

Joint Effect of Insulin-like Growth Factors and Sex Steroids on Breast Cancer Risk¹

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Abstract

Insulin-like growth factors (IGFs) and estrogens are essential hormones regulating the growth and differentiation of mammary cells. Studies have shown that IGFs and estrogens are strong mitogens for breast cancer cells and that high circulating IGF-I and estrogens are risk factors of breast cancer. Laboratory experiments further indicate that these hormones act synergistically on the pathogenesis of breast cancer. Estrogens increase the effect of IGF-I on breast cancer cells by stimulating the expression of IGF-I and IGF-I receptor; IGFs enhance the action of estrogens by regulating the production of estrogen receptor. Furthermore, the two systems have substantial signal transduction cross-talk. Despite the laboratory indications, there is no epidemiological evidence in support of the joint effect of IGFs and estrogens on breast cancer risk. To test this hypothesis in human populations, we compared plasma levels of IGFs and sex steroid hormones among 300 incident breast cancer patients and 300 age- and menopause-matched control women derived from a large population-based case-control study conducted in Shanghai, China between 1996 and 1998. Fasting morning blood samples were measured for plasma concentrations of IGF-I, IGF-II, IGF-binding protein (IGFBP)-3, estradiol, estrone, estrone sulfate, testosterone, and dehydroepiandrosterone sulfate using commercial immunoassay kits. Levels of these hormones were classified into two groups based on the median levels in the control group and combined between IGFs and steroid hormones. Conditional logistic regression analysis was performed to examine the association of breast cancer risk with the combined variables of sex steroids and IGFs. A synergistic effect on breast cancer

risk was suggested for IGF-I or IGFBP-3 with estrone or testosterone among both pre- and postmenopausal women. Compared with premenopausal women with low circulating levels of both IGF-I and estrone, the odds ratios were 1.21 [95% confidence interval (CI), 0.61–2.40] for high estrone, 1.50 (95% CI, 0.77–2.93) for high IGF-I, and 2.30 (95% CI, 1.21–4.37) for both high estrone and high IGF-I. Similar patterns of association were also seen for IGF-I with testosterone as well as for IGFBP-3 with estrone or testosterone, and some of the interactions were statistically significant or borderline significant ($P < 0.10$). No joint effects were found for IGF-I or IGFBP-3 with estradiol or estrone sulfate. In conclusion, the study suggests a possible synergy between IGF peptide hormone and sex steroid hormones, including both estrogen and androgen, in relation to breast cancer risk.

Introduction

Sex steroid hormones play a pivotal role in the development of breast cancer (1, 2). Women experiencing prolonged exposure to or having high circulating levels of estrogens are at increased risk for breast cancer. However, evidence also indicates that other regulatory molecules are involved and may work in concert with estrogens in facilitating carcinogenesis in breast tissue (3). Of those, IGFs,³ a group of peptide hormones with potent mitogenic and antiapoptotic effects on mammary cells, are found to be able to stimulate breast cancer growth both *in vitro* and *in vivo* (4, 5). Recent epidemiological studies further show that high circulating IGF-I is associated with increased risk of breast cancer, suggesting possible involvement of this molecule in the etiology of the disease (6–8).

Laboratory experiments have suggested substantial signal transduction cross-talk between mitogenic growth factors and sex steroid hormones (9, 10). Cell culture studies show that estrogens are able to enhance the actions of IGFs (11, 12) and stimulate the expression of IGF-I, IGF-IR, and its substrate, insulin receptor substrate 1 (13–15). Moreover, estrogens can suppress the production of IGFBPs that inhibit IGF action by blocking the interaction of IGFs with their receptors (16, 17). Estrogens also down-regulate the expression of type two IGF receptor, which degrades IGF-II, reducing its activity (18). Tamoxifen, an antiestrogen agent in the breast, counteracts the actions of IGFs in breast tissue by inhibiting the transcription of IGF-I and diminishing the response of IGF-IR to IGFs (19, 20). Similarly, IGFs also enhance the action of estrogens. IGF-I increases the activity of ER and stimulates the conversion of latent estrogen (estrone-S) to its active form, estrone (21, 22).

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³ The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF-binding protein; DHEA-S, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin; CV, coefficient of variance; OR, odds ratio; CI, confidence interval; IGF-IR, IGF-I receptor; ER, estrogen receptor; estrone-S, estrone sulfate.

Table 1 Plasma levels of IGFs and steroid hormones in breast cancer patients and controls

Variable	Case	Control	<i>P</i> ^a
Premenopausal women			
IGF-I (ng/ml)	163 (133, 201) ^b	146 (118, 179) ^b	0.018
IGF-II (ng/ml)	852 (688, 1043)	867 (653, 999)	0.248
IGFBP-3 (ng/ml)	4224 (3693, 5046)	3901 (3377, 4510)	0.009
Estradiol (pg/ml)	41.1 (23.1, 74.2)	45.3 (28.0, 74.0)	0.507
Estrone (pg/ml)	32.8 (23.5, 52.5)	28.9 (21.4, 42.3)	0.348
Estrone-S (pg/ml)	1412 (445, 3811)	1408 (415, 3400)	0.920
Testosterone (pg/ml)	207 (143, 294)	189 (133, 250)	0.036
DHEA-S (ng/ml)	907 (622, 1335)	826 (610, 1091)	0.300
SHBG (nmol/ml)	71.2 (42.2, 118.3)	73.9 (48.0, 121.0)	0.772
Progesterone (pg/ml)	1200 (600, 4500)	1200 (600, 5700)	0.498
Postmenopausal women			
IGF-I (ng/ml)	114 (91, 158)	106 (87, 131)	0.302
IGF-II (ng/ml)	867 (676, 1049)	810 (667, 1016)	0.848
IGFBP-3 (ng/ml)	4596 (3946, 5692)	4189 (3686, 5307)	0.316
Estrone (pg/ml)	19.6 (9.6, 25.4)	17.7 (9.1, 20.7)	0.004
Estrone-S (pg/ml)	1214 (456, 6677)	978 (464, 7262)	0.282
Testosterone (pg/ml)	199 (124, 282)	161 (121, 217)	0.034
DHEA-S (ng/ml)	680 (487, 1011)	611 (410, 828)	0.152
SHBG (nmol/ml)	73.1 (51.3, 108.0)	74.5 (53.8, 116.3)	0.198

^a χ^2 test based on the quartile distributions between cases and controls.

^b Values represent median (25th, 75th percentile).

The presence of ER in breast cancer cells enhances IGF signaling (23).

Animal experiments suggest that sex steroids affect the expression of IGF-II and IGFBPs, which act synergistically with steroid hormones on mammary tumor (17). Experiments further demonstrate that estrogens influence not only the expression of IGF ligands, receptors, and IGFBPs, but also IGF signal transduction. Estradiol is able to increase the expression of insulin receptor substrate 1, subsequently enhancing IGF signaling (24). ER- α can bind directly to the regulatory subunit of phosphatidylinositol 3'-kinase, which is involved in the signal transduction pathway activated by IGFs, and the binding activity is enhanced by estrogens (25). The presence of ER is important for IGF actions in breast cancer cells. It is reported that re-expressing ER- α in ER-negative breast cancer cells can restore both estrogen- and IGF-mediated signaling (23). IGF-I is able to enhance the interaction between ER and ER response element in the ER-activated genes even without the presence of ER ligand, estrogens (26).

Together, these peptide and steroid hormones exert strong mitogenic stimulation on breast cancer cells. Despite a large number of laboratory studies suggesting a strong synergy between estrogens and IGFs in initiating or promoting breast cancer growth, there is little epidemiological evidence in support of this interaction. In this report, we evaluated the joint effect of sex steroid hormones and IGFs on breast cancer risk in the Shanghai Breast Cancer Study, a population-based case-control study conducted among Chinese women in Shanghai.

Materials and Methods

Study Subjects. Between August 1996 and March 1998, 1459 incident breast cancer patients and 1556 community control women were enrolled in a population-based case-control study of breast cancer in Shanghai, China (27). The cases represented 91% of the newly diagnosed breast cancer patients, 25–64 years of age, identified for the study in urban Shanghai. The control women were randomly selected from the general population based on frequency-match to the age distribution of

Table 2 Correlations between IGFs and steroid hormones among controls

Variable	Spearman correlation coefficient (<i>P</i>)		
	IGF-I	IGF-II	IGFBP-3
Premenopausal women			
Estradiol (<i>n</i> = 154)	0.01 (0.944)	-0.06 (0.442)	-0.03 (0.696)
Estrone (<i>n</i> = 153)	0.07 (0.365)	-0.10 (0.204)	-0.04 (0.586)
Estrone sulfate (<i>n</i> = 170)	-0.10 (0.187)	-0.28 (<0.001)	-0.00 (0.985)
Testosterone (<i>n</i> = 147)	-0.06 (0.460)	-0.12 (0.152)	-0.12 (0.142)
DHEA-S (<i>n</i> = 169)	0.09 (0.246)	-0.15 (0.055)	-0.15 (0.052)
SHBG (<i>n</i> = 168)	-0.22 (0.004)	-0.21 (0.006)	0.10 (0.214)
Progesterone (<i>n</i> = 163)	0.13 (0.110)	-0.11 (0.145)	0.04 (0.608)
Postmenopausal women			
Estradiol (<i>n</i> = 56)	0.24 (0.081)	0.39 (0.003)	-0.36 (0.006)
Estrone (<i>n</i> = 92)	0.09 (0.413)	0.03 (0.799)	-0.09 (0.419)
Estrone sulfate (<i>n</i> = 128)	-0.12 (0.175)	0.30 (<0.001)	-0.35 (<0.001)
Testosterone (<i>n</i> = 112)	0.16 (0.095)	0.01 (0.953)	-0.23 (0.013)
DHEA-S (<i>n</i> = 126)	0.28 (0.002)	0.17 (0.064)	0.03 (0.743)
SHBG (<i>n</i> = 128)	-0.15 (0.099)	-0.02 (0.823)	-0.01 (0.938)

cases. A list of all permanent residents in urban Shanghai registered in the Shanghai Resident Registry was used for random selection of controls from the entire female population in the city. The number of controls in each age stratum (5-year interval) was determined in advance, based on the most recent data on age distribution of breast cancer cases reported to the Shanghai Cancer Registry. Once potential controls were identified from the resident registration list, our study interviewers visited the candidates to verify their eligibility and participation. Slightly over 90% of potential control women participated in the study.

Each subject enrolled in the study underwent an in-person interview using a structured questionnaire. Information collected in the questionnaire included demographic features, menstrual and reproductive history, use of sex steroid hormones, medical history, physical activity, alcohol and tobacco use, dietary habits, and family history of cancer. In addition to questionnaire information, blood samples were collected from the study participants. Of the 1459 cases and 1556 controls, morning fasting blood samples were taken from 1193 cases (82%) and 1310 controls (84%). The specimens were processed within 6 h of collection and stored at -70°C.

To improve the comparability of the study populations, we selected 300 cases and 300 individually matched controls for the current study. The selection of cases was based on the following conditions: (a) patient blood samples were collected before breast cancer treatment; and (b) patients were individually matched to controls on age (± 5 years), date of blood collection (± 30 days), and menopausal status (except for 14 pairs). For premenopausal women, the cases and controls were additionally matched on their menstruation day, which was either within the first 10 days of menstruation (mainly in follicular phase) or within 3 days after the first 10 days (in either follicular phase or luteal phase).

Specimen Measurement. Plasma concentrations of steroids, including testosterone, estradiol, estrone, estrone-S, DHEA-S, and progesterone, were measured directly without extraction. Commercial radioimmunoassays from Diagnostic Systems Laboratories (Webster, TX) were used for steroid measurement. An immunoradiometric assay from Diagnostic Systems Laboratories was used for SHBG. The intra- and interassay precisions for these methods were 6.7–8.1% and 5.7–10.5% of CV, respectively, for testosterone; 3.2–5.3% and 8.1–9.3% of CV for estradiol; 4.4–9.4% and 6.0–11.1% of CV for estrone;

Table 3 Associations of breast cancer with IGFs and steroid hormones^a

Variable	1 st quartile ^b (Reference)	2 nd quartile OR (95% CI)	3 rd quartile OR (95% CI)	4 th quartile OR (95% CI)	P for trend	Median cutoff
Premenopausal women						
IGF-I	1.00	1.41 (0.72–2.77)	1.48 (0.72–3.06)	2.66 (1.40–5.05)	0.003	1.78 ^c
IGF-II	1.00	1.88 (0.86–4.11)	1.15 (0.41–3.28)	1.69 (0.61–4.73)	0.378	0.82
IGFBP-3	1.00	1.17 (0.57–2.39)	2.17 (1.05–4.46)	4.26 (1.89–9.63)	<0.001	2.56 ^c
Estradiol	1.00	0.61 (0.31–1.18)	0.62 (0.33–1.15)	0.68 (0.37–1.26)	0.265	0.83
Estrone	1.00	1.02 (0.52–2.03)	1.44 (0.75–2.79)	1.46 (0.78–2.76)	0.149	1.44
Estrone-S	1.00	1.00 (0.53–1.91)	0.97 (0.47–1.99)	1.21 (0.65–2.27)	0.514	1.11
Testosterone	1.00	1.38 (0.62–3.06)	1.17 (0.51–2.66)	2.92 (1.27–6.70)	0.009	1.46
DHEA-S	1.00	0.80 (0.42–1.53)	0.92 (0.48–1.74)	1.43 (0.77–2.68)	0.187	0.85
SHBG	1.00	0.76 (0.39–1.48)	0.73 (0.37–1.43)	0.73 (0.36–1.49)	0.418	0.91
Progesterone	1.00	1.38 (0.70–2.72)	1.25 (0.62–2.55)	0.82 (0.40–1.70)	0.450	
Postmenopausal women						
IGF-I	1.00	1.34 (0.61–2.96)	1.05 (0.44–2.52)	2.11 (0.92–4.86)	0.108	1.29
IGF-II	1.00	0.89 (0.46–1.72)	1.63 (0.68–3.90)	2.21 (0.70–6.96)	0.259	1.78
IGFBP-3	1.00	1.17 (0.58–2.39)	1.43 (0.71–2.89)	2.86 (1.02–7.99)	0.059	1.55
Estrone	1.00	0.81 (0.33–1.98)	0.82 (0.35–1.93)	2.67 (1.17–6.07)	0.007	1.79 ^c
Estrone-S	1.00	0.56 (0.22–1.41)	1.41 (0.56–3.57)	0.72 (0.31–1.68)	1.000	1.32
Testosterone	1.00	0.58 (0.25–1.35)	1.04 (0.47–2.33)	1.87 (0.84–4.20)	0.034	1.89 ^c
DHEA-S	1.00	1.89 (0.87–4.11)	1.27 (0.61–2.65)	2.92 (1.28–6.67)	0.037	1.40
SHBG	1.00	1.00 (0.46–2.19)	0.86 (0.39–1.88)	0.79 (0.36–1.74)	0.487	0.92

^a Unadjusted conditional logistic regression analysis.^b Lowest quartile.^c $P < 0.05$.

4.6–9.2% and 5.1–8.8% of CV for estrone-S; 1.8–5.2% and 4.8–5.3% of CV for DHEA-S; 4.8–8.0% and 9.2–13.1% of CV for progesterone; and 1.1–3.7% and 8.7–11.5% of CV for SHBG.

Plasma concentrations of IGF-I, IGF-II, and IGFBP-3 were determined using commercial ELISA kits from Diagnostic Systems Laboratories. For IGF measurement, the plasma specimens were treated with acid-ethanol solution to separate IGFs from their binding proteins. The intra- and inter-assay precisions were 1.5–3.4 and 1.5–8.5% of CV for IGF-I, respectively; 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. Each assay does not have cross-reaction with other members of the IGF family. To eliminate measurement difference between cases and controls caused by plate-to-plate or batch-to-batch variation, we analyzed the samples of matched cases and controls in the same plate or batch. Technicians who performed the tests were unaware of the case-control status.

Statistical Analysis. Because most of the steroids and growth factors had a skewed distribution, nonparametric methods were used for data analysis. Spearman correlation coefficients were computed to assess the correlation between IGF and steroids. The evaluation was done among control subjects who had detectable levels of hormones. The associations of breast cancer risk with steroid and peptide hormones were evaluated by calculating ORs and their 95% CIs using conditional logistic regression analysis. In the regression model, IGFs and steroids were analyzed as categorical variables. The classification was based on the quartile distribution of a hormone in control women, and the cutoffs for each hormone are listed in Table 1. Conditional logistic regression analysis was also performed to examine the joint effect of IGFs and sex steroids after combining these hormones together. To create the combined variables, we first classified plasma levels of IGFs and steroids into two categories, high *versus* low, using the medians among control subjects as cutoffs ($<$ median *versus* \geq median). The four combinations of two dichotomous variables, including low/low,

high/low, low/high, and high/high, made up the categories of a combined variable. For instance, to combine IGF-I and estrone, we created a new variable with the following categories: women with low IGF-I and low estrone; women with low IGF-I and high estrone; women with high IGF-I and low estrone; and women with high IGF-I and high estrone. Multivariate analysis was performed to adjust for potential confounding factors, which included age at menarche, age at first live birth, a history of fibroadenoma, a family history of breast cancer, and SHBG. Interactions between interested peptide hormones and sex steroids were also examined in the regression analysis by fitting a logistic regression model with two independent variables of interest plus a product term of the two variables.

Results

The quartile distributions of peptide and steroid hormones among cases (left panel) and controls (right panel) stratified by their menopausal status are shown in Table 1. The distributions of IGF-I, IGFBP-3, and testosterone among premenopausal women and of estrone and testosterone among postmenopausal women were significantly different between the two groups ($P < 0.05$); cases had higher levels of these hormones than controls. Table 2 shows the correlations of IGF-I, IGF-II, and IGFBP-3 with steroid hormones in control women. Among premenopausal women, SHBG was inversely correlated with IGF-I ($P = 0.004$) and IGF-II ($P = 0.006$); estrone-S was inversely correlated with IGF-II ($P < 0.001$). Other steroids were not correlated significantly with IGF-I, IGF-II, or IGFBP-3. For postmenopausal women, estradiol and estrone-S were positively correlated with IGF-II ($P = 0.003$) but inversely correlated with IGFBP-3 ($P = 0.006$). Testosterone was inversely correlated with IGFBP-3 ($P = 0.013$), and DHEA-S was positively correlated with IGF-I ($P = 0.002$). However, all of the correlations were relatively weak, with correlation coefficients less than 0.4.

The associations between breast cancer risk and individual

Table 4 Associations of breast cancer with combined IGF and steroid hormones among premenopausal women^a

Variable	IGF-I		IGF-II		IGFBP-3	
	No. ^b	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
Testosterone						
Low/low ^c	41/33	1.00	36/32	1.00	35/26	1.00
High/low	44/32	0.97 (0.49–1.88)	49/56	1.23 (0.65–2.31)	50/30	0.84 (0.40–1.79)
Low/high	44/39	1.13 (0.57–2.24)	49/40	0.64 (0.25–1.66)	50/46	1.54 (0.74–3.21)
High/high	41/67	2.50 (1.25–5.01)	36/43	1.19 (0.50–2.83)	35/69	3.31 (1.53–7.17)
Estrone						
Low/low	43/28	1.00	37/37	1.00	41/27	1.00
High/low	42/37	1.21 (0.61–2.40)	47/50	1.04 (0.55–1.99)	44/29	0.84 (0.42–1.68)
Low/high	41/41	1.50 (0.77–2.93)	47/32	0.58 (0.25–1.33)	43/42	1.48 (0.69–3.18)
High/high	43/64	2.30 (1.21–4.37)	38/51	1.17 (0.52–2.66)	41/72	3.32 (1.61–6.82)
Estradiol						
Low/low	47/32	1.00	39/55	1.00	44/32	1.00
High/low	38/33	1.12 (0.60–2.08)	46/33	0.47 (0.26–0.87)	41/24	0.72 (0.36–1.40)
Low/high	38/61	2.39 (1.27–4.47)	46/38	0.44 (0.18–1.03)	41/61	2.24 (1.17–4.29)
High/high	47/45	1.44 (0.76–2.73)	39/45	0.72 (0.30–1.70)	44/61	2.19 (1.07–4.46)
Estrone-S						
Low/low	40/29	1.00	26/31	1.00	39/36	1.00
High/low	45/36	1.16 (0.60–2.25)	59/57	0.91 (0.46–1.81)	46/20	0.59 (0.29–1.21)
Low/high	45/56	1.86 (0.96–3.63)	59/54	0.75 (0.34–1.66)	46/49	1.53 (0.78–2.99)
High/high	40/50	1.97 (1.02–3.82)	26/29	0.94 (0.38–2.36)	39/66	3.00 (1.40–6.44)
DHEA-S						
Low/low	45/35	1.00	36/35	1.00	32/29	1.00
High/low	40/30	0.96 (0.50–1.85)	49/53	1.17 (0.63–2.18)	53/27	0.61 (0.30–1.26)
Low/high	40/39	1.37 (0.73–2.56)	49/39	0.76 (0.33–1.78)	53/45	1.21 (0.57–2.56)
High/high	45/67	2.25 (1.18–4.29)	36/44	1.11 (0.47–2.63)	32/70	2.92 (1.37–6.20)

^a Unadjusted conditional logistic regression analysis.

^b Number of control versus number of case.

^c Low/low, low steroids and low IGF-I or IGFBP-3; High/low, high steroids and low IGF-I or IGFBP-3; Low/high, low steroids and high IGF-I or IGFBP-3; High/high, high steroids and high IGF-I or IGFBP-3; Low, lower than median; High, higher than or equal to median.

peptide and steroid hormones are shown in Table 3. IGF-I, IGFBP-3, and testosterone were associated with breast cancer risk among premenopausal women. With increasing levels of these hormones, there was a significant trend of increasing risk ($P < 0.01$); women who were at the highest quartile of these hormones had a 2.66–4.26-fold increase in breast cancer risk compared with those who were at the lowest quartile. Among postmenopausal women, similar trends (*i.e.*, higher hormone associated with higher risk) were observed for estrone, testosterone, and DHEA-S ($P < 0.05$); IGFBP-3 had a borderline significant trend ($P = 0.059$). No disease associations were found for IGF-II and estrone-S in either pre- or postmenopausal women or for estradiol and progesterone in premenopausal women. Estradiol was not analyzed in postmenopausal women because a substantial number of subjects had undetectable levels. SHBG seemed to be inversely associated with the disease risk in both groups, but none of the associations was statistically significant.

Tables 4 and 5 show the associations of breast cancer risk with combined distributions of peptide and steroid hormones. Among premenopausal women, the ORs for IGF-I in combination with testosterone, estrone, or DHEA-S were much stronger than the ORs for each individual hormone (Table 4), suggesting possible joint effects between these hormones. Similar joint effects were also suggested for IGFBP-3 with testosterone, estrone, estrone-S, or DHEA-S (Table 4). Among postmenopausal women, the same joint effects were indicated for IGF-I with testosterone or estrone and for IGFBP-3 with testosterone (Table 5). Overall, women who had high levels of IGF-I or IGFBP-3 plus high circulating testosterone or estrone tended to have much higher risk for breast cancer compared with those who had high levels of only one hormone. However, the study

did not suggest any joint effects between estradiol and IGF-I or IGFBP-3. Interestingly, IGF-II appeared to have little interaction with any of the steroids among premenopausal women (Table 4), but among postmenopausal women, those with high IGF-II plus high testosterone or estrone had much higher risk for breast cancer than those with high levels of only one hormone (Table 5). None of these results changed substantially after adjustment for several potential confounding variables in the analysis, including age at menarche, age at first live birth, history of fibroadenoma, family history of breast cancer, and plasma levels of SHBG. For the hormones that had possible synergy, we also evaluated potential statistical interactions between these molecules in the logistic regression model. The analysis showed that the following combinations had significant or borderline significant interactions, including IGF-I and testosterone ($P = 0.078$), IGFBP-3 and estrone ($P = 0.061$), IGFBP-3 and testosterone ($P = 0.047$), IGFBP-3 and DHEA-S ($P = 0.005$), and IGFBP-3 and estrone-S ($P = 0.011$), all of which were in postmenopausal women.

Discussion

In this population-based case-control study, we found some indications that breast cancer risk was associated with IGF and sex steroids not only individually, but also synergistically. Breast cancer risk was increased with plasma levels of IGF-I in premenopausal women, estrone in postmenopausal women, and IGFBP-3 and testosterone in both pre- and postmenopausal women. Possible synergistic effects were suggested for IGF-I with estrone or testosterone, and the synergy seemed to be consistent between pre- and postmenopausal women. Similar joint effects were also suggested between IGFBP-3 and estrone

Table 5 Associations of breast cancer with combined IGF and steroid hormones among postmenopausal women^a

Variable	IGF-I		IGF-II		IGFBP-3	
	No. ^b	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
Testosterone						
Low/low ^c	35/24	1.00	30/21	1.00	32/20	1.00
High/low	30/31	1.67 (0.78–3.61)	35/35	1.35 (0.60–3.07)	33/29	1.63 (0.71–3.72)
Low/high	30/23	1.05 (0.46–2.41)	35/26	1.18 (0.39–3.54)	33/27	1.31 (0.55–3.12)
High/high	35/48	2.15 (1.02–4.50)	30/44	3.06 (1.10–8.53)	32/50	2.68 (1.18–6.06)
Estrone						
Low/low	33/22	1.00	25/18	1.00	39/22	1.00
High/low	31/32	1.84 (0.86–3.97)	38/37	1.71 (0.67–4.36)	26/27	2.13 (0.94–4.83)
Low/high	31/27	1.22 (0.54–2.77)	39/31	1.48 (0.47–4.70)	25/27	1.70 (0.75–3.88)
High/high	33/44	2.19 (1.06–4.56)	26/39	2.77 (1.00–7.63)	38/49	2.63 (1.21–5.73)
Estradiol						
Low/low	35/22	1.00	22/20	1.00	41/27	1.00
High/low	30/33	1.88 (0.73–4.82)	43/36	0.99 (0.39–2.58)	24/22	1.62 (0.62–4.25)
Low/high	29/35	1.93 (0.84–4.43)	42/37	1.28 (0.43–3.82)	23/30	1.77 (0.86–3.61)
High/high	36/36	1.67 (0.70–3.98)	23/33	2.24 (0.74–6.81)	42/47	2.23 (0.84–5.93)
Estrone-S						
Low/low	30/23	1.00	43/29	1.00	20/17	1.00
High/low	35/32	1.30 (0.60–2.82)	22/27	2.02 (0.87–4.70)	45/32	0.63 (0.25–1.58)
Low/high	35/32	1.27 (0.60–2.69)	22/26	2.62 (0.96–7.17)	45/38	0.80 (0.34–1.88)
High/high	30/39	1.72 (0.78–3.79)	43/44	2.33 (0.86–6.29)	20/39	1.78 (0.74–4.25)
DHEA-S						
Low/low	39/28	1.00	35/25	1.00	34/21	1.00
High/low	26/27	1.50 (0.74–3.04)	30/31	1.41 (0.67–2.96)	31/28	1.63 (0.73–3.62)
Low/high	26/24	1.37 (0.65–2.89)	30/27	1.79 (0.64–4.97)	31/31	1.81 (0.79–4.15)
High/high	39/47	1.72 (0.84–3.50)	35/43	2.47 (0.93–6.57)	34/46	2.07 (0.99–4.35)

^a Unadjusted conditional logistic regression analysis.

^b Number of control versus number of case.

^c Low/low, low steroids and low IGF-I or IGFBP-3; High/low, high steroids and low IGF-I or IGFBP-3; Low/high, low steroids and high IGF-I or IGFBP-3; High/high, high steroids and high IGF-I or IGFBP-3; Low, lower than median; High, higher than or equal to median.

or testosterone, and between IGF-II and these steroids in postmenopausal women. The study results indicate the possibility that women with high circulating levels of both sex steroids and IGF peptide hormones may have much higher risk for breast cancer than those with high levels of only one hormone. These indications appear to be in agreement with the results of laboratory experiments.

Although a large number of studies find synergistic interaction between estrogens and IGF in breast cancer, the findings are basically limited to cell culture experiments and animal studies. There are no population-based human studies assessing the interplay of IGF and sex steroids in relation to breast cancer. Our case-control study is the first human study to investigate the synergistic interaction, and its findings are in support of laboratory evidence. Moreover, the study suggests that androgen as well as estrogen is involved in the interaction with IGFs and their binding protein in breast cancer. A number of cohort studies evaluated the role of androgen in breast cancer (28–35), and interestingly, all of the studies found a positive association between circulating testosterone and breast cancer risk. The finding of our study on testosterone is consistent with those studies, suggesting that testosterone plays a role in the disease. In addition, our study indicates that testosterone interacts synergistically with IGF-I in association with breast cancer risk. Testosterone is the only steroid for which the association and interaction were seen in both pre- and postmenopausal women. These consistencies indicate that testosterone may be more important in the disease than other steroids. On the other hand, it may also be possible that androgen is a surrogate molecule for estrogens because among premenopausal women, circulating androgen tends to fluctuate less than estrogens during the menstrual cycle and thus can be measured more precisely than

estrogens. Although there are few data suggesting a synergistic interaction between androgen and IGF in breast cancer, laboratory experiments do find evidence that these hormones interact in other tissues. In prostate cancer cells, both androgen and androgen receptor are able to regulate the production of IGFBPs (36, 37). Dihydrotestosterone stimulates the expression of IGFBP-3, and the stimulation is associated with increases in cell proliferation (38). IGF-I and other growth factors can activate androgen receptor without the presence of androgens (39). In normal prostate gland, castration results in increased expression of IGFBPs, which facilitate apoptosis (40). In bone tissue, testosterone up-regulates the expression of IGF-I and IGF-IR (41). Both IGF-I and androgen stimulate the production of each other in the ovary (42, 43).

Several sex steroid hormones were correlated with the peptide hormones in our study, but none of the correlations was strong. IGF-I did not correlate with any steroids in premenopausal women, and in postmenopausal women, it correlated only with DHEA-S. Tissandier *et al.* (44) observed a similar correlation among elderly men; another study found a correlation between free IGF-I and DHEA-S among women aged 55–80 years (45). Besides the positive correlation with DHEA-S, IGF-I was negatively correlated with SHBG in our study, and this correlation appeared to be similar between pre- and postmenopausal women. Similar correlations were also seen in other studies (46, 47). Interestingly, IGFBP-3 was positively associated with breast cancer but negatively correlated with estrogens and testosterone, although these correlations were found only in postmenopausal women. IGF-II was positively correlated with estradiol and estrone-S in postmenopausal women, but there was an opposite correlation between IGF-II and estrone-S in premenopausal women. Because most

of the correlations were not consistent between pre- and postmenopausal women, menopausal status seems to have an important impact on the relationship of these hormones.

In the study, we found possible synergistic interplay between IGF-II and sex steroids among postmenopausal women, but not among premenopausal women. This menopause-specific interaction is intriguing because it may indicate some functional changes in the IGF system after menopause. It is known that IGF-II and IGF-I exert their actions at different stages of human growth and are subject to different regulatory systems (48). IGF-II levels are relatively stable during adulthood, whereas IGF-I levels rise and fall substantially with age due to the regulation of growth hormone. It remains to be elucidated whether IGF-II activity is elevated in compensation for the age-related decline of IGF-I.

Our study showed that IGFBP-3 was positively associated with breast cancer risk. Although this relationship was contrary to the findings of some earlier studies, the inconsistency is seen not only in our study but also in others (49) and not only in breast cancer but also in other cancer sites, all of which have been discussed in our previous paper (50). Because IGFBP-3 can either suppress or enhance the action of IGF, and this effect on IGF is regulated by a complex system, it is difficult to determine the actual impact of IGFBP-3 on breast cancer. This may also be true when we consider the joint effect of IGFBP-3 with steroid hormones on breast cancer risk. In our study, IGFBP-3 and IGF-I interact with the same steroid hormones, testosterone and estrone, and the interaction is affected by adjusting for each other's effect, suggesting that the two molecules have a similar impact on the disease and a similar relationship with sex steroids.

The major concern of case-control studies is the influences of disease status and treatment on the level of circulating hormones. To minimize the impact, patient blood collection in our study was done before cancer treatment, and for most of the patients, the specimens were collected very soon after diagnosis. We compared IGFs and IGFBP-3 among patients with different stages of the disease and status of steroid hormone receptors, and our results showed no significant associations between these growth factors and the disease characteristics (data not shown). It is possible that the impact of disease status on circulating hormones in our study is relatively small because most of the study results, such as those for IGF-I and steroid hormones, are generally in agreement with the findings of most cohort studies. Because menopause is an important determinant of steroid hormone production, it is important for us to assess the effects of steroids on the disease, including their interactions with peptide hormones, among women with the same menopausal status. Although we compromised the study power by analyzing the data separately in pre- and postmenopausal women, we still found clear indications of joint effect between sex steroids and peptide hormone IGFs in association with breast cancer risk. The assays for estrone and estradiol have a small cross-reactivity. The estradiol assay has 0.86% of cross-reaction with estrone, and the estrone assay has 1.25% of cross-reaction with estradiol. This cross-reaction might have a small impact on the results of estrogens in postmenopausal women because of low levels of sex steroids in their circulation.

In summary, the results of our study suggest possible synergy between IGF and sex steroid hormones in association with breast cancer risk. The study showed that women with high circulating levels of both IGF-I and estrone or testosterone were at much higher risk for breast cancer compared with those with high levels of only one hormone in the circulation. The joint effect was consistent in pre- and postmenopausal women

and sustained after adjustment for several potential confounding variables in the analysis. IGFBP-3 had similar interaction with sex hormones. Our finding of synergistic interplay between IGF and sex steroids supports the notion that the two groups of hormones may work in concert in promoting the development of breast cancer.

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