Published in final edited form as:

Cancer. 2004 February 15; 100(4): 694–700.

Evaluation of the Synergistic Effect of Insulin Resistance and Insulin-Like Growth Factors on the Risk of Breast Carcinoma

Alecia Malin, Dr.P.H., CHES¹, Qi Dai, M.D., Ph.D.¹, Herbert Yu, M.D., Ph.D.², Xiao-Ou Shu, M.D., Ph.D.¹, Fan Jin, M.D.³, Yu-Tang Gao, M.D.³, and Wei Zheng, M.D., Ph.D.¹

¹ Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee.

² Department of Epidemiology and Public Health Yale University School of Medicine, New Haven, Connecticut.

³ Shanghai Cancer Institute, Shanghai, People's Republic of China.

Abstract

BACKGROUND—The purpose of the current study was to investigate the association between insulin resistance (which was measured using fasting blood C-peptide) and its joint association with insulin-like growth factors (IGF-1, IGF-2, and IGF binding protein-3 [IGFBP-3]) on the risk of breast carcinoma.

METHODS—Included in the current study were 400 case-control pairs from the Shanghai Breast Cancer Study. Pretreatment biospecimens and interview data were collected from all breast carcinoma cases and their individually matched controls.

RESULTS—Breast carcinoma risk was found to be statistically significantly increased when higher blood levels of C-peptide and IGFs were noted in a dose-response manner. There was a statistically significant twofold to threefold increased risk of breast carcinoma for women in the highest quartile of C-peptide, IGF-1, or IG-FBP-3 compared with women in the lowest quartiles. Women with high levels of both C-peptide and IGF-1 or IGFBP-3 also were found to have a substantially higher risk of breast carcinoma than those women with a high level of only one of these molecules. The adjusted odds ratios (ORs) were 3.79 (95% confidence interval [95% CI], 2.03–7.08) for those with a higher level of both C-peptide and IGF-1 and 4.03 (95% CI, 2.06–7.86) for those with a higher level of both C-peptide and IGFBP-3.

CONCLUSIONS—The results of the current study suggest that insulin resistance and IGFs may synergistically increase the risk of breast carcinoma.

Keywords

insulin-like growth factors (IGF); C-peptide; insulin resistance; breast carcinoma

A recent area of interest in breast carcinoma research is the inter-play between insulin resistance and insulin-like growth factors (IGFs) in relation to breast carcinoma risk. Insulin has been shown to have a mitogenic effect in some in vitro systems, inducing a dose-dependent growth response in breast carcinoma cell lines. The IGF family includes the polypeptide ligands IGF-1 and IGF-2, their cognate receptors, six binding proteins (i.e., IGFBP-1 to IGFBP-6), and IGFBP proteases. A large number of in vitro studies have shown that IGFs are strong mitogens for a

Address for reprints: Wei Zheng, M.D., Ph.D., Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University, 6103 Medical Center East, Nashville, TN 37232-8300; Fax: (615) 936-1269; E-mail: wei.zheng@vanderbilt.edu.

Dr. Malin's current address: Department of Surgery, Meharry Medical College, Nashville, Tennessee.

variety of cancer cells, including many breast carcinoma cell lines.¹ IGFs also are reported to inhibit the cell apoptotic pathway to facilitate cell proliferation.^{2,3} The combination of these mitogenic and antiapoptotic effects is reported to have a profound impact on tumor growth.^{4–6} IGF-1 and IGF-2 are present in the circulation, in which the majority of them (> 90%) are bound to IGFBP-3.^{4,7,8} IGFBPs can inhibit or enhance the action of IGFs, resulting in either the suppression or stimulation of cell proliferation depending on the concentration of binding proteins, phosphorylation status, and proteolytic fragmentation.^{4,9,10} IGFBP-3 also may promote cell proliferation independently of IGF receptors.^{11,12}

Whereas insulin itself is an important growth factor that appears to influence breast carcinoma cells in vitro, it is believed that part of its growth-promoting effect in vivo may be through its role in regulating IGF-1 and IGF-2 production and bioavailability.⁶ Cumulative evidence suggests that insulin resistance and high IGF levels interact synergistically to increase a patient's risk of breast carcinoma.^{3,13,14} It has been suggested that insulin levels determine the bioavailable level of IGF-1 in the tissues by regulating the production and proteolysis of IGFBP-1.^{6,15} IGFs and insulin together have been shown to stimulate motility in human breast carcinoma cell lines, an effect that could enhance migration and invasion.^{9,16,17}

Despite evidence from in vitro and in vivo experiments, to our knowledge virtually no epidemiologic studies to date have evaluated the interplay of insulin resistance and IGFs in the etiology of breast carcinoma. There is growing epidemiologic evidence that high circulating levels of IGF-1 are associated with an increased risk of breast carcinoma in women,^{18–20} particularly at premenopausal ages.^{21–23} The association with IGFBP-3, however, has been inconsistent with regard to findings from previous studies and both positive and inverse associations have been reported.^{24,25} The conflicting results from epidemiologic studies are not unfounded, given the dual roles of IGFBP-3 protein in regulating the actions of IGF-1/2. In human studies, insulin resistance often is measured using blood C-peptide, a 31-amino acid peptide that is a byproduct of insulin production.¹² Several studies have shown that blood levels of insulin or C-peptide may be related to the risk of breast carcinoma. We recently reported a positive relation between blood IGF-1 and IGFBP-3²⁶ and C-peptide²⁷ levels with breast carcinoma risk in a small ancillary study performed within the Shanghai Breast Cancer study. In the current report, we reevaluated these associations using a larger sample size and explored possible interactions between C-peptide and IGF-1, IGF-2, and IGFBP-3.

MATERIALS AND METHODS

The Shanghai Breast Cancer Study was designed to recruit women ages 25–64 years who were newly diagnosed with breast carcinoma between August 1996 and March 1998, and a group of community controls for a population-based case-control study.²⁸ All study subjects were permanent residents of urban Shanghai. They had no prior history of cancer and were alive at the time of the interview. Through a rapid case-ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1602 eligible breast carcinoma cases were identified during the study period and in-person interviews were completed for 1459 of the eligible cases (91.1%). The controls were selected randomly from female residents in urban Shanghai, using the population-based Shanghai Resident Registry and frequency-matched to cases by age (at 5-year intervals). In-person interviews were completed with 1556 of the 1724 eligible controls identified (90.3%).

Trained interviewers measured each eligible subject for her weight, waist and hip circumference, and sitting and standing heights and conducted an in-person interview according to a standard protocol. A structured questionnaire was used to elicit detailed information concerning demographic factors, menstrual and reproductive history, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight,

and family history of cancer. Information regarding usual adult dietary intake was collected using a comprehensive quantitative food frequency questionnaire (FFQ) that covers > 85% of the foods consumed in Shanghai. Blood samples were collected from approximately 83% of the study participants.

An individually matched case-control substudy was built into the Shanghai Breast Cancer Study to increase the comparability of cases and controls in studying quantitative biomarkers. For each case whose samples were collected before any cancer treatment, a control was selected from the pool of subjects who completed the study and individually matched to the index case by age (± 3 years), menopausal status, and date of sample collection (± 30 days). Successful matches were completed for 400 case-control pairs for the current study. To eliminate between-assay variability in case-control comparisons, samples from a matched case-control pair were included in the same batch of assays of blood IGF and C-peptide levels. Three case-control pairs were excluded from all analyses because of laboratory failure with regard to biomarker assays.

Fasting blood samples (10 mL from each woman) were collected in the morning using either ethylenediamine tetraacetic acid (EDTA) or heparin Vacutainer[®] tubes (Becton Dickinson, San Jose, CA). Immediately after collection, the samples were placed in portable insulated cases with ice pads (4 °C) and transported to the Shanghai Cancer Institute for processing and storage. All samples were aliquoted and stored at -70 °C within 4 hours after collection. Plasma concentrations of IGF-1, IGF-2, and IGFBP-3 were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Systems Laboratories, Inc., Webster, TX). The calibrators used in the assays ranged from 4.5–640 ng/mL for IGF-1, 500–2000 ng/mL for IGF-2, 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 intraassay and inter-assay precisions were 1.5–3.4% and 1.5–8.5%, respectively, of coefficient of variation (CV) for IGF-1; 4.2–7.2% and 6.3–10.7%, respectively, of CV for IGF-2; and 0.5–1.9% and 1.8–3.9%, respectively, of CV for IG-FBP-3. Each assay had no cross-reaction with other members of the IGF family. Serum C-peptide was measured using an enzymatically amplified, one-step sandwich-type ELISA assay kit (Diagnostic Systems Laboratories, Inc.) and the ELISA assay was performed according to the manufacturer's instructions. Sample aliquots of 20 μ L were pipetted into microtiter wells coated with anti-C-peptide antibodies and incubated with 200 μ L of a buffered solution of anti-C-peptide antibody conjugated to horseradish peroxidase. Plasma samples were measured in duplicate to improve reliability. These methods were used in the majority of previous epidemiologic studies.^{26,27}

Both parametric and nonparametric methods were used to analyze the data collected from the current study. Log-transformed data were used in Student *t* tests for paired data to compare the mean differences between cases and controls. To evaluate the potential dose-response relation between biomarker level and risk of breast carcinoma, cases and controls were categorized into four groups according to the quartile distribution of the biomarker level among controls. Odds ratios (OR) and 95% confidence intervals (95% CI) for the upper three quartile groups were derived using conditional logistic regression, compared with the lowest quartile group. Multivariate analyses were performed to adjust for potential confounding variables. A score variable was created by summing the quintile level (0, 1, 2, 3, and 4) of C-peptide, IGF-1, and IGFBP-3 and used in the analysis to evaluate the joint association between these three biomarkers and risk of breast carcinoma. The assigned scores ranged from 0–12. Tests for trends were performed in logistic regressions by assigning the score “*j*” to the “*j*th” level of the variable selected. All statistical analyses were based on two-tailed probability.

RESULTS

Comparisons of selected demographics and risk factors between breast carcinoma patients and their matched controls are shown in Table 1. There were no statistically significant differences noted between cases and controls with respect to age and education. Although cases had a higher proportion of family history, a lower age at menarche, an older age at menopause, and were found to consume more energy and fat, the differences were not found to be statistically significant. Compared with controls, cases were more physically active, had a higher body mass index (BMI), an older age at the first live birth, and were found to consume more meats. Therefore, physical activity, BMI, age at first live birth, and total meat intake were considered potential confounders and adjusted for in some of the subsequent analyses. Cases were found to have significantly higher plasma levels of IGF-1, IGF-2, IGFBP-3, and C-peptide than controls.

Table 2 shows the association between breast carcinoma risk and IGF-1, IGF-2, IGFBP-3, and C-peptide. Because interactions did not exist between IGF-1, IGF-2, and IGFBP-3, mutual adjustment for these variables also was performed. A significant association was noted when IGF-1 was analyzed adjusting for traditional breast carcinoma risk factors (OR, 2.38; 95% CI, 1.44–3.93) (P value for trend = 0.01), but the association became insignificant when adjusting for other IGF molecules. A significant association between high IGFBP-3 and increased breast carcinoma risk was noted in both univariate and multivariate models. The ORs were 3.45 (95% CI, 1.72–6.93) when adjusting for traditional risk factors and 2.23 (95% CI, 0.89–5.56) when IGF-1 was adjusted. The association between C-peptide and breast carcinoma risk was found to be statistically significant; the OR was 2.64 (95% CI, 1.47–4.74) (trend test $P < 0.001$) after adjusting for traditional risk factors. There was no association noted between IGF-2 and risk of breast carcinoma; therefore, IGF-2 was not included in subsequent analyses.

The joint association between breast carcinoma risk and C-peptide and IGF-1 or IGFBP-3 was evaluated in Table 3. The risk of breast carcinoma was found to increase with the blood C-peptide level regardless of the level of IGF-1 or IGFBP-3. Women who had a high level of both C-peptide and IGF-1 or IGFBP-3 were at a particularly increased risk of developing breast carcinoma. However, the interaction between the IGF variables and C-peptide was not found to be statistically significant on the multiplicative scale.

The score variable of C-peptide, IGF-1, and IGFBP-3 was analyzed to evaluate a possible dose-response relation between breast carcinoma risk and a combined exposure level of these molecules, stratified by menopausal status (Table 4). Breast carcinoma risk was found to be strongly associated with the index; the highest risk was observed among women with the highest score for all 3 molecules, (OR, 4.07; 95% CI, 1.74–9.53). This association was found to be similar for both premenopausal and postmenopausal women.

DISCUSSION

In a previous small study of 143 case-control participants, we reported that the blood C-peptide level was positively associated with breast carcinoma risk.²⁷ The results of the current study, a larger study of 398 case-control pairs, confirm this finding. These results are supported by several previous epidemiologic studies. A case-control study in Amsterdam showed that the serum level of C-peptide was related positively to the risk of breast carcinoma and that this association was independent of general adiposity or abdominal obesity.¹⁹ Muti et al. reported a modest association between higher fasting insulin levels and breast carcinoma risk in a nested case-control study.²³ Fasting insulin was examined in a cohort of cases with early-stage breast carcinoma and was found to be positively associated with a threefold increased risk of death and a twofold increased risk of recurrence in both pre-menopausal and postmenopausal women.

²⁹ In a small case-control study of 45 postmenopausal breast carcinoma cases, blood levels of C-peptide, fasting insulin, and proinsulin were found to be somewhat higher in cases compared with controls, but the differences were not found to be statistically significant.³⁰ A nested case-control study conducted in New York City showed no association between risk of breast carcinoma and nonfasting levels of serum C-peptide.²² Two prospective studies could not detect a relation between nonfasting C-peptide levels and postmenopausal breast carcinoma.^{25,31}

The studies cited above used various biomarkers such as proinsulin as a proxy measure of insulin levels. The current study used serum C-peptide from a fasting blood sample, which to our knowledge is a more accurate measure of insulin levels. C-peptide is a byproduct of insulin production. The major advantages of measuring fasting serum C-peptide levels over insulin levels are twofold. First is the ability to readily distinguish endogenous insulin levels in the presence of exogenous insulin administration. Second, C-peptide can be measured in the presence of circulating insulin antibodies, which develop in most diabetic patients who have been treated with insulin injections for longer than a few weeks. Typically, circulating insulin antibodies interfere with the usual immunoassay for insulin. In this instance, these antibodies do not cross-react with human C-peptide.³²

Similar to the results of the current study, blood IGF-1 has been found fairly consistently to be positively associated with the risk of breast carcinoma. We also found levels of circulating IGFBP-3 to be elevated in women with breast carcinoma, which is congruent with other case-control studies.³³ However, in contrast to the findings of the current study regarding circulating IGFBP-3 (adjusted for IGF-1) are previous case-control studies in which there were inverse¹⁹ and null associations.²⁰ Prospective studies also have yielded inconsistent results with regard to circulating IGFBP-3. Some studies have shown a positive association in premenopausal women only²⁴ or in both premenopausal and postmenopausal women,^{23,28} whereas other studies reported an inverse association in premenopausal women age < 50 years²¹ or in post-menopausal women.²⁴ Null associations also have been reported.^{22,25} The conflicting results from epidemiologic studies are not unexpected, given the dual roles of IGFBP-3 protein in regulating the actions of IGF-1/IGF-2. In addition, blood levels of IGFBP-3 may not reflect the level of this protein in the target tissues, and to our knowledge, the majority of epidemiologic studies have no access to normal target tissue samples with which to evaluate the association between this protein and cancer risk.

Despite many studies concerning IGFs and C-peptide singly,^{19,22,23,28,30} no study published to date has evaluated the joint effect of these biomarkers on the risk of breast carcinoma. The dearth of literature in this area may be attributed to the use of a small sample size in which the statistical power was inadequate to evaluate the joint effects of multiple factors and blood samples from cancer cases collected after cancer treatment. These limitations would preclude studies that examine how insulin resistance and IGF activation are involved in breast malignancies. Signaling pathways involved in breast carcinoma cell growth are not to our knowledge simple or linear. Numerous divergent and similar pathways are stimulated after insulin-like growth factor receptor 1 (IGF-IR) activation, which then encroach on multiple other pathways that relate to the biology of breast carcinoma. The current study findings of a potentially synergistic effect of C-peptide and IGFs are consistent with the results from in vitro experiments showing the interplay of these molecules in the etiology of breast carcinoma.

As in any case-control study, the use of biologic samples collected after disease diagnosis for evaluating the association between biomarkers of interest with disease risk is a major concern in the current study. However, blood samples for this study were collected prior to any cancer treatment, and the majority of breast carcinoma patients were diagnosed at early stages of disease. Because samples from most breast carcinoma patients were collected within days of

the preliminary diagnosis of breast carcinoma, the lifestyle changes in a such short interval should not be appreciable, particularly for those patients with early-stage breast carcinoma. We further analyzed the association by disease stage of the cancer diagnosis and found that associations were somewhat stronger for early-stage breast carcinoma cases, indicating that the observed positive association is unlikely to be the consequence of tumor growth in breast carcinoma patients.

The current population-based case–control study conducted in Chinese women from Shanghai revealed a potential synergistic effect of C-peptide and IGF-1 or IGFBP-3 on the risk of breast carcinoma. These findings are biologically plausible and supported by studies in cell cultures suggesting the interactive effects of these molecules. Further studies with prospectively collected biologic samples are warranted to evaluate this association further.

References

1. Yee D. The insulin-like growth factor system as a treatment target in breast cancer. *Semin Oncol* 2002;29:86–95. [PubMed: 12138402]
2. Yu H, Levesque MA, Khosravi MJ, Papanastasiou-Diamandi A, Clark GM, Diamandis EP. Insulin-like growth factor binding protein-3 and breast cancer survival. *Int J Cancer* 1998;79:624–628. [PubMed: 9842972]
3. Smith GD, Gunnell D, Holly J. Cancer and insulin-like growth factor-I. A potential mechanism linking the environment with cancer risk. *BMJ* 2000;321:847–848. [PubMed: 11021847]
4. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* 2000;92:1472–1489. [PubMed: 10995803]
5. Westley BR, May FE. Insulin-like growth factors: the unrecognised oncogenes. *Br J Cancer* 1995;72:1065–1066. [PubMed: 7577447]
6. Stoll BA. Breast cancers: further metabolic-endocrine risk markers? *Br J Cancer* 1997;76:1652–1654. [PubMed: 9413957]
7. Rosen CJ. Serum insulin-like growth factors and insulin-like growth factor-binding proteins: clinical implications. *Clin Chem* 1999;45:1384–1390. [PubMed: 10430822]
8. Furstenberger G, Senn HJ. Insulin-like growth factors and cancer. *Lancet Oncol* 2002;3:298–302. [PubMed: 12067807]
9. Sachdev D, Yee D. The IGF system and breast cancer. *Endocr Relat Cancer* 2001;8:197–209. [PubMed: 11566611]
10. Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. *Int J Biochem Cell Biol* 1996;28:619–637. [PubMed: 8673727]
11. Schedlich LJ, Graham LD. Role of insulin-like growth factor binding protein-3 in breast cancer cell growth. *Microsc Res Tech* 2002;59:12–22. [PubMed: 12242693]
12. Steiner DF, Rubenstein AH. Proinsulin C-peptide—biological activity? *Science* 1997;277:531–532. [PubMed: 9254422]
13. Giovannucci E. Insulin-like growth factor-I and binding protein-3 and risk of cancer. *Horm Res* 1999;51(Suppl 3):34–41. [PubMed: 10592442]
14. Hadsell DL, Abdel-Fattah G. Regulation of cell apoptosis by insulin-like growth factor. *Adv Exp Med Biol* 2001;501:79–85. [PubMed: 11787734]
15. Holly J. Insulin-like growth factor-I and new opportunities for cancer prevention. *Lancet* 1998;351:1373–1375. [PubMed: 9593403]
16. Macaulay VM. Insulin-like growth factors and cancer. *Br J Cancer* 1992;65:311–320. [PubMed: 1313689]
17. Yee D. Are the insulin-like growth factors relevant to cancer? *Growth Horm IGF Res* 2001;11:339–345. [PubMed: 11914021]
18. Peyrat JP, Bonnetterre J, Hecquet B, et al. Plasma insulin-like growth factor-1 (IGF-1) concentrations in human breast cancer. *Eur J Cancer* 1993;29A:492–497. [PubMed: 8435198]

19. Bruning PF, Van Doorn J, Johannes MG. Insulin-like-growth-factor binding protein 3 is decreased in early-stage operable pre-menopausal breast cancer. *Int J Cancer* 1995;62:266–270. [PubMed: 7543079]
20. Bohlke K, Cramer DW, Trichopoulos D, Mantzoros CS. Insulin-like growth factor-I in relation to premenopausal ductal carcinoma in situ of the breast. *Epidemiology* 1998;9:570–573. [PubMed: 9730040]
21. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 1998;351:1393–1396. [PubMed: 9593409]
22. Toniolo P, Bruning PF, Akhmedkhanov A, et al. Serum insulin-like growth factor-I and breast cancer. *Int J Cancer* 2000;88:828–832. [PubMed: 11072255]
23. Muti P, Quattrin T, Grant BJ, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. *Cancer Epidemiol Biomarkers Prev* 2002;11:1361–1368. [PubMed: 12433712]
24. Krajcik RA, Borofsky ND, Massardo S, Orentreich N. Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2002;11:1566–1573. [PubMed: 12496045]
25. Kaaks R, Lundin E, Rinaldi S, et al. Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden. *Cancer Causes Control* 2002;13:307–316. [PubMed: 12074500]
26. Yu H, Jin F, Shu XO, et al. Insulin-like growth factors and breast cancer risk in Chinese Women. *Cancer Epidemiol Biomarkers Prev* 2002;11:705–712. [PubMed: 12163322]
27. Yang G, Lu G, Jin F, et al. Population-based, case-control study of blood C-peptide level and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;10:1207–1211. [PubMed: 11700270]
28. Gao YT, Shu XO, Dai Q, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. *Int J Cancer* 2000;87:295–300. [PubMed: 10861490]
29. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol* 2002;20:42–51. [PubMed: 11773152]
30. Jenstrom H, Barrett-Connor E. Obesity, weight change, fasting insulin, proinsulin, C-peptide, and insulin like growth factor level in women with and without breast cancer: The Rancho Bernardo Study. *J Womens Health Gend Based Med* 1999;8:1265–1272. [PubMed: 10643834]
31. Keinan-Boker L, Bas Bueno De Mesquita H, Kaaks R, et al. Circulating levels of insulin-like growth factor I, its binding proteins-1,-2,-3, C-peptide and risk of postmenopausal breast cancer. *Int J Cancer* 2003;106:90–95. [PubMed: 12794762]
32. Horowitz DL, Rubenstein AH, Mako ME, Cruz A. C-peptide conditions other than diabetes mellitus. *Diabetes* 1978;27(Suppl):267–271. [PubMed: 631444]
33. Del Giudice ME, Fantus IG, Ezzat S, McKeown-Eyssen G, Page D, Goodwin PJ. Insulin and related factors in premenopausal breast cancer risk. *Breast Cancer Res Treat* 1998;47:111–120. [PubMed: 9497099]

Comparison of Cases and Controls to Demographics and Selected Breast Carcinoma Risk Factors: The Shanghai Breast Cancer Study, 1996–1998

TABLE 1

Characteristics	Cases ^d (n = 397)	Controls ^d (n = 397)	P value ^b
Age (yrs)	47.8 ± 7.8	47.6 ± 7.9	0.20
Education, elementary or lower (%)	12.56	14.61	0.32
Breast cancer in first-degree relatives (%)	1.5	0.75	0.16
Physically active (%)	39.6	35.0	0.005
Body mass index	23.5 ± 3.3	22.9 ± 3.2	0.0186
Age at first live birth (yrs) ^c	26.9 ± 4.1	26.3 ± 3.9	0.01
Menarcheal age (yrs)	14.7 ± 1.7	14.9 ± 1.7	0.11
Menopausal age (yrs) ^d	48.5 ± 4.5	47.8 ± 4.5	0.12
Energy intake (kcal/day)	1905.7 ± 470.3	1862.3 ± 481.9	0.16
Total fat intake (g/day)	37.1 ± 19.3	36.8 ± 15.9	0.79
Total meat intake (g/day)	93.1 ± 69.2	84.3 ± 53.0	0.04
IGF-I ^e	150.6 (144.52–156.92)	138.5 (133.48–143.80)	< 0.001
IGF-II ^e	820.5 (797.38–844.19)	798.6 (779.46–820.64)	0.034
IGFBP-3 ^e	3963.9 (3813.56–4119.54)	3718.2 (3586.50–3854.76)	< 0.0001
C-peptide ^e	1.43 (1.34–1.52)	1.19 (1.12–1.26)	< 0.0001

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3. Subjects with missing values were excluded from the analysis.

^aUnless otherwise specified, the mean ± the standard deviation are presented.

^bP values were derived from chi-square tests for categorical variables and Student t tests for paired data for continuous variables.

^cAmong women who had live births.

^dAmong postmenopausal women.

^eThe geometric means and 95% confidence intervals are presented.

Adjusted ORs and 95% CIs for the Association between Breast Carcinoma and Blood Levels of C-Peptide, IGF-1, IGF-2, and IGFBP-3: The Shanghai Breast Cancer Study, 1996–1998

	No. of cases	No. of controls	OR (95% CI) ^a	OR (95% CI) ^{b,c}
IGF-1 (ng/mL)				
Q1 (low)	85	101	1.00	1.00
Q2	77	98	1.19 (0.74–1.92)	1.00 (0.60–1.64)
Q3	91	99	1.37 (0.83–2.26)	1.01 (0.58–1.74)
Q4	145	99	2.38 (1.44–3.93)	1.54 (0.87–2.73)
Trend test			$P < 0.01$	$P < 0.12$
IGF-2 (ng/mL)				
Q1 (low)	83	100	1.00	1.00
Q2	99	99	1.36 (0.85–2.17)	0.87 (0.52–1.47)
Q3	94	99	1.79 (1.01–3.18)	0.96 (0.50–1.86)
Q4	122	99	2.47 (1.28–4.75)	0.99 (0.44–2.22)
Trend test			$P = 0.007$	$P = 0.97$
IGFBP-3 (ng/mL)				
Q1 (low)	99	100	1.00	1.00
Q2	57	99	0.74 (0.42–1.32)	0.59 (0.31–1.06)
Q3	98	99	1.40 (0.76–2.57)	0.96 (0.49–1.86)
Q4	144	99	3.45 (1.72–6.93)	2.12 (0.99–4.55)
Trend test			$P = 0.001$	$P = 0.0045$
C-peptide				
Q1 (low)	57	93	1.00	
Q2	85	118	1.32 (0.77–2.30)	
Q3	133	94	2.80 (1.62–4.87)	
Q4	123	92	2.64 (1.47–4.74)	
Trend test			$P = 0.001$	

ORs: odds ratios; 95% CI: 95% confidence interval; IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; Q: quartile.

^a Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.
^b Adjusted for physical activity, age at first live birth, body mass index, total meat intake, and either insulin-like growth factor binding protein 3 or insulin-like growth factor-1.
^c Adjusted for physical activity, age at first live birth, body mass index, total meat intake, and both insulin-like growth factor binding protein-3 and insulin-like growth factor-1.

Joint Associations between Blood Levels of C-Peptide and IGF-1, IGF-2, and IGFBP-3 with the Risk of Breast Carcinoma: The Shanghai Breast Cancer Study, 1996–1998

TABLE 3

IGF levels (by median)	Serum level of C-peptide by tertile			
	< 0.090 (ng/mL)		0.090–1.443 (ng/mL)	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
IGF-1				
≤ 141 (ng/mL)	44/86	1.00	57/66	1.88 (1.01–3.51)
> 141 (ng/mL)	34/37	2.02 (1.00–4.11)	85/83	2.54 (1.41–4.56)
			<i>P</i> value for interaction, 0.74	
IGFBP-3				
≤ 3741 (ng/mL)	32/58	1.00	46/71	1.04 (0.55–1.98)
> 3741 (ng/mL)	46/65	1.43 (0.76–2.70)	96/78	2.72 (1.45–5.10)
			<i>P</i> value for interaction, 0.19	

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; OR: odds ratio; 95% CI: 95% confidence interval.

^a Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.

TABLE 4
Combined Association of C-Peptide, IGF-1, and IGFBP-3 Stratified by Menopausal Status with Breast Carcinoma Risk: The Shanghai Breast Cancer Study, 1996–1998

Score variable	Q1 (0–1)	Q2 (2–3)	Score variable of C-peptide, IGF-1, and IGFBP-3 (by quartile)		Q5 (8–9)	P value
			Q3 (4–5)	Q4 (6–7)		
Case/control OR (95% CI)	18/28 1.00	58/94 1.14 (0.55–2.38)	88/127 1.25 (0.61–2.59)	All subjects 163/110 2.52 (1.23–5.20)	71/38 4.07 (1.74–9.53)	<0.001
Case/control OR (95% CI)	8/16 1.00	31/46 1.11 (0.37–3.30)	48/84 1.12 (0.41–3.10)	Premenopausal women 112/78 2.17 (0.78–6.06)	51/28 4.64 (1.44–14.97)	<0.001
Case/control OR (95% CI)	10/12 1.00	27/48 0.93 (0.30–2.86)	40/43 2.04 (0.58–7.14)	Postmenopausal women 51/52 3.20 (0.91–11.22)	20/10 5.30 (1.13–24.89)	0.0011

IGF: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein-3; Q: quartile; OR: odds ratio; 95% CI: 95% confidence interval.

^a Adjusted for physical activity, age at first live birth, body mass index, and total meat intake.