SERUM CONCENTRATIONS OF IGF-I, IGFBP-3 AND C-PEPTIDE AND RISK OF HYPERPLASIA AND CANCER OF THE BREAST IN POSTMENOPAUSAL WOMEN

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Experimental evidence suggests that insulin and insulin-related growth factors may play a role in breast pathology through their mitogenic and anti-apoptotic effects on breast cells. Our objective was to assess the relationship between serum concentrations of insulin-like growth factor-I (IGF-I), its major binding protein (IGFBP-3), the ratio IGF-I:IGFBP-3, c-peptide (a marker of insulin secretion) and the ratio c-peptide:fructosamine (a marker of insulin resistance) and the risk of epithelial hyperplasia (an established breast cancer risk factor) and localized breast cancer among postmenopausal women. Study subjects were patients who provided serum before breast biopsy or mastectomy in 3 hospitals in Grand Rapids, MI between 1977 and 1987. Two case groups, 186 subjects with epithelial hyperplasia of the breast and 185 subjects with localized breast cancer, were compared to 159 subjects with nonproliferative breast changes that have not been associated with increased breast cancer risk. Serum concentrations of IGF-I, IGFBP-3 and the ratio IGF-I:IGFBP-3 were not related to risk of either hyperplasia or breast cancer. For women in the highest quartile of c-peptide or of c-peptide:fructosamine compared to those in the lowest quartile, the odds ratios (ORs) for hyperplasia were 3.0 (95% confidence interval [CI] 1.4–6.5) and 3.3 (95% CI 1.5–7.3), respectively (p trend = 0.02 and 0.02, respectively). The corresponding ORs for breast cancer were 1.5 (95% CI 0.7–3.0) and 1.6 (95% CI 0.8–3.2), respectively (p trend = 0.35 and 0.25, respectively). Our results suggest that insulin and insulin resistance may play a role in breast pathology in postmenopausal women.

Key words: *IGF-I; IGFBP-3; c-peptide; breast cancer; hyperplasia*

Experimental evidence suggests that insulin and insulin-like growth factors (IGF) may play a role in breast pathology through their mitogenic and anti-apoptotic effects on normal, benign and malignant breast epithelial cells.[1–4] Chronic hyperinsulinemia resulting from insulin resistance might also contribute to the association between obesity and breast cancer risk in postmenopausal women.[5] The binding proteins for the IGFs, on the other hand, are postulated to reduce growth stimulus by binding circulating IGFs and through a direct anti-proliferative effect on cells.[6,7]

Epidemiologic studies have consistently shown high serum levels of IGF-I to be associated with increased risk of breast cancer in premenopausal women,[8–14] but in only 2[8,13] of several studies[9,11,12,14–17] in postmenopausal women has high serum IGF-I been related to breast cancer risk. Results for IGFBP-3 have been mixed, with studies showing increased risk,[13] reduced risk[14] or no change in risk[12,16,17] of breast cancer associated with this analyte in postmenopausal women. C-peptide, a marker of insulin secretion, has been associated with increased breast cancer risk in postmenopausal women in 2[18,19] of several studies that have examined either insulin or markers of insulin secretion in relationship to breast cancer risk.[16–17,20–21] To our knowledge, there have been no published epidemiologic studies of the relationship between these factors and breast epithelial hyperplasia, a benign process that has been associated with up to a doubling in risk of breast cancer in the absence of atypia and a 5-fold increase in risk when atypia is present.[22]

In our study, we assessed the relationship of serum concentrations of IGF-I, IGF binding protein (IGFBP-3), c-peptide and the ratio c-peptide:fructosamine (a high ratio suggests hyperinsulinemia associated with insulin resistance[19]) to risk of breast cancer and benign breast epithelial hyperplasia in postmenopausal women. Women with nonproliferative breast conditions unrelated to increased risk of breast cancer served as the control group.

MATERIAL AND METHODS

Between 1977 and 1987, all patients about to undergo breast biopsy or mastectomy in 3 hospitals in Grand Rapids, MI (henceforth referred to as hospitals A, B and C) were invited to provide serum as part of a study to assess putative new breast cancer markers.[23] A total of 5,358 women agreed to participate in our study. Each participant provided written informed consent and participated in an in-person interview assessing breast cancer risk factors. Information on diagnosis and extent of disease was abstracted from medical records. Before surgery, volunteers donated 30 mL of nonfasting blood that was collected in sterile vacutainers, immediately chilled and allowed to clot within the hour. The serum was separated within 2 hr and was then divided into 1 mL aliquots and stored at –70° C in sealed glass vials. The serum samples were then shipped in containers with dry ice first to a central repository at the Mayo Foundation (Rochester, MN) and subsequently to the National Cancer Institute (NCI) and stored at –70° to –76° C at both locales.[23]

Selection criteria for study subjects are shown in Table I. The table reflects the order in which the inclusion criteria were administered. Thus, the last row indicates women who met the final criterion as well as all preceding criteria. Our analyses were restricted to postmenopausal women (those with a natural menopause whose blood collection occurred more than 1 year after their last menstrual period, those who experienced menopause due to bilateral oophorectomy or radiation and those at least 54 years old with a hysterectomy) with no self-reported history of diabetes or cancer and who were not taking menopausal estrogens or oral contraceptives at the time of the blood draw. Those taking hormones were excluded because additional analyses with included subjects will examine associations with endogenous estrogen lev-

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els. Information on past hormone use was not available. Among breast cancer cases, we further restricted the analysis to women with cancer confined to the breast in order to minimize any possible effect of systemic disease on the analyte levels. In addition, study subjects were restricted to those whose blood draw occurred on or before the date of diagnosis. In order to assure that enough serum was available to complete all assays planned for our study (including those not discussed in this article), study subjects also had to have at least 7 mL of serum available. Sufficient serum was available for 92% ($n = 707$) of eligible subjects with benign breast changes and 78% ($n = 197$) of eligible subjects with breast cancer.

We sought pathology slides and corresponding pathology reports from study hospitals for the 904 (707 with benign changes, 197 breast cancers) eligible subjects. Slides and pathology reports were successfully retrieved for 607 subjects with benign changes (86%) and 174 subjects with breast cancer (88%) (Table I). Histopathologic slides were reviewed by one of the authors (M.E.S.) to verify the breast cancer diagnosis and to determine the histologic characteristics of the benign changes.

Our study consisted of 2 case groups and one control group. A total of 185 women with either carcinoma *in situ* or invasive carcinoma with no regional lymph node metastases were studied as one case group (Table I). We were able to confirm the diagnosis of breast cancer based on the pathology material for 97% of the 174 women for whom we had at least one slide. Based on this confirmation rate, we included the 23 cases for which we were unable to obtain slides. Of the breast cancers, 11% were *in situ* disease only.

The second case group consisted of 186 subjects with benign changes who were determined to have ductal or lobular hyperplasia upon slide review. A total of 43 of these subjects had atypia (23% of those with hyperplasia; 7% of all subjects with benign changes for whom slides and pathology reports were retrieved).

The control group was selected from subjects not included in either case group. It included all 159 women who upon slide review had nonproliferative benign changes that have not been associated with increased breast cancer risk.[22] These included *(i)* nonspecified nonproliferative changes (48.4%), *(ii)* atrophic lobules (30.2%) and *(iii)* apocrine metaplasia (19.5%). A total of 261 subjects with microscopic papilloma, cysts $\geq$ 1 cm, sclerosing adenosis, adenosis and fibroadenoma were excluded from the control group because these nonproliferative conditions have been linked to an increased risk of breast cancer.[22]

Discrepancies between the original diagnosis made by the community pathologists in MI and the retrospective slide review by one of the authors (M.E.S.) that would have caused a study subject to be classified in a different group (i.e., control, case with hyperplasia, case with cancer) were noted for 1% of the control group ($n = 2$), 12% of the case group with hyperplasia ($n = 22$) and 3% of the breast cancer case group ($n = 4$). The overall agreement was 95% and the kappa statistic for agreement based on the 530 study subjects included in the analysis was 0.92. A retrospective review by a second pathologist (C.M.) agreed with the first retrospective review (M.E.S.) for 68% of the 28 subjects with discrepancies. The kappa statistic for agreement between the first and second reviewers (M.E.S. and C.M.) for these 28 subjects was 0.31. In the case of discrepancies, the final study groupings (control, case with hyperplasia and case with breast cancer) were determined by the majority among the original diagnoses and the 2 retrospective slide reviews. In the case of no majority agreement and 3 discrepant reports along a spectrum (e.g., nonproliferative disease, hyperplasia and carcinoma *in situ*) the intermediate report was chosen (in this example, hyperplasia). Because the original diagnoses were made by a number of pathologists between 1977 and 1987, it was not possible to standardize the histologic criteria for classifying the benign changes among the diagnosing pathologists and the pathologists doing the retrospective slide review.

Of the study subjects, 97% included in the analysis were white. There were only minor differences in mean age at diagnosis, age at menopause, height, Quetelet Index, age at menarche, year of diagnosis and hour of blood draw between the 607 eligible subjects with benign changes and subjects otherwise eligible but who were excluded from the analyses because serum or slides were not available ($n = 162$). Otherwise eligible breast cancer cases excluded because serum was not available ($n = 55$) were on average 61.6 years of age at diagnosis, while those included in the analyses ($n = 185$) were on average 67.0 years of age. However, there were only minor differences in mean age at menopause, height, Quetelet Index, age at menarche, year of diagnosis and hour of blood draw between the breast cancer cases included and those excluded.

Serum levels of IGF-I and IGFBP-3 were assayed in 2001 by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX) at the Lady Davis Research Institute of the Jewish General Hospital and McGill University, Montreal, Quebec, Canada (M.P.). The limits of detection for these assays were 0.03 and 0.04 ng/mL, respectively. We measured c-peptide, which is cleaved from proinsulin in equimolar amounts to insulin within the pancreatic beta cell, as a marker of pancreatic insulin secretion.[24] C-peptide was assayed in 2001 using radioimmunoassay techniques at Quest Diagnostics (Van Nuys, CA). The limit of detection for this assay was 0.5 ng/mL. Fructosamine, which reflects the average blood glucose concentrations over the previous 2–3 weeks,[25] was assayed in 2001 using colorimetric methods at Quest

TABLE I – SELECTION CRITERIA FOR STUDY SUBJECTS

	Benign breast disease subjects		Breast cancer cases
Postmenopausal	1,375		874
No diabetes or prior cancer	1,115		637
Not taking HRT[1] or OCs[2]	837		549
Intraductal carcinoma (TIS) or T1-3, N0 invasive carcinoma based on pathology reports abstracted by study hospitals	N/A		266
Blood drawn on or several days before diagnosis, but > one year after LMP[3]	769		252
7 ml serum available	707		197
Slides and pathology reports retrieved	607		174
Classified as intraductal carcinoma (TIS) or T1-3, N0 invasive cancer after slide review	N/A		167
Meet all inclusion criteria for breast cancer	N/A		190[4]
Meet inclusion criteria for benign changes based on histology[5]	Control group 159	Benign case group 187	N/A
Analytes successfully measured	159	186	185

[1]Hormone replacement therapy.–[2]Oral contraceptives.–[3]Last menstrual period.–[4]190 = 167 classified as early stage cancer after the slide review plus 23 with no slide review.– [5]Excluded were 261 subjects with benign histologies that were not included in the control group or the benign case group.

Diagnostics (Van Nuys, CA). The limit of detection for this assay was 5 nmol/mL.

For each assay, samples from study subjects were randomly assigned to batches, with approximately equal numbers of samples from each of the 2 case groups and the control group per batch. Two aliquots from each of 2 pooled quality control sera obtained from the Breast Cancer Serum Bank were inserted randomly in each batch. Laboratory personnel were unable to distinguish among case, control and quality-control samples. Using a nested components of variance analysis, with logarithmically transformed quality control measurements,[26] the estimated coefficients of variation (CV) of the assays for IGF-I, IGFBP-3, c-peptide and fructosamine were 5.3%, 8.5%, 9.7% and 1.9%, respectively.

Unconditional logistic regression was used to estimate odds ratios (OR) and compute 95% confidence intervals (CIs) for the analytes defined by quartiles of the frequency distribution in controls. Trends were assessed by including the categorical variable coded 1–4 in the model as a continuous variable and assessing the *p*-value from the Wald chi-square statistic. The $\log_e$-transformed values for the serum measurements of selected analytes were also included in the logistic regression models as continuous variables. Quadratic terms for selected analytes were added to assess additional improvement in fit, but none were statistically significant. Wald chi-square statistics were used to assess the *p*-value of coefficients in the logistic regression models. We tested whether coefficients for the 2 case groups were significantly different from each other using the CATMOD procedure in SAS 8.2. All regression analyses were adjusted for study hospital, age at diagnosis, year of diagnosis, hour of the blood draw, age at menopause, Quetelet Index and nulliparity. Study hospital and nulliparity were included in the analyses as categorical variables; age at diagnosis, year of diagnosis, hour of the blood draw, age at menopause and Quetelet Index were examined as both categorical and continuous variables, but were included in the final models as continuous variables. None of these variables had missing data. Pearson correlation coefficients (r) were calculated for the $\log_e$-transformed values of the serum measurements. Analysis of covariance was used to estimate geometric mean levels of the analytes adjusted to the mean age of the study subjects combined.

RESULTS

Of the study subjects, 27% of controls, 32% of cases with hyperplasia and 22% of breast cancer cases were identified at hospital A; 48%, 27% and 38%, respectively, were diagnosed at hospital B and 24%, 40% and 41%, respectively, were diagnosed at hospital C. The mean year of diagnosis (and blood draw) for the controls was 1981, for the cases with hyperplasia was 1983 and for the breast cancer cases was 1982. The corresponding mean times from blood draw to assaying the analytes were 20 years, 18 years and 19 years, respectively. The mean hours of blood collection for the controls, cases with hyperplasia, and breast cancer cases were 11 AM, 10 AM and 11 AM, respectively. Blood was collected in the morning (through noon) for 70% of controls compared to 83% of cases with hyperplasia and 64% of cases with breast cancer.

Other selected characteristics of the cases and controls are shown in Table II. The average ages at diagnosis of controls, cases with hyperplasia, and cases with breast cancer were 60.9 years (range 23–83), 62.6 years (range 34–86) and 67.0 years (range 48–92), respectively. Women with hyperplasia were slightly older at menopause, had a higher Quetelet Index and were less likely to be nulliparous or to have a family history of breast cancer (1st or 2nd degree) than the controls. Breast cancer cases had an older age at menopause and a slightly older age at first full-term pregnancy than the controls.

Geometric mean concentrations of IGF-I, IGFBP-3 and c-peptide in the controls according to levels of selected potential confounding factors are shown in Table III. IGF-I concentrations were inversely associated with age, but there were no consistent patterns of variation by age at menopause, Quetelet Index or height, and little difference by nulliparity or family history of breast cancer. IGFBP-3 concentrations were somewhat higher in parous than nulliparous women and slightly higher in women with an older age at menopause. Concentrations of c-peptide were higher among the oldest women and increased with increasing Quetelet Index. C-peptide levels were inversely associated with year of diagnosis (correlation = −0.23) and were higher in the afternoon than in the morning (mean levels of 2.0 and 1.4, respectively; data not shown in Table). Concentrations of IGF and IGFBP-3 did not vary systematically by year of diagnosis or hour of blood draw.

Estimated age-adjusted geometric mean concentrations for the analytes for cases and controls are presented in Table IV. Mean concentrations of IGF-I and IGFBP-3 were somewhat higher in cases with hyperplasia than controls, while mean values of the ratio IGF-I:IGFBP-3, c-peptide concentration and the ratio c-peptide:fructosamine were similar in the 2 groups. Among subjects with hyperplasia that was mild or moderate ($n = 106$), florid ($n = 37$) and atypical ($n = 43$), the geometric mean levels of c-peptide were 1.6 (95% CI 1.4–1.8), 1.6 (95% CI 1.3–1.9) and 1.9 (95% CI 1.6–2.2), respectively. Mean levels of other analytes did not vary consistently according to degree of hyperplasia. There was little difference between mean concentrations of these factors in the breast cancer cases and controls.

Log_e-transformed values of the serum concentrations for IGF-I and IGFBP-3 were significantly correlated among all study subjects ($r = 0.62$; $p < 0.0001$). None of the correlation coefficients among the other individual analytes was greater than 0.06 and none was statistically significant.

Logistic regression results for breast hyperplasia are summarized in Table V. Serum concentrations of IGF-I and IGFBP-3 and the ratio IGF-I:IGFBP-3 were not related to risk of hyperplasia. Results were similar when analyses were restricted to women in the 2 highest quartiles of Quetelet Index, who might be expected to have higher endogenous estrogen levels (data not shown). C-peptide was associated with a statistically significant increase in

TABLE II – SELECTED CHARACTERISTICS OF THE STUDY POPULATION

	Controls ($n = 159$); mean (SD)	Cases with hyperplasia ($n = 186$); mean (SD)	Cases with breast cancer ($n = 185$); mean (SD)
Age at diagnosis (years)	60.9 (9.9)	62.6 (9.1)	67.0 (8.3)
Age at menopause (years)	45.6 (7.7)	46.1 (7.2)	47.3 (6.0)
Age at menarche (years)[1]	12.9 (1.7)	13.1 (1.6)	13.1 (1.6)
Height (cm)	163.1 (6.2)	163.4 (6.5)	162.4 (6.8)
Quetelet Index[2]	25.1 (3.9)	26.0 (4.5)	25.6 (5.5)
Nulliparous (%)	19.5	9.7	17.8
Number of full-term pregnancies	2.6 (2.2)	3.0 (1.8)	2.6 (2.0)
Age at first full-term pregnancy[3]	23.7 (4.8)	23.5 (4.3)	24.8 (4.5)
Family history of breast cancer (%)[4]	27.7	25.8	28.1

[1]Age at menarche was unknown for 3 controls, 2 cases with hyperplasia, and 2 cases with breast cancer.–[2]Weight (kg)/height (meters)^2.–[3]Among parous women.–[4]Mother, grandmother, sister, aunt. Family history was unknown for 5 controls, 6 cases with hyperplasia, and 8 cases of breast cancer.

TABLE III – GEOMETRIC MEAN CONCENTRATIONS OF IGF-I, IGFBP-3 AND C-PEPTIDE ACCORDING TO LEVELS OF POTENTIAL CONFOUNDING VARIABLES AMONG CONTROLS

	IGF-I (ng/ml) geometric mean	IGFBP-3 (ng/ml) geometric mean	C-peptide (ng/ml) geometric mean
Age at diagnosis[1]			
≤55.8	155.2	2,900.1	1.5
55.9–61.2	150.8	2,946.3	1.4
61.3–67.6	130.6	2,901.6	1.5
>67.6	134.3	2,670.6	2.0
Age at menopause[1]			
≤41	147.8	2,739.4	1.7
42–46	131.7	2,789.6	1.6
47–52	148.0	2,983.9	1.4
>52	139.8	2,965.9	1.6
Parity			
Nulliparous	138.3	2,713.7	1.6
Parous	143.2	2,903.7	1.5
Quetelet index[1]			
≤22.7	141.4	2,768.2	1.4
22.8–24.6	139.9	2,851.2	1.4
24.7–27.2	150.5	3,005.1	1.6
>27.2	137.4	2,846.5	1.8
Family history of breast cancer			
Yes	140.2	2,929.5	1.5
No	143.3	2,857.8	1.6
Height (cm)[1]			
≤160	139.5	2,879.1	1.6
161–163	143.6	3,003.0	1.5
164–168	149.8	2,993.7	1.5
>168	135.7	2,527.4	1.5

[1]Quartiles.

TABLE IV – AGE-ADJUSTED GEOMETRIC MEAN SERUM CONCENTRATIONS FOR IGF-I, IFGBP-3, IGF-I:IGFBP-3, C-PEPTIDE, AND C-PEPTIDE:FRUCTOSAMINE FOR CASES AND CONTROLS

	Controls (n = 159)	Cases with hyperplasia (n = 186)	Cases with breast cancer (n = 185)
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
IGF-I (ng/ml)	139.1 (133.7–144.8)	146.4 (141.1–151.9)	139.1 (133.9–144.6)
IGFBP-3 (ng/ml)	2,840 (2,731–2,952)	2,991 (2,938–3,046)	2,899 (2,792–3,011)
IGF-I:IGFBP-3	0.049 (0.047–0.051)	0.049 (0.047–0.051)	0.048 (0.046–0.050)
C-peptide (ng/ml)	1.6 (1.5–1.7)	1.65 (1.52–1.72)	1.7 (1.5–1.8)
C-peptide: fructosamine	0.0069 (0.0063–0.0075)	0.0072 (0.0066–0.0077)	0.0072 (0.0066–0.0078)

risk, with the OR in the highest quartile being 3.0 (95% CI 1.4–6.5; p trend = 0.02). The difference between the age-adjusted and fully-adjusted estimates was largely due to inclusion of year of diagnosis and time of the blood draw. The coefficient for $\log_e$ c-peptide considered as a continuous variable in the logistic model was 0.65 (95% CI 0.14–1.16). Although the ratio c-peptide:fructosamine was also associated with a statistically significant increase in risk (p trend = 0.02), there was no consistent dose-response relationship. Again, much of the difference between the age-adjusted and fully-adjusted estimates was due to inclusion of year of diagnosis and hour of the blood draw. The coefficient for $\log_e$ ratio c-peptide:fructosamine considered as a continuous variable was 0.62 (95% CI 0.12–1.12).

We also examined associations for hyperplasia with and without atypia separately. Results for IGF-I, IGFBP-3 and the ratio IGF-I:IGFBP-3 according to presence of atypia were similar to the overall results. For hyperplasia with atypia, the coefficient for $\log_e$ c-peptide included as a continuous variable in the logistic model was 1.31 (95% CI 0.43–2.19), while that for hyperplasia without atypia was 0.57 (95% CI 0.02–1.12). These coefficients did not differ significantly (p = 0.11). The corresponding coefficients for $\log_e$ ratio of c-peptide:fructosamine were 1.19 (95% CI 0.34–2.05) and 0.54 (95% CI –0.001 to 1.08). Again, the coefficients did not differ significantly (p =0.12).

Serum concentrations of IGF-I, IGFBP-3 and the ratio IGF-I:IGFBP-3 were not associated with risk of breast cancer (Table VI). Results were similar when analyses were restricted to women in the upper 2 quartiles of Quetelet Index and when controls younger than age 48 (the youngest age of the cases) were excluded (data not shown). The ORs associated with the highest quartiles of c-peptide and c-peptide:fructosamine were 1.5 (95% CI 0.7–3.0) and 1.6 (95% CI 0.8–3.2), respectively (p trend = 0.35 and 0.25, respectively). The coefficients in the logistic model for the $\log_e$ transformations of c-peptide and the ratio of c-peptide:fructosamine considered as continuous variables were 0.37 (95% CI –0.09 to 0.84) and 0.35 (95% CI –0.11 to 0.81), respectively. These coefficients did not differ significantly from those for benign hyperplasia (p = 0.27 and 0.26, respectively).

DISCUSSION

We examined serum concentrations of IGF-I, its major carrier protein (IGFBP-3) and measures of insulin secretion and insulin resistance in relationship to risk of postmenopausal breast cancer and benign breast hyperplasia. The lack of association between breast cancer risk and IGF-I or the ratio IGF-I:IGFBP-3 in our study is consistent with most other studies in postmenopausal women.[9–12,14–16] Two studies, however, report some evidence of an association in postmenopausal women.[8,13] Because estrogen activity may be necessary for maximal IGF-I signaling,[27,28] the absence of an association with IGF-I in postmenopausal women may be due to the relatively low endogenous estrogen levels after

TABLE V – ODDS RATIOS (95% CIs) FOR BREAST HYPERPLASIA ACCORDING TO QUARTILES OF SERUM CONCENTRATIONS OF IGF-I, IGFBP-3, IGF-I:IGFBP-3, C-PEPTIDE, AND C-PEPTIDE:FRUCTOSAMINE IN POSTMENOPAUSAL WOMEN

	Q_1[8]	Q_2	Q_3	Q_4	*p*-value for trend test
IGF-I ng/ml	≤119.15	≤138.68	≤167.7	> 167.7	
(cases/controls)	(41/40)	(39/40)	(57/40)	(49/39)	
OR[1] (95% CI)	1.0	1.0 (0.5–1.9)	1.6 (0.9–2.9)	1.5 (0.8–2.8)	
OR[1,2,3] (95% CI)	1.0	1.0 (0.5–2.0)	1.2 (0.5–2.5)	1.0 (0.4–2.2)	.87
IGFBP-3 ng/ml	≤2,523.6	≤2,937.1	≤3,345.3	> 3,345.3	
(cases/controls)	(42/40)	(42/39)	(39/40)	(63/40)	
OR[1] 95% CI)	1.0	1.2 (0.6–2.2)	1.0 (0.5–1.9)	1.7 (0.9–3.1)	
OR[1,2,4] (95% CI)	1.0	1.1 (0.5–2.2)	0.8 (0.4–1.7)	1.2 (0.6–2.8)	.80
IGF-I:IGFBP-3	≤0.0428	≤0.0491	≤0.0574	>0.0574	
(cases/controls)	(54/39)	(39/40)	(46/41)	(47/39)	
OR[1] (95% CI)	1.0	0.7 (0.4–1.3)	0.9 (0.5–1.6)	0.9 (0.5–1.7)	
OR[1,2,5] (95% CI)	1.0	0.7 (0.4–1.4)	1.0 (0.5–1.9)	0.9 (0.4–1.7)	.99
C-peptide (ng/ml)	≤1	≤1.5	≤2.3	> 2.3	
(cases/controls)	(40/41)	(55/44)	(36/37)	(55/37)	
OR[1] (95% CI)	1.0	1.2 (0.7–2.2)	0.9 (0.5–1.7)	1.4 (0.8–2.6)	
OR[1,2,6] (95% CI)	1.0	1.5 (0.8–3.0)	1.1 (0.5–2.4)	3.0 (1.4–6.5)	.02
C-peptide:fructosamine	≤0.0043	≤0.0066	≤0.0096	> 0.0096	
(cases/controls)	(30/39)	(65/40)	(32/41)	(59/39)	
OR[1] (95% CI)	1.0	2.0 (1.1–3.8)	1.0 (0.5–1.9)	1.8 (1.0–3.5)	
OR[1,2,7] (95% CI)	1.0	2.1 (1.0–4.2)	1.1 (0.5–2.4)	3.3 (1.5–7.3)	.02

[1]Adjusted for age at diagnosis.–[2]Adjusted for age at menopause, Quetelet Index, nulliparity, year of diagnosis, hour of blood draw and study hospital.–[3]Adjusted for c-peptide:fructosamine and IGFBP-3.–[4]Adjusted for c-peptide:fructosamine and IGF-I.–[5]Adjusted for c-peptide:fructosamine.–[6]Adjusted for IGF-I, IGFBP-3, and fructosamine.–[7]Adjusted for IGF-I, and IGFBP-3.–[8]Reference category.

TABLE VI – ODDS RATIOS (95% CIs) FOR BREAST CANCER ACCORDING TO QUARTILES OF SERUM CONCENTRATIONS OF IGF-I, IGFBP-3, IGF-I:IGFBP-3, C-PEPTIDE, AND C-PEPTIDE:FRUCTOSAMINE IN POSTMENOPAUSAL WOMEN

	Q_1[8]	Q_2	Q_3	Q_4	*p*-value for trend test
IGF-I (ng/ml)	≤119.15	≤138.68	≤167.7	>167.7	
(case/controls)	(57/40)	(46/40)	(45/40)	(37/39)	
OR[1] (95% CI)	1.0	1.1 (0.6–2.0)	1.1 (0.6–2.2)	1.1 (0.5–2.0)	
OR[1,2,3] (95% CI)	1.0	1.1 (0.6–2.4)	1.0 (0.5–2.2)	0.9 (0.4–2.0)	.56
IGFBP-3 (ng/ml)	≤2,523.6	≤2,937.1	≤3,345.3	> 3,345.3	
(cases/controls)	(55/40)	(38/39)	(41/40)	(51/40)	
OR[1] (95% CI)	1.0	0.9 (0.5–1.7)	0.8 (0.4–1.5)	1.4 (0.7–2.6)	
OR[1,2,4] (95% CI)	1.0	0.8 (0.4–1.7)	0.7 (0.3–1.6)	1.2 (0.6–2.8)	.59
IGF-I:IGFBP-3	≤0.0428	≤0.0491	≤0.0574	> 0.0574	
(cases/controls)	(56/39)	(46/40)	(45/41)	(38/39)	
OR[1] (95% CI)	1.0	0.8 (0.5–1.6)	0.9 (0.5–1.7)	0.8 (0.4–1.5)	
OR[1,2,5] (95% CI)	1.0	0.9 (0.4–1.6)	0.9 (0.5–1.8)	0.9 (0.5–1.9)	.91
C-peptide (ng/ml)	≤1	≤1.5	≤2.3	> 2.3	
(cases/controls)	(47/41)	(39/44)	(38/37)	(61/37)	
OR[1] (95% CI)	1.0	0.7 (0.4–1.3)	0.6 (0.3–1.2)	1.2 (0.6–2.1)	
OR[1,2,6] (95% CI)	1.0	0.7 (0.4–1.4)	0.6 (0.3–1.3)	1.5 (0.7–3.0)	.35
C-peptide:fructosamine	≤0.0043	≤0.0066	≤0.0096	> 0.0096	
(cases/controls)	(42/39)	(40/40)	(36/41)	(67/39)	
OR[1] (95% CI)	1.0	0.8 (0.4–1.5)	0.6 (0.3–1.2)	1.3 (0.7–2.3)	
OR[1,2,7] (95% CI)	1.0	0.7 (0.4–1.5)	0.6 (0.3–1.3)	1.6 (0.8–3.2)	.25

[1]Adjusted for age at diagnosis.–[2]Adjusted for age at menopause, Quetelet Index, and nulliparity, year of diagnosis, hour of blood draw and study hospital.–[3]Adjusted for c-peptide:fructosamine and IGFBP3.–[4]Adjusted for c-peptide:fructosamine and IGF-I.–[5]Adjusted for c-peptide: fructosamine.–[6]Adjusted for IGF-I, IGFBP-3, and fructosamine.–[7]Adjusted for IGF-I and IGFBP-3.–[8]Reference category.–

menopause. However, we also found no association among heavier women, who were likely to have higher endogenous estrogen levels, although still substantially lower than those of premenopausal women.

We are unaware of any published epidemiologic studies of the relationship between IGF-I and risk of breast hyperplasia. The null associations we found for IGF-I are consistent with one experimental study showing that mean IGF-I immunoreactivity in breast epithelial and stromal cells did not increase with progression from normal tissue to hyperplasia.[29] Other experimental data, however, show IGF-I to be a potent mitogen in epithelial cell lines established from proliferative breast diseases[3] and to increase mammary gland hyperplasia in rhesus monkeys.[30] Higher levels of IGF-I specific binding sites have also been reported in epithelial hyperplasia than in adenofibromas or dystrophic diseases of the breast.[31]

We found no association between serum IGFBP-3 and breast cancer risk, in accord with several other studies of postmenopausal women.[12,16,17] At least one study reported lower levels of IGFBP-3 in postmenopausal breast cases compared to controls.[14] We also found no association between IGFBP-3 and breast hyperplasia, but are unaware of other published studies addressing this association.

Although we found no statistically significant associations between c-peptide or the ratio c-peptide:fructosamine and breast cancer risk, risk was elevated in the highest quartile of each analyte. Null associations between c-peptide or insulin and breast cancer risk have been reported in several studies of pre-[12] and postmenopausal women,[12,16–17,20–21] but others have reported an increased risk with increasing concentrations of c-peptide in both menopausal groups.[18,19] In our study, both c-peptide and the ratio c-peptide:fructosamine were significantly associated with risk of

benign breast hyperplasia. We are unaware of other published data on such associations. The stronger associations with hyperplasia than breast cancer are plausible if some, but not all, breast cancer progresses from hyperplasia. Whether hyperplasia reflects a generalized predisposition to breast cancer or a direct precursor to malignancy is a matter of debate.[32]

Several methodologic issues warrant mention in assessing our findings. In our present study, we used a control group consisting of women who, like the cases, had undergone a breast biopsy. This is a strength of our study for several reasons. First, we were able to ensure that the controls did not have the diseases under study, particularly breast hyperplasia. In addition, we were able to select controls that had nonproliferative breast conditions that have not been associated with increased breast cancer risk. Finally, to the extent possible, we excluded from our control group benign conditions that have been significantly associated with IGF-I or IGFBP-3.[33] We are not aware of any published data identifying those benign breast conditions that may be associated with c-peptide/insulin.

We were unable to document how the volunteers who gave blood for the breast cancer marker study carried out in the 1970s and 1980s differed from those who did not because no information was collected from those who did not participate; however, it is likely that any characteristics favoring participation were similar for all study subjects included in this analysis. We also excluded some women who met the eligibility criteria for our study but did not have serum or slides (benign conditions only) available. There were only minor differences between included cases and cases that had to be excluded with the exception that excluded breast cancer cases were on average 5 years younger than those included. However, our results, which are age-adjusted, would not be affected by these exclusions, assuming that the levels of analytes in those excluded were similar to levels of included subjects of the same age.

Because this is a case-control study, it is possible that the disease process affected concentrations of the analytes measured. It has been noted that circulating concentrations of IGF-I generally fall after a diagnosis of cancer, particularly advanced disease.[2] We addressed this issue by limiting the breast cancer cases to those localized to the breast; in addition, blood specimens were collected immediately before diagnosis and thus before any subsequent treatment. However, it remains possible that IGF-I levels were lower due to the disease and diagnostic process among the cases included in our analysis, in which case the ORs would be attenuated. It is less likely that the benign breast disease process significantly affected circulating concentrations of IGF-I or other analytes measured. Finally, null associations between IGF-I and postmenopausal breast cancer risk similar to ours have been reported in cohort studies,[11,12,14,16] in which the disease process is less likely to affect levels of analytes.

C-peptide is frequently measured as a surrogate for insulin because it has a longer half-life (30 min *vs*. 3–7 min) and is thus thought to provide a more reproducible index of insulin secretion than insulin itself.[34] Like insulin, however, c-peptide is ideally measured in fasting serum samples as the major determinant of its concentration is glucose.[34] Because we did not have fasting serum samples, we adjusted for the hour of blood collection as the best available proxy for food intake. However, it is likely that the values of c-peptide we report reflect recent food intake by the individual women as well as basal levels. Thus, it is possible that we underestimated the associations due to nondifferential misclassification. It is notable that both positive[19] and null associations[8,21] with c-peptide have been found in studies with fasting samples.

Our study was similar in size to most studies that have examined the relationship between IGF-I, IGFBP-1 or c-peptide and breast cancer risk in postmenopausal women.[8,9,11–15,17–20] Similar to other studies, however, we were limited in our power to detect associations of small magnitudes. For instance, our study had power of only 26% to reject the null hypothesis of no association between c-peptide or c-peptide:fructosamine and breast cancer risk, assuming ORs of the magnitude we observed in our study.

In summary, our results add to the accumulating evidence that serum concentrations of IGF-I and IGFBP-3 do not contribute to postmenopausal breast cancer risk. Furthermore, our data suggest no role for these analytes in the etiology of breast hyperplasia in postmenopausal women. We did not address the possibility that premenopausal levels of these analytes might influence postmenopausal risk of breast cancer or hyperplasia. We found that serum c-peptide concentrations and the ratio of c-peptide:fructosamine were associated with increased risk of breast hyperplasia, whereas levels were only weakly associated with breast cancer and may have reflected chance. These results suggest that insulin and insulin resistance may play a role in breast pathology in postmenopausal women.

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