Premenopausal levels of circulating insulin-like growth factor I and the risk of postmenopausal breast cancer

Dana E. Rollison[1,2], Craig J. Newschaffer[1]*, Yuzhen Tao[3], Michael Pollak[3] and Kathy J. Helzlsouer[1,4]

[1]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD*
[2]*Division of Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL*
[3]*Department of Oncology, McGill University-Jewish General Hospital, Montreal, Canada*
[4]*Center for Prevention and Research, Mercy Medical Center, Baltimore, MD*

Increased levels of insulin-like growth factor I (IGF-I) may directly stimulate breast cell proliferation and promote growth and survival of transformed cells. Higher levels of IGF-I have been associated with increased risk of premenopausal breast cancer but not postmenopausal breast cancer. We investigated whether circulating levels of IGF-I prior to menopause are associated with breast cancer diagnosed after menopause in a population-based nested case-control study. Female cohort participants were enrolled in 1974 (n = 15,192) and 1989 (n = 18,724) and blood was drawn. Cases were women diagnosed with primary breast cancer at ages ≥50, of whom 152 were premenopausal at blood draw. One control was matched to each case on cohort participation, age, ethnic group, menopausal status and date of blood draw. Levels of IGF-I and IGF binding protein 3 (IGFBP-3) were measured using enzyme-linked immunoabsorbant assays assays. The association between IGF-I and breast cancer was determined using conditional logistic regression, adjusting for IGFBP-3. IGF-I levels decreased with age (p = 0.0001). Prior to age-stratification, IGF-I levels neither measured before nor after menopause were associated with postmenopausal breast cancer. After age-stratification, associations were suggested in the youngest premenopausal age group (upper *vs.* lowest third: odds ratio (OR) = 5.31, 95% confidence intervals (CI) = 0.85–33.13; p trend = 0.06) and oldest postmenopausal age group (upper *vs.* lowest third: OR = 3.41, 95% CI = 0.66–17.71; p trend = 0.13). The association between circulating levels of IGF-I and postmenopausal breast cancer risk may be modified by age. Increased levels of circulating IGF-I may be of particular interest in the younger premenopausal women and older postmenopausal women. Age-stratification should be undertaken in larger investigations of IGF-I levels as predictors of postmenopausal breast cancer.

Key words: breast cancer; insulin-like growth factor I; hormones; case-control; epidemiology

Insulin-like growth factor I (IGF-I) is an important component of the growth hormone (GH)-IGF axis, a complex system that contributes to the regulation of apoptosis and cell growth. Increased levels of IGF-I have been implicated in the development of several types of cancer, including breast, prostate and colon.[1–5] IGF-I directly stimulates the proliferation of breast epithelial cells and promotes the growth and survival of transformed cells.[6,7] One of 6 insulin-like growth factor binding proteins (IGFBP), IGFBP-3, binds most of the IGF-I in circulation.[3,5] IGFBP-3 may also independently promote apoptosis through pathways outside of the GH-IGF axis, including enhancement of p53 activity.[1,3,7] The role of IGF-I and the GH-IGF axis in breast carcinogenesis, specifically, has been previously reviewed.[6–8]

Four case-control studies have found positive associations between higher circulating levels of IGF-I and premenopausal breast cancer,[9–12] while 2 others have observed no association.[13,14] Four of 5 prospective studies have also observed an increased risk of premenopausal breast cancer associated with higher levels of IGF-I,[15–19] including 1 study that observed a stronger association between IGF-I levels and breast cancer among premenopausal women who were less than 50 years at baseline, as compared to all premenopausal women combined.[15] As opposed to the positive associations generally noted with premenopausal breast cancer, IGF-I has typically not been found to be associated with increased risk of postmenopausal breast cancer. While 3[11,12,20] of 5[11,12,20–22] case-control studies have reported increased levels of IGF-I in postmenopausal breast cancer cases *vs.* controls, 5 of 6 prospective studies have found null[15,17–19,23] associations. The 6th prospective study found a positive association, but it was not statistically significant.[16]

None of the previous prospective studies of IGF-I and postmenopausal breast cancer were designed to investigate the association between premenopausal levels of IGF-I and postmenopausal breast cancer, and when conducted, analyses of this subgroup were limited by small sample size. Among women determined to be premenopausal at blood draw, Muti *et al.* reported a statistically significant 15-fold risk of breast cancer diagnosed at ages greater than 48 years, presumably postmenopausal cases, associated with the highest thirds of IGF-I.[18] Krajcik *et al.* observed no association between IGF-I and postmenopausal breast cancer among 31 case-control pairs who were younger than 50 years, presumably premenopausal, at blood draw.[17] Both studies acknowledged limited sample sizes for these analyses.

We investigated whether circulating levels of IGF-I prior to menopause were associated with breast cancer diagnosed after menopause in a population-based nested case-control study. Cohort participants were enrolled in 1974 and 1989 and blood was drawn. Breast cancer cases were ascertained through 2001, incorporating 27 years of follow-up and allowing for adequate accrual of postmenopausal cases who were premenopausal at the time of blood draw. Circulating IGF-I and IGFBP-3 levels were measured and compared among case-control pairs who were premenopausal at blood draw and postmenopausal at breast cancer diagnosis. For comparison, we also investigated associations with IGF-I and IGFBP-3 among case-control pairs who were postmenopausal at both blood draw and breast cancer diagnosis. Because previous results have suggested that age at blood draw may be an important modifying factor, we also explored the effect of age at blood draw on the association between IGF-I levels and postmenopausal breast cancer.

Material and methods

Study population

Two research serum banks were established in Washington County, Maryland: the first in 1974 (CLUE I) and the second in 1989 (CLUE II). Residents of Washington County, MD, were

Abbreviations: BMI, body mass index; CI, confidence interval; ELISA, enzyme-linked immunoabsorbant assay; GH, growth hormone; IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein 3; OR, odds ratio.

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recruited to donate a blood sample to be used in future research studies investigating "clues to heart disease and cancer." Female residents (15,192) of Washington County donated serum samples in CLUE I, and 18,724 donated plasma samples in CLUE II, of whom 5,269 participated in both CLUE I and CLUE II. Samples were frozen and stored at −70°C. All participants gave informed consent at the time of blood draw and completed a brief questionnaire to obtain information on ethnic group, sex, age, education, cigarette smoking and current oral contraceptive use. Height and weight were also recorded at CLUE II baseline. Subsequent follow-up questionnaires were mailed out to CLUE II participants in 1996, 1998 and 2000. These questionnaires obtained more detailed information on person medical history, family history of cancer and reproductive history.

Protocols for the CLUE cohort studies and this case-control analysis were approved by the institutional review board at the Johns Hopkins Bloomberg School of Public Health. Cases were defined as women, aged 50 and older at the time of diagnosis with primary malignant tumors of the breast (ICD-9 174, 233, C50.0 or D05.9), having no prior history of cancer, except possibly for nonmelanoma skin cancer and cervical cancer *in situ*. Cases diagnosed through May 31, 2001 were identified using the Washington County Cancer Registry and, since 1992, the Maryland Cancer Registry. One case diagnosed within 1 year of blood draw was excluded.

One control was individually matched to each case on participation in 1 or both the CLUE cohorts, age within 1 year, menopausal status at blood draw, ethnic group, date of blood draw within 14 days and freezer/thaw history of the serum sample. Postmenopausal status at baseline was defined as age $\geq$50 and self-reported last menstrual period greater than 1 year prior to blood draw. In rare situations, age and date of blood draw criteria were expanded to facilitate matching. Controls were cancer-free up to the time of cases' diagnosis, with the possible exceptions of nonmelanoma skin cancer and cervical cancer *in situ*.

Cohort participation was included as a matching factor because of the complexity of the study design. Final sample size comprised 83 cases who participated only in CLUE I (58 premenopausal and 25 postmemopausal), 50 cases only in CLUE II (25 premenopausal and 25 postmenopausal), and 69 cases who participated in both CLUE I and CLUE II (25 premenopausal at both CLUE I and II and 44 premenopausal at CLUE I and postmenopausal at CLUE II). Both CLUE I and CLUE II blood samples were included for these cases unless there was missing CLUE II data ($n = 2$) or the breast cancer diagnosis came less then 1 year from the CLUE II blood draw date ($n = 3$).

Laboratory assays

Levels of IGF-I and IGFBP-3 were measured using enzyme-linked immunoabsorbant assays (ELISA), as previously described.[15] Fifteen masked sets comprising 30 serum samples and 12 masked sets comprising 24 plasma samples were assayed for quality control. The mean interset coefficients of variation for IGF-I/IGFBP-3 measured in serum and plasma were 5.9%/6.0% and 5.9%/6.5%, respectively. Among duplicate pairs of samples, mean intraset coefficients of variation for IGF-I/IGFBP-3 were 4.0%/5.9% among 9 duplicate pairs of serum and 3.2%/3.7% among 7 duplicate pairs of plasma.

Statistical analysis

Baseline characteristics were described for cases and controls, including age at blood draw, education, smoking, oral contraceptive use and body mass index (BMI) (<25, 25–29, $\geq$30 kg/m^2). Duplicate IGF-I and IGFBP-3 measures were averaged for each individual. Mean IGF-I and IGFBP-3 levels were calculated separately for women who were premenopausal or postmenopausal at blood draw and compared among baseline characteristics. Cross-sectional relationships between the natural log of IGF-I, IGFBP-3 and age were investigated using Pearson correlation coefficients.

To examine the association between levels of IGF-I and IGFBP-3 and investigate a dose-response, levels of IGF-I and IGFBP-3 were divided into thirds, based on the distributions in the controls, and calculated separately for women who were premenopausal *vs.* postmenopausal at blood draw. The associations between levels of IGF-I, IGFBP-3 and postmenopausal breast cancer were modeled using conditional logistic regression. Matched odds ratios (OR) and 95 percent confidence intervals (CI) were obtained separately for premenopausal and postmenopausal serum levels. To account for the repeated measures of IGF-I and IGFBP-3 obtained for the subset of 23 case-control pairs who donated 2 blood samples and were premenopausal at both donations (1974 and 1989), we used conditional logistic regression programmed to generate sandwich estimates of variance that are robust to the non-independence of these observations.[24] *p*-values for trends across thirds of IGF-I and IGFBP-3 were assessed by including these variables as continuous measures. Adjustment for BMI, family history of breast cancer, age at menarche, parity, age at first live birth and oral contraceptive use in those women for whom data were available did not appreciably change the risk estimates for IGF-I and postmenopausal breast cancer, thus they were not included in the final models.

Analyses were restricted to postmenopausal breast cancer cases who were diagnosed after the of age 55 years, so as to exclude perimenopausal cases, and results obtained for this subgroup were compared to results obtained from the entire study population. The relationships between premenopausal levels of IGF-I, IGFP-3 and postmenopausal invasive breast cancer were investigated further by excluding from analyses 20 cases of ductal carcinoma *in situ*. To estimate levels of free IGF-I, or the amount of IGF-I not bound to IGFBP-3, we calculated the ratio of IGF-I to IGFBP-3 levels. Values of this ratio were divided into thirds based on the controls, separately for women who were premenopausal or postmenopausal at blood draw.

Because previous studies have shown that IGF-I levels correlate strongly with age, we investigated differences in the association between IGF-I and postmenopausal breast cancer by the age at IGF-I measurement (*i.e.* age at blood draw). Early life exposures may be important in breast cancer etiology, thus, women in the youngest 10-year age category were analyzed separately from all other premenopausal women (ages 25–35 *vs.* ages 36–50). Postmenopausal women were divided into 2 equal-length age categories (ages 46–60 *vs.* 61–74). OR for IGF-I levels and postmenopausal breast cancer risk were calculated for each of the 4 groups defined by menopausal status and age at blood draw.

Age at blood draw and latency (time between blood draw and diagnosis) were inversely correlated among breast cancer cases (Spearman correlation coefficient = -0.63, $p < 0.0001$). To separate effects of age at blood draw from latency on the risk of postmenopausal breast cancer associated with increased premenopausal IGF-I levels, we conducted a residual analysis.[25] Specifically, IGF-I and IGFBP-3 levels were regressed on time from blood draw to diagnosis,[25] and the resulting residuals were categorized into thirds based on cutpoints from the control distribution. The postmenopausal breast cancer OR for the upper two thirds, with the lower third as the reference, were then estimated separately for subjects under age 36 and age 36 or older at blood draw.

All statistical tests were 2 sided, and *p*-values less than 0.05 were considered statistically significant. All analyses were conducted using SAS (version 8.1, Cary, NC).

Results

Baseline characteristics of postmenopausal cases and matched controls are presented by menopausal status at blood draw as shown in Table I. We identified 202 case-control pairs who con-

TABLE I – BASELINE CHARACTERISTICS OF POSTMENOPAUSAL BREAST CANCER CASES AND CONTROLS, WASHINGTON COUNTY, MD, 1975–2001

Baseline characteristic (measured at the time of blood draw)	Premenopausal at blood draw[1]		Postmenopausal at blood draw[2]	
	Cases *n* (%)	Controls *n* (%)	Cases *n* (%)	Controls *n* (%)
Total individuals[2]	152	152	91	91
Total observations[1]	175	175	91	91
Age at blood draw (years)[3]				
Mean (standard deviation)	42.4 (6.7)	42.0 (6.7)	60.5 (6.0)	60.2 (6.1)
Education (grades)[4]				
<12	45 (25.7)	34 (19.5)	25 (27.5)	28 (31.1)
=12	81 (46.3)	86 (49.4)	35 (38.5)	42 (46.7)
>12	49 (28.0)	54 (31.0)	31 (34.1)	20 (22.2)
Smoking				
Never	102 (58.3)	106 (60.6)	53 (58.2)	57 (62.6)
Former	29 (16.6)	26 (14.9)	23 (25.3)	20 (22.0)
Current	44 (25.1)	43 (24.6)	15 (16.5)	14 (15.4)
Oral contraceptive use[4,5]				
Never (1989)	24 (13.7)	26 (14.9)	51 (56.7)	57 (63.3)
Former (1989)	22 (12.6)	19 (10.9)	14 (15.6)	8 (8.9)
Current (1974, 1989)	15 (8.6)	14 (8.0)		
Not current (1974)	114 (65.1)	116 (66.3)	25 (27.8)	25 (27.8)
Body mass index (kg/m^2)[5]				
<25	24 (50.0)	23 (47.9)	29 (43.9)	45 (47.9)
25–29	12 (25.0)	16 (33.3)	21 (31.8)	16 (33.3)
≥30	12 (25.0)	9 (18.8)	16 (24.2)	9 (18.8)

[1]Premenopausal observations include 23 pairs of observations for the 23 case-control pairs who participated in both CLUE I (1974) and CLUE II (1989); baseline characteristics were measured twice for these individuals (at CLUE I and II baseline).–[2]Case-control pairs (41) contributed a premenopausal blood sample at CLUE I and a postmenopausal sample at CLUE II.–[3]Matching factor.–[4]Data on education were missing for 1 premenopausal control and 1 postmenopausal control; data on oral contraceptive use were missing for 1 postmenopausal case and 1 postmenopausal control.–[5]Oral contraceptive use was assessed as current or not current at CLUE I baseline (1974) and as never, former or current at CLUE II baseline (1989); BMI collected only in 1989 (CLUE II).

TABLE II – IGF-I AND IGFBP-3 IN RELATION TO AGE FOR POSTMENOPAUSAL BREAST CANCER CASES AND MATCHED CONTROLS, WASHINGTON COUNTY, MD, 1974 (CLUE I) AND 1989 (CLUE II)

Age at blood draw (years)	Total observations		IGF-I (ng/ml)		IGFBP-3 (ng/ml)	
	Premenopausal[1,2] *n* (%)	Postmenopausal[1] *n* (%)	Premenopausal mean (SD)	Postmenopausal mean (SD)	Premenopausal mean (SD)	Postmenopausal mean (SD)
20–29	19 (5.4)		300.5 (68.2)		2086.1 (219.3)	
30–39	104 (29.7)		251.8 (83.2)		2034.1 (321.5)	
40–49	194 (55.4)	7 (3.8)	196.1 (70.5)	106.2 (42.8)	1952.8 (294.6)	1740.3 (225.6)
50–59	33 (9.4)	74 (40.7)	201.4 (81.7)	175.2 (74.1)	2097.1 (392.3)	2062.2 (330.8)
60–69		89 (48.9)		169.0 (70.4)		2049.1 (381.8)
70–79		12 (6.6)		146.1 (88.0)		1892.6 (511.1)
p-value for trend[3]			<0.01	0.74	0.05	0.54

[1]Refers to menopausal status at blood draw (all cases are postmenopausal at breast cancer diagnosis).–[2]Premenopausal observations include 23 pairs of observations for the 23 cases and 23 controls who participated in both CLUE I (1974) and CLUE II (1989).–[3]*p*-values were obtained from linear regression models using the natural logarithm of IGF-I or IGFBP-3 as the dependent variable and age at blood draw (continuous) as the dependent variable; for premenopausal women, the linear regression models were corrected for the repeated measures obtained for 23 of the 152 case-control pairs.

tributed 266 pairs of blood samples, as described earlier. All cases but 1 were White, reflective of the underlying Washington County population at the time of the CLUE campaigns. No differences between cases and controls were observed for education, smoking or oral contraceptive use in CLUE I or CLUE II. Postmenopausal breast cancer was associated with increased BMI only in those women who were postmenopausal at baseline.

Circulating levels of IGF-I and IGFBP-3 were positively correlated in both the CLUE I serum samples ($r = 0.57$, $p < 0.0001$) and the CLUE II plasma samples ($r = 0.59$, $p < 0.0001$). Both IGF-1 and IGFBP-3 tended to decrease with age at blood draw, although the trends were much stronger among women who were premenopausal at blood draw (IGF-I: $p < 0.01$; IGFBP-3: $p = 0.05$) than women who were postmenopausal at blood draw (IGF-I: $p = 0.74$; IGFBP-3: $p = 0.54$) (Table II). Levels of IGF-I and IGFBP-3 were not associated with other baseline characteristics (data not shown).

Results for IGF-I and IGFBP-3 in relation to postmenopausal breast cancer, stratified by menopausal status at blood draw, are presented in Table III. Overall, the highest *vs.* lowest third of IGF-I was associated with an approximate 60% increase in risk among women who were premenopausal at blood draw (OR = 1.60, 95% CI = 0.85, 3.02) and postmenopausal at blood draw (OR = 1.55, 95% CI = 0.61, 3.94), after adjustment for IGFBP-3 (Table III). Results did not substantially change after exclusion of perimenopausal cases diagnosed at ages 50–55 or cases of ductal carcinoma *in situ* (data not shown). When main analyses were repeated using the ratio of IGF-I to IGFBP-3 levels, similar results were observed for the IGF-I/ IGFBP-3 ratio and postmenopausal breast cancer as for IGF-I levels adjusted for IGFBP-3 (data not shown).

In analyses stratified by age at blood draw, there was a trend in postmenopausal breast cancer risk with increasing levels of IGF-I measured prior to menopause in the women 25–35 years old (p for trend = 0.01). Young women in the in the highest third of IGF-I had postmenopausal breast cancer risk 5 times greater than women in the lowest third (OR = 5.31, 95% CI = 0.85–33.13) (Table IV). IGFBP-3 levels were inversely related to postmenopausal

TABLE III – CIRCULATING IGF-I, IGFBP-3 AND SUBSEQUENT POSTMENOPAUSAL BREAST CANCER RISK BY MENOPAUSAL STATUS AT BLOOD DRAW, WASHINGTON COUNTY, MD

Thirds of IGF-I and IGFBP-3	Premenopausal at blood draw (175 case samples/175 control samples)				Thirds of IGF-I and IGFBP-3	Postmenopausal at blood draw (91 cases/91 controls)			
	Cases	Controls	Unadjusted[1] OR (95% CI)[2]	Adjusted[1] OR (95% CI)		Cases	Controls	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
IGF-I (ng/ml)					IGF-I (ng/ml)				
49.36–170.20	51	58	1.00	1.00	45.72–117.43	21	30	1.00	1.00
170.21–242.87	58	59	1.14 (0.67–1.96)	1.18 (0.68–2.07)	117.44–184.22	33	31	1.42 (0.69–2.94)	1.31 (0.59–2.93)
242.88–447.90	66	58	1.36 (0.77–2.39)	1.60 (0.85–3.02)	184.23–482.82	37	30	1.73 (0.82–3.64)	1.55 (0.61–3.94)
p trend			0.32	0.30				0.13	0.48
IGFBP-3 (ng/ml)					IGFBP-3 (ng/ml)				
961.12–1882.03	60	58	1.00	1.00	1240.58–1809.72	21	30	1.00	1.00
1882.04–2123.47	63	59	1.03 (0.59–1.77)	0.94 (0.53–1.66)	1809.73–2163.07	36	31	1.63 (0.77–3.45)	1.40 (0.62–3.16)
2123.48–3074.54	52	58	0.85 (0.48–1.54)	0.69 (0.36–1.34)	2163.08–3197.57	34	30	1.51 (0.75–3.06)	1.17 (0.48–2.84)
p trend			0.82	0.73				0.11	0.36

[1]Both "adjusted" and "unadjusted" models are adjusted for matching factors: age, ethnic group and date of blood draw; in the "adjusted" model, IGF-I and IGFBP-3 are adjusted for each other.

breast cancer risk among this group (Table IV). Similar risks were not observed for women who were 36 years and older at blood draw (p interaction = 0.07). The risk of postmenopausal breast cancer associated with IGF-I levels measured prior to age 36 increased in magnitude after adjustment for latency period using residual analysis (middle *vs*. lower third of IGF-I: OR = 3.50, 95% CI = 0.57–21.43; upper *vs*. lower third of IGF-I: OR = 5.70, 95% CI = 1.01–32.32, p for trend = 0.01), as did the inverse association between IGFBP-3 and postmenopausal breast cancer (upper *vs*. lower third of IGFBP-3: OR = 0.34, 95% CI = 0.08–1.45, p for trend = 0.14).

Results are similarly presented for postmenopausal levels of IGF-I and IGFBP-3 in relation to postmenopausal breast cancer in Table V. There was no association observed between high postmenopausal levels of IGF-I and subsequent breast cancer among women aged 46–60 years at blood draw, with or without adjustment for IGFBP-3 (Table V). Among women who were 61–74 years at blood draw, an increased risk of breast cancer was observed for the highest *vs*. lowest thirds of IGF-I (OR = 3.41, 95% CI = 0.66–17.71), while no association was observed for the highest *vs*. lowest thirds of IGFBP-3 (OR = 0.98, 95% CI = 0.24–3.97) (p interaction = 0.92).

Discussion

The present study was the first designed to examine the association of IGF-I measured premenopausally and postmenopausal breast cancer risk. In analyses that did not consider age at blood draw, IGF-I levels measured premenopausally were not significantly associated with increased postmenopausal breast cancer risk. However, analyses also considering age at blood draw suggest that this association may depend on age. While the age-stratified analyses reported here should be considered exploratory given small sample size, there were indications that increased levels of premenopausal IGF-I at young ages may be associated with risk of subsequent postmenopausal breast cancer.

Four previous studies have commented briefly on the relationship between premenopausal IGF-I levels and postmenopausal breast cancer.[15,17–19] Krajcik *et al*. stated that they observed no increased risk of postmenopausal breast among 31 pairs who were aged 50 years or younger, presumably premenopausal at blood draw.[17] Similarly, Toniolo *et al*. reported no association between IGF-I and breast cancer among an unstated number of cases who were <50 years at blood draw and ≥50 years at diagnosis.[19] Hankinson *et al*. stated that they were limited by sample size to look at this group, and that an association could not be ruled out.[15] Only 1 study presented an OR associated with presumably premenopausal IGF-I levels in relation to postmenopausal breast cancer: Muti *et al*. observed a 15-fold risk of breast cancer associated with higher levels of IGF-I among 33 cases who were premenopausal at blood draw and diagnosed after the age of 48 years (OR = 15.43, 95% CI = 3.25–73.19).[18] Although we did not find an increased risk of similar magnitude, the data were consistent with stronger associations between IGF-I and postmenopausal breast cancer among younger women. None of the previous studies stratified postmenopausal women, premenopausal at blood draw, by age at blood draw.

As mentioned earlier, it is possible that the association between IGF-I measured at young ages and postmenopausal breast cancer may be a chance occurrence in this data set, since the sample sizes within age strata were fairly small. However, the observed association does not appear to be a result of confounding, since adjustment for known breast cancer risk factors did not change effect estimates appreciably. Selection and information bias also are unlikely, given the nested study design. Cohort participants who were younger at blood draw may have been more likely to be lost to follow-up, although it is unlikely that this loss would be differential by either IGF-I levels or breast cancer diagnosis decades later.

TABLE IV – CIRCULATING IGF-I AND IGFBP-3 MEASURED PRIOR TO MENOPAUSE AND SUBSEQUENT POSTMENOPAUSAL BREAST CANCER RISK BY AGE AT BLOOD DRAW, WASHINGTON COUNTY, MD

Thirds of IGF-I and IGFBP-3	Age at blood draw: 25–35 years (37 cases/37 controls)				Age at blood draw: 36–50 years (138 cases/138 controls)			
	Cases	Controls	Unadjusted[1] OR (95% CI)[2]	Adjusted[1] OR (95% CI)	Cases	Controls	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
IGF-I (ng/ml)								
49.36–170.20	3	8	1.00	1.00	48	50	1.00	1.00
170.21–242.87	11	10	3.13 (0.61–16.02)	2.88 (0.54–15.50)	47	49	1.00 (0.56–1.79)	1.02 (0.57–1.85)
242.88–447.90	23	19	4.07 (0.75–22.24)	5.31 (0.85–13.13)	43	29	1.16 (0.62–2.16)	1.23 (0.61–2.48)
p trend			0.02	0.01			0.99	0.76
IGFBP-3 (ng/ml)								
961.12–1882.03	8	7	1.00	1.00	52	51	1.00	1.00
1882.04–2123.47	18	13	1.49 (0.31–7.23)	1.04 (0.19–5.73)	45	46	0.96 (0.54–1.70)	0.93 (0.52–1.67)
2123.48–3074.54	11	17	0.58 (0.14–2.38)	0.37 (0.08–1.78)	41	41	0.98 (0.53–1.82)	0.88 (0.44–1.76)
p trend			0.71	0.14			0.65	0.59

[1]Both "adjusted" and "unadjusted" models are adjusted for matching factors: age, ethnic group and date of blood draw; in the "adjusted" model, IGF-I and IGFBP-3 are adjusted for each other.

TABLE V – CIRCULATING IGF-I AND IGFBP-3 MEASURED AFTER MENOPAUSE AND SUBSEQUENT POSTMENOPAUSAL BREAST CANCER RISK BY AGE AT BLOOD DRAW, WASHINGTON COUNTY, MD

Thirds of IGF-I and IGFBP-3	Age at blood draw:46–60 years (51 cases/51 controls)				Age at blood draw: 61–74 years (40 cases/40 controls)			
	Cases	Controls	Unadjusted[1] OR (95% CI)	Adjusted[1] OR (95% CI)	Cases	Controls	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
IGF-I (ng/ml)								
45.72–117.43	15	15	1.00	1.00	6	15	1.00	1.00
117.44–184.22	16	18	0.85 (0.32–2.27)	0.65 (0.19–2.20)	17	13	3.09 (0.91–10.45)	3.02 (0.84–10.93)
184.23–482.82	20	18	1.18 (0.44–3.17)	0.95 (0.29–3.12)	17	12	3.45 (0.98–12.12)	3.41 (0.66–17.71)
p trend			0.60	0.91			0.10	0.34
IGFBP-3 (ng/ml)								
1240.58–1809.72	12	15	1.00	1.00	9	15	1.00	1.00
1809.73–2163.07	21	19	1.45 (0.50–4.22)	1.67 (0.52–5.40)	15	12	1.79 (0.62–5.22)	1.12 (0.31–4.01)
2163.08–3197.57	18	17	1.32 (0.49–3.59)	1.62 (0.44–6.02)	16	13	1.71 (0.63–4.70)	0.98 (0.24–4.70)
p trend			0.44	0.56			0.14	0.54

[1]Both "adjusted" and "unadjusted" models are adjusted for matching factors: age, ethnic group and date of blood draw; in the "adjusted" model, IGF-I and IGFBP-3 are adjusted for each other.

Given that results from our study, the first to compare IGF-I measured earlier in life with postmenopausal breast cancer risk, did find an indication of increased risk, replication of this analysis is warranted in other samples.

If the association is confirmed in other studies, further thought must be given to the underlying mechanism. Elevated exposure to IGF-I at younger ages may be of particular etiological importance, perhaps because of concurrent physiologic events specific to that point in the life course. For example, estrogen levels are greater at younger ages, and some evidence suggests a joint effect of IGF-I and estrogen on breast cancer risk.[8,26–28] Alternatively, an association with IGF-I levels at young ages may be a function of a long latency period, since age at blood draw was inversely associated with time between blood draw and diagnosis in our study (*i.e.*, women who were younger at blood draw tended to have longer times between blood draw and diagnosis). However, when residual analysis was conducted to separate the effects of age at exposure and latency period in our study population, no appreciable changes in risk estimates for higher levels of IGF-I and postmenopausal breast cancer were observed among women who were younger than 36 years at blood draw. Thus, the observed association cannot be entirely attributed to latency.

Postmenopausal IGF-I levels were also associated with breast cancer risk in our study population. However, this effect may also potentially be modified by age, since the association persisted only in those women older than 60 years at blood draw. Five of 6 previous prospective studies did not observe associations between postmenopausal IGF-I and breast cancer,[15,17–19,23] but none of these studies focused on women above age 60. Additionally, the 6th prospective study observed an association between postmenopausal IGF-I levels and breast cancer among postmenopausal women older than 55 at blood draw,[16] 5 years older than the age cutoff of 50 used in the other studies. Age-related endogenous or exogenous factors may affect the relationship between IGF-I and breast cancer risk differently throughout the postmenopausal period, such as use of hormone replacement therapy.[16] Alternatively, these results could also be due to chance, given the small samples sizes in the age-stratified analyses.

The observation that the relationship between IGF-I levels and postmenopausal breast cancer risk depends on age suggests that IGF-I levels do not track over long periods of time within individuals. Previous studies have demonstrated concordance in repeated measures of IGF-I within individuals over time, but the time periods investigated were relatively short (1–5 years).[29,30] In our data on the 128 women (64 cases, 64 controls) who donated blood in both 1974 and 1989, IGF-I levels decreased over time in the majority of women ($n = 104$ or 81%), as expected. When we explored changes in women's IGF level rank over the 15-year time period, IGF-I levels in 1974 were only mildly correlated with IGF-I levels in 1989 (Spearman's correlation coefficient = 0.48), and less than half ($n = 51$ or 40%) of women were categorized in the same 4th of IGF-I at both time points (data not shown). However, change in rank over time was not associated with case-control status, suggesting that the measurement of IGF-I levels at older ages as a surrogate marker of IGF-I levels at younger ages may be subject to nondifferential misclassification and result in breast cancer risk estimates that are biased toward the null.

The data from our study support the notion that circulating levels of IGF-I, particularly during earlier life, may be important for breast carcinogenesis. However, we emphasize that the initial evidence provided here should be interpreted cautiously and needs replication in future studies. The strong correlation be-

tween IGF-I levels and age, the potential influence of age-related internal and external factors on IGF-GH axis activity and age-stratified findings from this and previous studies suggest the need for future studies examining the effects of age at IGF-I measurement on the observed associations between IGF-I levels and postmenopausal breast cancer. Although the CLUE cohort provided a unique opportunity to investigate this hypothesis, larger samples, such as that being assembled through the Endogenous Hormones Breast Cancer Collaborative Group,[31] should be able to provide additional epidemiologic evidence on what could be a potentially important and modifiable breast cancer risk factor.

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