer.

Although we did not include IGF-II in our multivariate analysis, the potential interaction between IGF-II and IGF-I or IGFBP-3 indicates that the association between breast cancer and IGF-I or IGFBP-3 may vary by IGF-II levels in the circulation. To preliminarily assess the impact of IGF-II on the relationship between the disease and IGF-I or IGFBP-3, we performed additional analysis on subgroups of women stratified by their IGF-II levels. On the basis of IGF-II median level in the control group, the study subjects were classified into two groups, low IGF-II *versus* high IGF-II. The associations between breast cancer and IGF-I and IGFBP-3 were analyzed in each IGF-II category. The results showed that the disease association with IGF-I was more evident in women with low IGF-II, whereas the association with IGFBP-3 was more significant in high IGF-II subjects (Table 6). Although these associations were significant only in one IGF-II group, we found no evidence that conflicting associations existed in women with different levels of IGF-II, suggesting that the interaction between IGF-II and IGF-I or IGFBP-3 has a minimal impact on the association of breast cancer with IGF-I or IGFBP-3.

Table 6 Interaction between IGF-II and IGF-I/IGFBP-3 in relation to breast cancer

Variable	Low IGF-II		High IGF-II	
	OR[a]	95% CI	OR[a]	95% CI
IGF-I Low	1.00		1.00	
IGF-I Medium	1.89	0.87–4.09	1.16	0.52–2.56
IGF-I High	4.05	1.66–9.88	1.85	0.80–4.23
Test for trend	$P = 0.002$		$P = 0.125$	
IGFBP-3 Low	1.00		1.00	
IGFBP-3 Med	1.00	0.44–2.30	2.34	1.10–4.96
IGFBP-3 High	2.37	0.91–6.18	4.32	1.71–10.93
Test for trend	$P = 0.098$		$P = 0.002$	

[a] Adjusted for BMI, age at menarche, age at first live birth, total energy intake, WHR, history of fibroadenoma, and family history of breast cancer.

Both human and animal studies have shown that malnutrition or restriction on total energy or protein intake causes declines in serum concentrations of IGF-I and IGFBP-3. Levels of these molecules can quickly normalize when the condition is improved (37–41). Furthermore, high energy or high protein intake results in increases in the production of IGF-I and IGFBP-3 (42). However, these relationships have not been seen in epidemiological studies (1). The inconsistency may be because of the homogeneity of the study population in lifestyle and the insensitive methods used in measuring dietary nutrients in epidemiological studies. To assess the potential impacts of diet and lifestyle on the IGF system, we compared IGF-I concentrations in plasma between healthy Caucasian women and their Chinese counterparts using the data published in literature (15). Among premenopausal women, the tertile cutoffs were 158 ng/ml and 207 ng/ml for Caucasian women, whereas for Chinese women the corresponding levels were much lower, only 126 ng/ml and 170 ng/ml. The same trend also existed for postmenopausal women when the quartile classifications were compared between the two groups. The quartile cutoffs were 110 ng/ml, 145 ng/ml, and 220 ng/ml for Caucasians and 87 ng/ml, 106 ng/ml, and 131 ng/ml for Chinese. Overall, Chinese women had 20–40% lower IGF-I levels in plasma than Caucasians. The discrepancies in IGF-I are unlikely to be explained by the differences in laboratory methods because the same assay from the same company is used in both studies, or by the differences in the type of specimens analyzed because both studies measure the concentration of IGF-I in plasma, or by the differences in the controls because both control groups are healthy women. Also, because this is a population-based study, which has a high recruiting rate, 90%, and the mobility of people permanently living in the city of Shanghai is low because of better living conditions and tight government control, the control women in our study should well represent the general population in the city.

In the study, we found no correlation between total calorie intake and serum levels of IGF-I and IGFBP-3, and no joint effect of these factors on breast cancer risk. However, measuring total calorie intake at one time point may not reflect the overall long-term dietary habit and lifestyle; BMI and WHR may be more relevant to these factors. We evaluated the joint effect of IGFs and IGFBP-3 with BMI and WHR. The results showed that women with both high IGF and high BMI or WHR had much higher risk of breast cancer compared with those with only one high variable. These findings suggest that in Chinese women, there is a possible synergistic interaction among IGF, diet, and lifestyle in relation to breast cancer risk.

In summary, we found that high IGF-I was associated with increased risk of breast cancer in Chinese women. The association was dose-dependent and was more evident in premenopausal women. These results were in agreement with the findings in Caucasians. However, our study also showed that high IGFBP-3 was associated with elevated risk of breast cancer; this finding was contradictory to those seen in some of the Caucasian studies in which IGFBP-3 was found to be inversely associated with breast cancer risk after adjusting for IGF-I. The reason for the inconsistency remains to be determined. The study also suggested possible synergistic interplay among IGF, diet, and lifestyle in relation to breast cancer risk. In conclusion, our results confirm that high circulating IGF-I is a risk factor for breast cancer.

References

1. Yu, H., and Rohan, T. Role of the insulin-like growth factor family in cancer development and progression. J. Natl. Cancer Inst., *92:* 1472–1489, 2000.

2. Khandwala, H. M., McCutcheon, I. E., Flyvbjerg, A., and Friend, K. E. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr. Rev., *21:* 215–244, 2000.

3. Sara, V. R., and Hall, K. Insulin-like growth factors and their binding proteins. Physiol. Rev., *70:* 591–614, 1990.

4. Jones, J. I., and Clemmons, D. R. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev., *16:* 3–34, 1995.

5. Furlanetto, R. W., and DiCarlo, J. N. Somatomedin-C receptors and growth effects in human breast cells maintained in long-term tissue culture. Cancer Res., *44:* 2122–2128, 1984.

6. Karey, K. P., and Sirbasku, D. A. Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17b-estradiol. Cancer Res., *48:* 4083–4092, 1988.

7. Arteaga, C. L., and Osborne, C. K. Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. Cancer Res., *49:* 6237–6241, 1989.

8. Dunn, S. E., Hardman, R. A., Kari, F. W., and Barrett, J. C. Insulin-like growth factor 1 (IGF-I) alters drug sensitivity of HBL 100 human breast cancer cells by inhibition of apoptosis induced by diverse anticancer drugs. Cancer Res., *57:* 2687–2693, 1997.

9. Gooch, J. L., Van Den Berg, C. L., and Yee, D. Insulin-like growth factor (IGF)-I rescues breast cancer cells from chemotherapy-induced cell death-proliferative and anti-apoptotic effects. Breast Cancer Res. Treat., *56:* 1–10, 1999.

10. Stewart, A. J., Johnson, M. D., May, F. E., and Westley, B. R. Role of insulin-like growth factor and the type I insulin-like growth factor receptor in the estrogen-stimulated proliferation of human breast cancer cells. J. Biol. Chem., *265:* 21172–21178, 1990.

11. Thorsen, T., Lahooti, H., Rasmussen, M., and Aakvaag, A. Oestradiol treatment increases the sensitivity of MCF-7 cells for the growth stimulatory effect of IGF-I. J. Steroid Biochem. Mol. Biol., *41:* 537–540, 1992.

12. Huynh, H., Yang, X., and Pollak, M. Estradiol and antiestrogens regulate a growth inhibitory insulin-like growth factor binding protein 3 autocrine loop in human breast cancer cells. J. Biol. Chem., *271:* 1016–1021, 1996.

13. Mukherjee, P., Sotnikov, A. V., Mangian, H. J., Zhou, J. R., Visek, W. J., and Clinton, S. K. Energy intake and prostate tumor growth, angiogenesis, and vascular endothelial growth factor expression. J. Natl. Cancer Inst., *91:* 512–523, 1999.

14. Dunn, S. E., Kari, F. W., French, J., Leininger, J. R., Travlos, G., Wilson, R., and Barrett, J. C. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res., *57:* 4667–4672, 1997.

15. Hankinson, S. E., Willett, W. C., Colditz, G. A., Hunter, D. J., Michaud, D. S., Deroo, B., Rosner, B., Speizer, F. E., and Pollak, M. Circulating concentrations of insulin-like growth factor-1 and risk of breast cancer. Lancet, *351:* 1393–1396, 1998.

16. Bruning, P. F., Van Doorn, J., Bonfrer, J. M., Van Noord, P. A., Korse, C. M., Linders, T. C., and Hart, A. A. Insulin-like growth-factor binding protein 3 is decreased in early-stage operable pre-menopausal breast cancer. Int. J. Cancer, *62:* 266–270, 1995.

17. Bohlke, K., Cramer, D. W., Trichopoulos, D., Mantzoros, C. S. Insulin-like growth factor-1 in relation to premenopausal ductal carcinoma *in situ* of the breast. Epidemiology, *9:* 570–573, 1998.

18. Toniolo, P., Bruning, P. F., Akhmedkhanov, A., Bonfrer, J. M., Koenig, K. L., Lukanova, A., Shore, R. E., and Zeleniuch-Jacquotte, A. Serum insulin-like growth factor-I and breast cancer. Int. J. Cancer, *88:* 828–832, 2000.

19. Agurs-Collins, T., Adams-Campbell, L. L., Kim, K. S., and Cullen, K. J. Insulin-like growth factor-1 and breast cancer risk in postmenopausal African-American women. Cancer Detect. Prev., *24:* 199–206, 2000.

20. Byrne, C., Colditz, G. A., Willett, W. C., Speizer, F. E., Pollak, M., and Hankinson, S. E. Plasma insulin-like growth factor (IGF) I. IGF-binding protein 3, and mammographic density. Cancer Res., *60:* 3744–3748, 2000.

21. Gao, Y. T., Shu, X. O., Dai, Q., Potter, J. D., Brinton, L. A., Wen, W., Sellers, T. A., Kushi, L. H., Ruan, Z., Bostick, R. M., Jin, F., and Zheng, W. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai breast cancer study. Int. J. Cancer, *87:* 295–300, 2000.

22. Chan, J. M., Stampfer, M. J., Giovannucci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H., and Pollak, M. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science (Wash. DC), *279:* 563–566, 1998.

23. Manousos, O., Souglakos, J., Bosetti, C., Tzonou, A., Chatzidakis, V., Trichopoulos, D., Adami, H. O., and Mantzoros, C. IGF-I and IGF-II in relation to colorectal cancer. Int. J. Cancer, *83:* 15–17, 1999.

24. Yu, H., Mistry, J., Nicar, M. J., Khosravi, M. J., Diamandis, A., van Doorn, J., and Juul, A. Insulin-like growth factors (IGF-I, free IGF-I, and IGF-II) and

insulin-like growth factor binding proteins (IGFBP-2. IGFBP-3, IGFBP-6, and ALS) in blood circulation. J. Clin. Lab. Anal., *13:* 166–172, 1999.

25. Del Giudice, M. E., Fantus, I. G., Ezzat, S., McKeown-Eyssen, G., Page, D., and Goodwin, P. J. Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res. Treat., *47:* 111–120, 1998.

26. Stattin, P., Bylund, A., Rinaldi, S., Biessy, C., Dechaud, H., Stenman, U. H., Egevad, L., Riboli, E., Hallmans, G., and Kaaks, R. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. J. Natl. Cancer Inst., *92:* 1910–1917, 2000.

27. Kaaks, R., Toniolo, P., Akhmedkhanov, A., Lukanova, A., Biessy, C., Dechaud, H., Rinaldi, S., Zeleniuch-Jacquotte, A., Shore, R. E., and Riboli, E. Serum c-peptide, insulin-like growth factor (IGF)-I. IGF-binding proteins, and colorectal cancer risk in women. J. Natl. Cancer Inst., *92:* 1592–1600, 2000.

28. Yu, H., Levesque, M. A., Khosravi, M. J., Papanastasiou-Diamandi, A., Clark, G. M., and Diamandis, E. P. Associations between insulin-like growth factors and their binding proteins and other prognostic indicators in breast cancer. Br. J. Cancer, *74:* 1242–1247, 1996.

29. Rocha, R. L., Hilsenbeck, S. G., Jackson, J. G., Lee, A. V., Figueroa, J. A., and Yee, D. Correlation of insulin-like growth factor-binding protein-3 messenger RNA with protein expression in primary breast cancer tissues: detection of higher levels in tumors with poor prognostic features. J. Natl. Cancer Inst., *88:* 601–606, 1996.

30. Rocha, R. L., Hilsenbeck, S. G., Jackson, J. G., VanDenBerg, C. L., Weng, C., Lee, A. V., and Yee, D. Insulin-like growth factor binding protein-3 and insulin receptor substrate-1 in breast cancer: correlation with clinical parameters and disease-free survival. Clin. Cancer Res., *3:* 103–109, 1997.

31. Yu, H., Levesque, M. A., Khosravi, M. J., Papanastasiou-Diamandi, A., Clark, G. M., and Diamandis, E. P. Insulin-like growth factor-binding protein-3 and breast cancer survival. Int, J. Cancer, *79:* 624–628, 1998.

32. Katsaros, D., Yu, H., Levesque, M. A., Danese, S., Genta, F., Richiardi, G., Fracchioli, S., Khosravi, M. J., Diamandi, A., Gordini, G., Diamandis, E. P., and Massobrio, M. IGFBP-3 in epithelial ovarian carcinoma and its association with clinico-pathological features and patients survival. Eur. J. Cancer, *37:* 478–485, 2001.

33. Collett-Solberg, P. F., and Cohen, P. The role of the insulin-like growth factor binding proteins and the IGFBP proteases in modulating IGF action. Endocrinol. Metab. Clin. N. Am., *25:* 591–614, 1996.

34. Kelley, K. M., Oh, Y., Gargosky, S. E., Gucev, Z., Matsumoto, T., Hwa, V., Ng, L., Simpson, D. M., and Rosenfeld, R. G. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int. J. Biochem. Cell Biol., *28:* 619–637, 1996.

35. Clemmons, D. R. Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine Growth Factor Rev., *8:* 45–62, 1997.

36. Stewart, C. E. H., and Rotwein, P. Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol. Rev., *76:* 1005–1026, 1996.

37. Soliman, A. T., Hassan, A. E., Aref, M. K., Hintz, R. L., Rosenfeld, R. G., and Rogol, A. D. Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. Pediatr. Res., *20:* 1122–1130, 1986.

38. Devine, A., Rosen, C., Mohan, S., Baylink, D., and Prince, R. L. Effects of zinc and other nutritional factors on insulin-like growth factor I and insulin-like growth factor binding proteins in postmenopausal women. Am. J. Clin. Nutr., *68:* 200–206, 1998.

39. Smith, W. J., Underwood, L. E., and Clemmons, D. R. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J. Clin. Endocrinol. Metab., *80:* 443–449, 1995.

40. Straus, D. S., and Takemoto, C. D. Effect of dietary protein deprivation on insulin-like growth factor (IGF)-I and -II. IGF binding protein-2, and serum albumin gene expression in rat. Endocrinology, *127:* 1849–1860, 1990.

41. VandeHaar, M. J., Moats-Staats, B. M., Davenport, M. L., Walker, J. L., Ketelslegers, J. M., Sharma, B. K., and Underwood, L. E. Reduced serum concentrations of insulin-like growth factor-I (IGF-I) in protein-restricted growing rats are accompanied by reduced IGF-I mRNA levels in liver and skeletal muscle. J. Endocrinol., *130:* 305–312, 1991.

42. Thissen, J. P., Ketelslegers, J. M., and Underwood, L. E. Nutritional regulation of the insulin-like growth factors. Endocr. Rev., *15:* 80–101, 1994.

Insulin-like Growth Factors and Breast Cancer Risk in Chinese Women

Herbert Yu, Fan Jin, Xiao-Ou Shu, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:705-712.