Circulating Levels of Insulin-like Growth Factors, their Binding Proteins, and Breast Cancer Risk

Eva S. Schernhammer,[1,2] Jeff M. Holly,[3] Michael N. Pollak,[4] and Susan E. Hankinson[1,5]

[1]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; [2]Ludwig Boltzmann Institute for Applied Cancer Research, KFJ-Spital, Vienna, Austria; [3]Department of Surgery, Bristol Royal Infirmary, Bristol, United Kingdom; [4]Departments of Medicine and Oncology, McGill University, Montreal, Quebec, Canada; and [5]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

Abstract

Background: **Earlier data support the hypothesis that the relation between circulating insulin-like growth factor-I (IGF-I) levels and breast cancer risk differs by menopausal status. The strong association of IGF-I with height in childhood and weak or no association between adult levels and adult height also suggest that IGF levels in young women may better reflect an exposure time period of importance to breast cancer. Few studies have assessed IGF binding protein-1 (IGFBP-1) or free IGF and breast cancer risk.**

Materials and Methods: **We conducted a large case-control study nested within the prospective Nurses' Health Study. Plasma concentrations of IGF-I, free IGF, IGFBP-3, and IGFBP-1 were measured in blood samples collected in 1989 to 1990. Eight hundred women were identified who had a diagnosis of invasive or *in situ* breast cancer after blood collection, up to 1998, 27% of whom were premenopausal at blood collection. To those 800 women, one to two controls were age-matched for a total of 1,129 controls. We used logistic regression models to estimate the relative risk (RR) of breast cancer associated with IGF levels.**

Findings: **Among postmenopausal women, neither IGF-I, IGFBP-3, IGFBP-1, nor free IGF was associated with breast cancer risk [RRs, top versus bottom quintile: IGF-I, 1.0; 95% confidence interval (95% CI), 0.7-1.4; IGFBP-3, 0.8; 95% CI, 0.6-1.1; IGFBP-1, 0.9; 95% CI, 0.6-1.5; and free IGF, 1.0; 95% CI, 0.6-1.4]. Among premenopausal women, IGFBP-3, IGFBP-1, and free IGF similarly were not associated with breast cancer risk (RRs, top versus bottom quintile: IGFBP-3, 1.2; 95% CI, 0.8-2.3; IGFBP-1, 1.5; 95% CI, 0.8-3.0; and free IGF, 1.1; 95% CI, 0.7-2.1). Higher IGF-I plasma levels, however, were associated with a modestly elevated breast cancer risk (RR, 1.6; 95% CI, 1.0-2.6) among the premenopausal women, with a stronger association among premenopausal women ages ≤50 (RR, 2.5; 95% CI, 1.4-4.3); further adjustment for IGFBP-3 did not greatly change these estimates.**

Interpretation: **Circulating IGF-I levels seem to be modestly associated with breast cancer risk among premenopausal women, but not among postmenopausal women. IGFBP-3, IGFBP-1, and free IGF are not associated with breast cancer risk in either premenopausal or postmenopausal women in this cohort.**

Introduction

Factors that have the potential to either prevent cell death or enhance the survival of abnormal cells influence a cell's fate. Such control over cells has been ascribed to several members of the insulin-like growth factor (IGF) family (1). IGF-I, for example, is a peptide hormone that is involved in controlling proliferation and differentiation. Although most of the IGF present in circulation is protein bound, a small fraction of IGF-I is ''free''; this component may be more bioavailable, but assays specific for free ligand are controversial. The IGF binding proteins IGFBP-3 and IGFBP-1 both affect IGF-I bioavailability and, in addition, seem to exert independent effects on the growth control of malignant cells (2) as part of a comprehensive regulation system of cell survival and death.

Although circulating levels of IGF-I generally have not been associated with breast cancer risk among postmenopausal women, a positive association was observed in premenopausal women (3). However, results from observational studies have not been consistent (4, 5) and considerable uncertainty remains regarding the true association between IGF-I and premenopausal breast cancer risk. Furthermore, the association between IGFBP-3 and breast cancer risk is also inconsistent. In part, these inconsistencies may be attributed to technical variation in performance of assays for IGFs, particularly IGFBP-3, the primary IGF-I binding protein. Other potential explanations for inconsistencies in results include differing blood sampling and storage methods, different definitions of cancer ''cases,'' differences in age at blood sampling, and the possibility of differences between populations in factors that may influence the IGF-I risk relation.

We studied the potential of IGF-I and its binding proteins as biomarkers for breast cancer by menopausal status. Utilizing a nested case-control design within the large, prospective Nurses' Health Study cohort, we investigated associations between various plasma IGF levels and breast cancer risk. In the current study, we update our previous analyses (6), with now double the number of cases, and for the first time report on the associations of IGFBP-3 (as assayed by both ELISA and RIA), IGFBP-1, and free IGF-I levels with breast cancer risk.

Grant support: National Cancer Institute (NCI) grants CA49449 and CA87969, NCI Specialized Program of Research Excellence grant in breast cancer at the Channing Laboratory, and Streams of Excellence Program of the Canadian Breast Cancer Initiative (M.N. Pollak).

Materials and Methods

Study Population. In 1976, 121,700 female registered nurses from 11 large U.S. states, ages 30 to 55, and of primarily Caucasian descent were enrolled in the Nurses' Health Study. Since baseline, they have completed biennial mailed questionnaires that comprise items about their health status, medical history, and known or suspected risk factors for cancer (7) and

heart disease (8). Every 2 years, follow-up questionnaires have been sent to cohort members to update the information on potential risk factors and to identify new diagnoses of cancer and other major medical events. Between 1989 and 1990, blood samples were collected from 32,826 women. Further details of the Nurses' Health Study blood collection methods have been previously published (8-10). Follow-up of this subcohort was 98.5% as of 1998.

Samples were not available for 47 cases and controls because of either low plasma volume or assay problems; thus, there were 800 incident breast cancer cases and 1,129 matched controls available for these analyses. Cases in this analysis are women with no cancer diagnosis (with the exception of nonmelanoma skin cancer) before blood collection and for whom breast cancer was reported any time after blood collection up to June 1, 1996, for postmenopausal women, and up to June 1, 1998, for premenopausal women. Pathology records were obtained for 99.8% of the cases. In total, 671 were pathologically confirmed invasive breast cancers and 129 were *in situ* cancers. Although these 800 cases included two women whose pathology reports had not yet been obtained, we based our analyses on the total because the accuracy of self-reported breast cancer in this cohort is extremely high (99%; ref. 11). To each case, we matched one to two controls on their year of birth, menopausal status (postmenopausal versus not), recent postmenopausal hormone use defined as use within 3 months of blood collection versus not (because these hormones tend to lower IGF-I concentrations; ref. 12), month and time of day of blood collection, and fasting status at blood draw (≥10 hours since a meal versus not). Blood samples were randomly collected; however, previous reports (13-15) suggest that circulating IGF levels do not range substantially by phase of menstrual cycle. We defined menopausal status at the time of blood collection in our main analyses. In subanalyses, we also stratified by menopausal status defined at the time of diagnosis. A woman was defined as premenopausal if she had had at least one natural menstrual cycle in the previous 12 months or was younger than 48 years (if she was a nonsmoker) or younger than 46 years (if a current smoker) after hysterectomy without bilateral oophorectomy—at these ages, <10% of the cohort had had a natural menopause. A woman was defined as postmenopausal if she reported a natural menopause or bilateral oophorectomy or if she was at least 56 years old (if a nonsmoker) or 54 years (if a current smoker) after hysterectomy without bilateral oophorectomy—by these ages, natural menopause had occurred in 90% of the cohort. For each of the 237 postmenopausal cases in the study who were not on hormone-replacement therapy at blood collection, a second control also was selected.

The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

The data set we used in this study is a combination of the 397 invasive breast cancer cases and their 620 controls that we have previously described in detail (6) and a new set of cases that were diagnosed with breast cancer more recently (after June 1, 1994, to May 31, 1996, for postmenopausal women and after June 1, 1994, to May 31, 1998, for premenopausal women) and their age-matched controls. Specifically, we have added 330 breast cancer cases (both *in situ* and invasive) that were diagnosed between 1994 and 1998 and their 427 controls. In addition, we include here 73 cases with *in situ* breast cancer diagnosed from 1990 to 1994 that were not included in our earlier publication (6). In sum, a total of 800 cases and 1,129 controls (470 cases diagnosed between 1990 and 1994 and 330 cases diagnosed between 1994 and 1998) formed the study population for the current analyses. This represents an addition of 2 more years of postmenopausal cases (261 additional cases) and 4 more years of premenopausal cases (142 additional cases), compared with our previous data set (6).

IGF Measurements. IGF-I, free IGF, and IGFBP-1 levels were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX).

Because of inconsistent findings for the association between IGFBP-3 and breast cancer risk from the literature, and the possibility that these resulted at least in part from differences in the type of assay used, we measured IGFBP-3 levels using both an ELISA (Diagnostic Systems Laboratory) and an RIA (J.M. Holly). IGFBP-3 levels via RIA are presented as the primary IGFBP-3 measure in this study (unless explicitly noted otherwise) as RIA has been more extensively used and validated for these assays. The ELISA assays were conducted in three different batches: (*a*) 1990 to 1994 cases and controls (as previously described; ref. 6); (*b*) 1994 to 1996 premenopausal cases and controls; (*c*) 1994 to 1996 postmenopausal and 1996 to 1998 premenopausal cases and controls. IGFBP-3 results for the 1994 to 1996 premenopausal data set were markedly different from all other batches in terms of both absolute value and distribution; these changes may have arisen because of a change in the IGFBP-3 standards used in the assays produced by Diagnostic Systems. Therefore, IGFBP-3 ELISA values from the 1996 premenopausal data set are not included here. In comparisons between the two IGFBP-3 assays, all data from the RIA are used (i.e., including the 1994-1996 premenopausal data set) because exclusion of this batch did not alter the differences observed between assay methods.

All samples were masked as to case or control status. Masked split specimens included within each batch were used to calculate the coefficient of variation within batches; for IGF-I and IGFBP-3, coefficients of variation ranged from 4% to 9% and for free IGF and IGFBP-1 coefficients of variation ranged from 6% to 13%.

Whereas all of the women included in this study had IGF-I values, a few (n = 5) had ELISA IGFBP-3 values, but not RIA IGFBP-3 values, because of insufficient plasma volumes. For those women, we created an indicator variable for missing IGFBP-3 values and retained them in the analyses. In addition, because of assay cost considerations, only the 1,410 women (1990-1996 set) who had provided fasting blood samples (441 cases and 631 controls) had IGFBP-1 levels assayed and only 1,646 women (677 cases and 969 controls, 1990-1996) had free IGF levels; similarly, we created indicator variables for missing data on free IGF and IGFBP-1 and kept all study subjects in the analyses.

Quintiles of IGF levels were defined based on the IGF levels of all controls for postmenopausal women, and tertiles were defined for premenopausal women based on IGF levels of all premenopausal controls.

Statistical Analyses. To test for differences in hormone levels between case and control subjects, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and for any residual correlation between case and control subjects within the matched set (16). To estimate the relative risks (RR, odds ratios) and 95% confidence intervals (95% CI), we used unconditional logistic regression models adjusted for the matching factors. We controlled for age in 5-year age groups among postmenopausal women and in tighter age categories among the premenopausal women (≤46, 47-48, 49-50, and >50 years). When we additionally adjusted for other breast cancer risk factors [age at menarche (<12, 12, 13, and 14+ years), parity (0, 1-2, 3-4, and >4 births), age at birth of first child (0, <25, 25-29, and 30+ years), family history of breast cancer, body mass index (<21, 21-22.9, 23-24.9, 25-28.9, and 29+ kg/m^2), age at menopause (continuous), and postmenopausal hormone use (never, past, and current user)], the RRs were essentially unchanged; thus, estimates adjusted only for the matching factors are presented in this study. Because of several broken

pairs due to low plasma volume for some cases or controls, unconditional logistic regression allowed us to retain all study subjects in the analyses thereby increasing power, which was particularly relevant for the analyses among premenopausal women. However, the results of the conditional analyses were essentially identical to those from the unconditional analyses. We conducted tests for trends by calculating the Wald statistics for a continuous distribution of IGF values. We used the square root of IGF-I because of its skewed distribution.

Results

Table 1 shows baseline characteristics of the 800 cases and 1,129 controls by menopausal status. The mean time between blood collection and diagnosis was 46 months (SD 25) with a range of 1 to 106 months.

Among all controls, IGF-I and IGFBP-3 were strongly and positively correlated with each other ($r = 0.57$, $P < 0.001$), as were IGF-I and free IGF ($r = 0.44$, $P < 0.001$), whereas IGFBP-1 was inversely correlated with free IGF ($r = -0.41$, $P < 0.001$). The two different measures of IGFBP-3 (RIA and ELISA) were also reasonably well correlated ($r = 0.73$, $P < 0.001$, Table 2). IGF-I levels and age were significantly correlated ($r = -0.33$, $P < 0.001$).

Neither IGF-I nor IGFBP-3, IGFBP-1, or free IGF levels varied significantly between cases and controls as a whole or among premenopausal or postmenopausal women separately (Table 3). Among premenopausal women ages <50, however, there was a significant difference in plasma IGF-I levels between cases and controls [median IGF-I plasma levels, 187 ng/mL (cases) versus 176 ng/mL (controls), $P = 0.01$; Table 3].

When evaluating the entire study population, circulating IGF-I levels were not associated with breast cancer risk, after adjustment for matching factors and IGFBP-3 levels (IGF-I, RR, top versus bottom quintile, 1.1; 95% CI, 0.8-1.5); additional adjustment for breast cancer risk factors, such as body mass index and family history of breast cancer, also did not alter these estimates (data not shown). The RRs remained essentially unchanged after the exclusion of *in situ* breast cancer cases (1.1 top versus bottom quintile; 95% CI, 0.8-1.6) and they were very similar for postmenopausal women (Table 4) even after exclusion of current or recent users of postmenopausal hormones (data not shown). Furthermore, the exclusion of women with extreme IGF-I values (i.e., below 50 ng/mL, $n = 14$) also did not alter these estimates (data not shown). These risks also remained essentially unchanged when we stratified on body mass index: heavier women (with a body mass index of ≥25) had only slightly increased RRs (top versus bottom quintile, 1.3; 95% CI, 0.8-2.1), when compared with the RRs of leaner women with a body mass index of <25 (RR, top versus bottom quintile, 1.0; 95% CI, 0.6-1.5). Neither IGFBP-3 (assayed by either method), IGFBP-1, nor free IGF were associated with breast cancer risk in these postmenopausal women. Analyses conducted among invasive breast cancer cases only were essentially unchanged.

We observed a positive association between circulating IGF-I levels and breast cancer risk among premenopausal women: higher circulating IGF-I levels were associated with a higher breast cancer risk (1.6 top versus bottom tertile; 95% CI, 1.0-2.5; Table 5), and this association was strongest among women who were both premenopausal and younger than age 50 at blood collection (2.5 top versus bottom tertile; 95% CI, 1.4-4.5, $P_{trend} = 0.01$). The positive association was driven largely by our earlier findings; in the more recent follow-up period (1994-1998), the relationship was weakly positive with RRs of 1.2 (premenopausal) and 1.4 (premenopausal ages <50) for the top versus bottom tertile of IGF-I levels. In subanalyses, when we restricted our premenopausal set of women to those who were not only premenopausal at blood collection but also premenopausal at the date of their breast cancer diagnosis, these associations became even stronger (2.2 top versus bottom tertile; 95% CI, 1.1-4.4; premenopausal and age <50 both at blood collection and time of diagnosis: 3.8 top versus bottom tertile; 95% CI 1.7-8.3, all without adjustment for IGFBP-3).

Further adjustment for IGFBP-3 (RIA) levels left these risks essentially unchanged and they remained statistically significant. When controlling for ELISA IGFBP-3, however, the RRs became slightly stronger (Table 5).

Overall, the exclusion of *in situ* breast cancer cases only slightly increased RRs (premenopausal, RR, 1.8 top versus bottom tertile; 95% CI, 1.0-3.1; premenopausal and age <50: RR, 3.3; 95% CI, 1.6-6.8, both after adjustment for IGFBP-3). Similarly, the RR of breast cancer among premenopausal women who were diagnosed with breast cancer between 1990 and 1994 (the data set we used for our previous publication; ref. 17) was slightly higher (RR, 2.9; 95% CI, 1.2-6.9) without *in situ* cases, compared with the combined data set, including both invasive and *in situ* breast cancer cases (RR, 2.4; 95% CI, 1.1-5.2, all after adjustment for ELISA IGFBP-3).

Because we had two measures of IGFBP-3, assessed by both ELISA and RIA assays, we compared the RRs obtained with either measure and they varied slightly (Tables 4 and 5). The association between plasma IGFBP-3 levels and breast cancer risk, by both methods, was overall null (all women combined, 0.8 top versus bottom quintile; 95% CI, 0.6-1.1, after adjustment for IGF-I). These associations were similar among postmenopausal women (Table 4). Among premenopausal women, IGFBP-3 by RIA was positively associated with breast cancer; this was slightly stronger in the subset of women who were also <50 years of age (1.4; 95% CI, 0.8-2.3; Table 5). Further

Table 1. Baseline characteristics

	Cases (n = 800)	Controls (n = 1,129)
Postmenopausal women		
Age (y)	60.5 (5.2)	61.0 (5.1)
Age at menopause (y)*	48.9 (4.9)	49.1 (4.7)
Age at menarche (y)	12.4 (1.9)	12.5 (1.8)
Parity†	3.4 (1.6)	3.6 (1.7)
Family history of breast cancer (%)	16.9	11.9
Premenopausal women		
Age (y)	48.5 (3.2)	48.5 (3.2)
Age at menarche (y)	12.4 (2.4)	12.4 (1.5)
Parity†	2.9 (1.3)	2.7 (1.2)
Family history of breast cancer (%)	12.8	8.5

NOTE: Data are mean (SD).
*Among parous women only.
† At natural menopause or bilateral oophorectomy.

Table 2. Correlation matrix of IGFs, all women combined (controls only)

	Spearman correlation coefficient (r) between the continuous measures of various IGFs*				
	IGF-I	IGFBP-3 (ELISA)	IGFBP-3 (RIA)	IGFBP-1	Free IGF
IGF-I	1.0	0.46	0.57	−0.11	0.44
IGFBP-3 (ELISA)		1.0	0.73	0.20	0.09
IGFBP-3 (RIA)			1.0	0.02	0.13
IGFBP-1				1.0	−0.42
Free IGF					1.0

*All P values <0.01, except for correlation between IGFBP-3 (RIA) and IGFBP-1 ($P = 0.58$).

Table 3. Plasma IGF-I, IGFBP-3 (RIA), IGFBP-1, and free IGF concentrations by case or control status

	Cases		Controls		P*
	n	Median (range†)	n	Median (range†)	
IGF-I					
All cases or controls	800	150 (92.6-241)	1,129	161 (95.3-236)	0.46
Postmenopausal	514	137 (85.7-230)	754	144 (89.7-231)	0.74
Premenopausal	218	182 (126-260)	281	174 (122-248)	0.06
Premenopausal, age ≤50	155	187 (135-264)	193	176 (128-253)	0.01
IGFBP-3 (RIA)					
All cases or controls	798	4,506 (3,262-6,044)	1,129	4,544 (3,302-5,901)	0.77
Postmenopausal	512	4,457 (3,216-6,062)	751	4,557 (3,222-5,911)	0.51
Premenopausal	218	4,592 (3,437-5,913)	281	4,533 (3,458-5,996)	0.71
Premenopausal, age ≤50	155	4,623 (3,547-5,760)	193	4,508 (3,478-5,738)	0.30
IGFBP-1					
All cases or controls	441	40.8 (8.63-106)	631	39.7 (9.06-110)	0.69
Postmenopausal	318	42.8 (9.62-119)	451	41.0 (10.5-116)	0.25
Premenopausal	98	32.5 (7.22-92.0)	136	28.8 (5.73-78.8)	0.37
Premenopausal, age ≤50	72	38.5 (7.42-92.6)	99	33.1 (5.73-83.0)	0.54
Free IGF					
All cases or controls	677	1.12 (0.50-1.88)	969	1.11 (0.54-1.96	0.86
Postmenopausal	473	1.03 (0.45-1.78)	684	1.08 (0.50-1.90)	0.71
Premenopausal	155	1.33 (0.71-2.05)	213	1.22 (0.70-2.06)	0.46
Premenopausal, age ≤50	109	1.29 (0.71-2.07)	148	1.19 (0.67-2.10)	0.39

*P values for comparison of mean natural IGF plasma levels between cases and controls, based on mixed-effects regression models with adjustment for the matching variables.

† Range of plasma values, 10th to 90th percentile.

control for IGF-I attenuated these associations. In contrast, the association with IGFBP-3 ELISA and risk tended to be inverse, although not statistically significant and further control for IGF-I increased the magnitude of the association.

Neither IGFBP-1 levels nor free IGF levels (Tables 4 and 5) seemed to be strongly associated with breast cancer risk in our analyses. Because IGFBP-1 was correlated with IGF-I ($r = -0.11$), and free IGF with IGF-I ($r = 0.44$) and IGFBP-1 ($r = -0.42$), we adjusted for those factors in subanalyses; however, the RRs remained essentially unchanged (Tables 4 and 5).

The RRs comparing the top and bottom tertile of plasma IGFs were similar in analyses that excluded the overall 200 women diagnosed in the first 2 years after blood collection. For example, among premenopausal women ages <50 (30 cases excluded), the RR for breast cancer associated with IGF-I levels, after adjustment for IGFBP-3, was 2.6 (top versus bottom tertile; 95% CI, 1.3-5.3).

Discussion

In a matched, nested case-control study of breast cancer, we found a modest but statistically significant association between IGF-I levels and premenopausal breast cancer risk. However, neither free IGF nor the two binding proteins, IGFBP-3 and IGFBP-1, seemed to be associated with breast cancer risk in this cohort. Additionally, we continued to see no association between IGF-I and breast cancer risk among postmenopausal women.

The potent proliferative effects of IGF-I influence both normal and transformed breast epithelial cells (18, 19). In rodents, the overexpression of growth hormone increased the frequency of breast tumors (20), and treatment with growth hormone or IGF-I led to mammary gland hyperplasia in monkeys (18). The expression of IGFBP-3 in many tissues, on the other hand, suggests that it locally modulates the action of

Table 4. RR of breast cancer (invasive and *in situ*) by plasma IGF quintiles, postmenopausal women only, 1990 to 1996, unless noted otherwise

Protein	Q_1	Q_2	Q_3	Q_4	Q_5	P_{trend}
IGF-I						
IGF-I (1990-1994)	1.0	1.2 (0.8-1.8)	0.8 (0.5-1.2)	0.9 (0.6-1.4)	0.8 (0.5-1.3)	0.41
IGF-I (1994-1996)*	1.0	1.4 (0.9-2.4)	1.2 (0.6-2.1)	1.2 (0.7-2.3)	1.2 (0.6-2.4)	0.80
IGF-I	1.0	1.2 (0.9-1.6)	1.2 (0.8-1.7)	0.9 (0.6-1.3)	1.0 (0.7-1.4)	0.59
IGF-I + IGFBP-3 (ELISA)†	1.0	1.4 (1.0-1.9)	1.4 (0.9-2.0)	1.1 (0.7-1.6)	1.3 (0.8-1.9)	0.82
IGF-I + IGFBP-3 (RIA)	1.0	1.3 (0.9-1.8)	1.3 (0.9-1.9)	1.0 (0.6-1.5)	1.2 (0.8-1.5)	0.68
IGFBP-3						
IGFBP-3 (ELISA)†	1.0	1.1 (0.8-1.6)	0.9 (0.6-1.2)	0.7 (0.5-1.1)	1.1 (0.8-1.5)	0.49
IGFBP-3 (ELISA)† + IGF-I	1.0	1.1 (0.7-1.5)	0.8 (0.5-1.2)	0.7 (0.4-1.0)	0.8 (0.5-1.2)	0.56
IGFBP-3 (RIA)	1.0	0.8 (0.6-1.2)	0.9 (0.6-1.3)	0.7 (0.5-1.0)	0.8 (0.6-1.1)	0.54
IGFBP-3 (RIA) + IGF-I	1.0	0.8 (0.5-1.1)	0.9 (0.6-1.3)	0.7 (0.5-1.0)	0.8 (0.5-1.2)	0.72
IGFBP-1						
IGFBP-1	1.0	1.2 (0.7-1.9)	1.2 (0.7-1.9)	1.0 (0.6-1.6)	0.9 (0.6-1.5)	0.25
IGFBP-1 + adjustment‡	1.0	1.2 (0.7-1.9)	1.2 (0.7-1.9)	1.0 (0.6-1.6)	0.9 (0.6-1.5)	0.24
Free IGF						
Free IGF	1.0	0.7 (0.5-1.0)	1.1 (0.8-1.6)	0.9 (0.7-1.4)	1.0 (0.6-1.4)	0.72
Free IGF + adjustment§	1.0	0.7 (0.5-1.0)	1.0 (0.7-1.5)	0.9 (0.6-1.3)	0.9 (0.6-1.4)	0.68

NOTE: All RRs are adjusted for the matching variables.

*One hundred seventy-eight cases and 272 controls.

† For ELISA IGFBP-3, batch-specific cut points for quintiles were created (two different laboratory batches: 1990-1994 and 1994-1996).

‡ Relative risks were, in addition to matching variables, further adjusted for plasma IGF-I.

§Relative risks were, in addition to matching variables, further adjusted for plasma IGF-I and IGFBP-1; 336 cases and 482 controls.

Table 5. Relative risk of breast cancer (invasive and *in situ*) by plasma IGF tertiles, premenopausal women only, 1990 to 1998, unless noted otherwise

Protein	Premenopausal women			P_{trend}	Premenopausal women ages <50			P_{trend}
	T_1	T_2	T_3		T_1	T_2	T_3	
IGF-I								
IGF-I (1990-1994)	1.0	1.4 (0.7-2.8)	1.9 (1.0-3.9)	0.08	1.0	2.3 (0.9-5.4)	3.9 (1.7-9.2)	0.01
IGF-I (1994-1998)	1.0	1.1 (0.6-2.0)	1.2 (0.7-2.2)	0.66	1.0	1.0 (0.5-2.2)	1.4 (0.6-3.0)	0.56
IGF-I	1.0	1.2 (0.8-1.9)	1.6 (1.0-2.5)	0.07	1.0	1.4 (0.8-2.4)	2.5 (1.4-4.5)	0.01
IGF-I + IGFBP-3 (ELISA)*	1.0	1.5 (0.9-2.7)	2.0 (1.1-3.8)	0.03	1.0	1.9 (0.9-3.9)	3.6 (1.6-7.7)	0.01
IGF-I + IGFBP-3 (RIA)	1.0	1.2 (0.8-2.0)	1.6 (1.0-2.8)	0.14	1.0	1.4 (0.8-2.6)	2.9 (1.5-5.7)	0.01
IGFBP-3								
IGFBP-3 (ELISA)*	1.0	0.9 (0.5-1.6)	0.8 (0.5-1.4)	0.12	1.0	1.0 (0.5-1.9)	1.0 (0.5-1.8)	0.10
IGFBP-3 (ELISA) + IGF-I†	1.0	0.8 (0.4-1.3)	0.6 (0.4-1.1)	0.38	1.0	0.8 (0.4-1.6)	0.5 (0.3-1.1)	0.70
IGFBP-3 (RIA)	1.0	1.3 (0.9-2.1)	1.2 (0.8-1.9)	0.71	1.0	1.4 (0.8-2.3)	1.4 (0.8-2.4)	0.35
IGFBP-3 (RIA) + IGF-I‡	1.0	1.2 (0.7-1.8)	0.9 (0.5-1.6)	0.41	1.0	1.0 (0.6-1.8)	0.8 (0.4-1.5)	0.32
IGFBP-1								
IGFBP-1	1.0	1.1 (0.6-2.1)	1.5 (0.8-2.8)	0.34	1.0	0.6 (0.3-1.4)	1.2 (0.6-2.5)	0.51
IGFBP-1 + adjustment§	1.0	1.1 (0.5-2.1)	1.6 (0.8-3.0)	0.25	1.0	0.6 (0.3-1.3)	1.4 (0.6-2.9)	0.31
Free IGF								
Free IGF	1.0	1.1 (0.6-1.8)	1.3 (0.8-2.1)	0.46	1.0	1.2 (0.7-2.3)	1.4 (0.8-2.6)	0.42
Free IGF + adjustment‖	1.0	1.0 (0.6-1.7)	1.2 (0.7-2.1)	0.95	1.0	1.0 (0.5-2.0)	1.1 (0.5-2.3)	0.62

NOTE: All RRs are adjusted for the matching variables.
*For ELISA IGFBP-3, batch-specific cut points for tertiles were created (two different laboratory batches: 1990-1994 and 1996-1998; see text for more details).
† Breast cancer cases diagnosed between 1990-1994 and 1996-1998 and their matched controls.
‡ Breast cancer cases diagnosed between 1990-98 and their matched controls.
§Relative risks were, in addition to matching variables, further adjusted for plasma IGF-I.
‖Relative risks were, in addition to matching variables, further adjusted for plasma IGF-I and IGFBP-1.

IGF peptides. Retinoic acid–induced expression of IGFBP-3, for example, inhibits the growth-promoting effects of IGF-I in breast cancer cells (21). IGFBP-3 may also have other, not yet fully understood, physiologic roles. In addition, several groups have reported a positive association between plasma IGF-I levels in premenopausal women and the percent breast density observed on a mammogram (22, 23). Mammographic breast density is strongly and consistently related to breast cancer risk (24). IGFBP-1 is another binding protein of a family of six high-affinity IGF binding proteins (IGFBP 1-6), which regulates IGF actions (1).

To date, six prospective analyses have evaluated associations between IGFs and breast cancer risk (4, 6, 25-28). In four prior nested case-control studies (5, 20-22), a positive association was observed between IGF levels and breast cancer risk in premenopausal women, but no association was observed among postmenopausal women. The largest study to date analyzed data from two large Swedish cohorts and no noteworthy associations were observed between plasma IGF levels and breast cancer risk regardless of menopausal status (4). In this study, 116 cases and 330 controls were premenopausal at blood collection. The most recent prospective analysis was conducted among postmenopausal women only (28) and confirmed previous reports of no association. In the current analysis, among premenopausal women, we observed that, although the overall association between IGF-I and breast cancer risk remained statistically significant, the association with the more recent follow-up was weak and nonsignificant. However, based on the small numbers of subjects in each time period and given that excluding cases diagnosed within the first 2 years after blood collection did not alter substantially our findings, it would seem most likely that the summary RR over the 8 years provides the best estimate of the association. As with most prior studies, we confirm no association between IGF-I and breast cancer risk in postmenopausal women.

Many of the studies also investigated the relation between IGFBP-3 and breast cancer risk. All four prospective studies published (4, 25-27) observed a positive association with breast cancer risk, although the magnitude of the association varied substantially and only one of the estimates was statistically significant. RRs ranged from 1.4 to 5.3 when comparing the top to the bottom category of levels. In postmenopausal women, findings for IGFBP-3 and breast cancer risk have been less consistent with RRs for the top versus bottom category comparison ranging from 0.3 to 1.5 (4, 26-28).

Two of the prospective studies also examined associations of other IGF binding proteins, specifically IGFBP-1, with breast cancer risk (4, 26). Both studies report no association of IGFBP-1 with breast cancer; however, serum IGFBP-1 levels are responsive to insulin, and fasting status of the study subjects—an important requirement for valid assessments of IGFBP-1—was not optimal in either cohort. Three retrospective studies report on associations of IGFBP-1 with breast cancer and they were all conducted under optimal fasting conditions (29-31). Two of them did not observe a notable association, whereas in the third study (31) IGFBP-1 levels were used to predict survival rather than risk of breast cancer.

Whereas IGF-I was modestly associated with breast cancer risk among our premenopausal women, free IGF did not seem to be associated with breast cancer risk in this cohort. The total serum IGF concentration is a measure of a pool of IGF that is potentially available to the tissues (32). In contrast, the interpretation that bound hormone is inactive and free therefore equates active—a reasonable approximation for other hormones, such as steroids—cannot be extrapolated to the IGF system. In fact, being bound to specific IGFBPs may facilitate the transfer of IGFs from the vasculature to tissues and to its sequestration within certain tissue compartments (33, 34). Furthermore, at the cellular level, IGFBPs can enhance cellular actions through a variety of mechanisms, including increasing concentrations at the cell surface for receptor interactions and preventing receptor down-regulation (32). Thus, bound IGF can be more active than unbound, which makes the interpretation of the component that can be measured as free within the circulation very complex.

The design of our study is prospective and of fairly large size, adding to the strength of our findings. Furthermore, with a fairly large proportion of premenopausal women, we were able to address associations by menopausal status, with sufficiently large numbers of cases in each quantile. Nonetheless, the youngest of our premenopausal women is >42 years old, which compromised our ability to assess risks among very young women. The strong association of IGF-I with height in childhood, and weak or no association between adult levels

and adult height, also suggests that IGF levels in young women may better reflect an exposure time period of importance to breast cancer. Thus, larger cohorts of premenopausal women with a lower median age are needed to address questions whether IGF-I associations with breast cancer risk may vary by age, and whether IGF levels in young women may better reflect the exposure time period of importance. In addition, we only had a single blood sample per subject to represent long-term IGF levels, which may have introduced some misclassification, thus biasing our findings to the null. However, evidence for IGF-I and IGFBP-3 suggest that a single plasma measurement reflects longer term circulating levels reasonably well with correlations of 0.9 for samples measured over 8 weeks (35) and 0.81 for IGF-I and 0.60 for IGFBP-3 measured over a 1-year period (27).

Finally, to date, it is still not clear if the observed association in younger women can be attributed to the possibility that IGF levels earlier in life are important, or if the association is specific to premenopausal breast cancer. To distinguish these two possibilities, one would need to have women who were premenopausal at blood collection and then either premenopausal or postmenopausal at diagnosis; with our short follow-up, we have little ability to address this. In addition, premenopausal data sets (including ours) have generally still been relatively small (case numbers ranged from 66 to 172 and the combined evidence for premenopausal women is based on a total of 499 breast cancer cases only), and further confirmation of our results in larger premenopausal cohorts is needed.

In summary, our study supports previous reports indicating that the relation between IGF-I and breast cancer risk may differ by menopausal status. The prospective studies, including our own, have been very consistent in observing no association between circulating IGF-I levels and breast cancer risk among postmenopausal women. In contrast, our results give further support to a positive association among premenopausal women. Our data do not support an important role for circulating levels of IGFBP-3, IGFBP-1, or free IGF as estimated by a specific ELISA method in breast cancer etiology.

Acknowledgments

We thank the participants of the ongoing Nurses' Health Study for their continuing outstanding dedication to the study, and David Coppola, Karen Corsano, Victor Pontes, and Jeanne Sparrow for technical assistance.

References

1. Medical Intelligence Unit. Insulin-like growth factors. New York: Kluwer Academic; 2003.
2. Baxter RC. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. Am J Physiol Endocrinol Metab 2000;278:E967–76.
3. Hankinson SE, Schernhammer ES. Insulin-like growth factor and breast cancer risk: evidence from observational studies. Breast Dis 2003;17:27–40.
4. 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;13:307–16.
5. Hankinson SE, Schernhammer ES. Insulin-like growth factor and breast cancer risk: evidence from observational studies. Breast Dis 2003;17:27–40.
6. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351: 1393–6.
7. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Hennekens CH, Speizer FE. Dietary fat and the risk of breast cancer. N Engl J Med 1987;316:22–8.
8. Stampfer MJ, Willett WC, Colditz GA, Rosner B, Speizer FE, Hennekens CH. A prospective study of postmenopausal estrogen therapy and coronary heart disease. N Engl J Med 1985;313:1044–9.
9. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.
10. Stampfer MJ, Willett WC, Speizer FE, et al. Test of the national death index. Am J Epidemiol 1984;119:837–9.
11. Willett WC, Browne ML, Bain C, et al. Relative weight and risk of breast cancer among premenopausal women. Am J Epidemiol 1985;122:731–40.
12. Campagnoli C, Biglia N, Altare F, et al. Differential effects of oral conjugated estrogens and transdermal estradiol on insulin-like growth factor 1, growth hormone and sex hormone binding globulin serum levels. Gynecol Endocrinol 1993;7:251–8.
13. Blake EJ, Adel T, Santoro N. Relationships between insulin-like growth hormone factor-I and estradiol in reproductive aging. Fertil Steril 1997;67:697–701.
14. Wang HS, Lee JD, Soong YK. Serum levels of insulin-like growth factor I and insulin-like growth factor-binding protein-1 and -3 in women with regular menstrual cycles. Fertil Steril 1995;63:1204–9.
15. Thierry van Dessel HJ, Chandrasekher Y, Yap OW, et al. Serum and follicular fluid levels of insulin-like growth factor I (IGF-I), IGF-II, and IGF-binding protein-1 and -3 during the normal menstrual cycle. J Clin Endocrinol Metab 1996;81:1224–31.
16. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988;44:1049–60.
17. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.
18. Ng ST, Zhou J, Adesanya OO, Wang J, LeRoith D, Bondy CA. Growth hormone treatment induces mammary gland hyperplasia in aging primates. Nat Med 1997;3:1141–4.
19. Yang XF, Beamer WG, Huynh H, Pollak M. Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. Cancer Res 1996;56:1509–11.
20. Bates P, Fisher R, Ward A, Richardson L, Hill DJ, Graham CF. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). Br J Cancer 1995;72:1189–93.
21. Shang Y, Baumrucker CR, Green MH. Signal relay by retinoic acid receptors α and β in the retinoic acid-induced expression of insulin-like growth factor-binding protein-3 in breast cancer cells. J Biol Chem 1999;274:18005–10.
22. Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankinson SE. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000;60:3744–8.
23. Guo YP, Martin LJ, Hanna W, et al. Growth factors and stromal matrix proteins associated with mammographic densities. Cancer Epidemiol Biomarkers Prev 2001;10:243–8.
24. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J Natl Cancer Inst 1995;87:1622–9.
25. Toniolo P, Bruning PF, Akhmedkhanov A, et al. Serum insulin-like growth factor-I and breast cancer. Int J Cancer 2000;88:828–32.
26. Krajcik RA, Borofsky ND, Massardo S, Orentreich N. Insulin-like growth factor (IGF-I), IGF-binding proteins, and breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1566–73.
27. Muti P, Quattrin T, Grant BJB, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2002; 11:1361–8.
28. Keinan BL, Bueno de Mesquita HB, 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 2002;106:90–5.
29. 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;47:111–20.
30. Favoni RE, de Cupis A, Perrotta A, et al. Insulin-like growth factor-I (IGF-I) and IGF-binding proteins blood serum levels in women with early- and late-stage breast cancer: mutual relationship and possible correlations with patients' hormonal status. J Cancer Res Clin Oncol 1995;121:674–82.
31. Goodwin PJ, Ennis M, Pritchard KI, et al. Insulin-like growth factor binding proteins 1 and 3 and breast cancer outcomes. Breast Cancer Res Treat 2002;74:65–76.
32. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;47:505–18.
33. Perks CM, Holly JM. Insulin-like growth factor binding proteins (IGFBPs) in breast cancer. J Mammary Gland Biol Neoplasia 2000;5:75–84.
34. Marshman E, Streuli CH. Insulin-like growth factors and insulin-like growth factor binding proteins in mammary gland function. Breast Cancer Res 2002;4:231–9.
35. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo study. Am J Epidemiol 1997;145:970–6.