Circulating Insulin-Like Growth Factor-I and Binding Protein-3 and the Risk of Breast Cancer

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Abstract

Four meta-analyses and literature reviews have concluded that a positive association exists between circulating levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) and breast cancer risk for premenopausal but not postmenopausal women. Recently, a large prospective study reported an association with IGF-I and IGFBP-3 concentration for breast cancer diagnosed after, but not before, the age of 50 years; and in a large cohort of primarily premenopausal women, IGF-I and IGFBP-3 were not associated with breast cancer risk. We did a case-cohort study within the Melbourne Collaborative Cohort Study, which included a random sample of 1,901 women (subcohort) and 423 breast cancer cases diagnosed during a mean of 9.1 years of follow-up. IGF-I and IGFBP-3 concentrations were measured in plasma collected at baseline. The association between quartiles of IGF concentration and breast cancer risk was tested using a Cox model adjusted for known and potential confounders. The hazard ratio (HR) for breast cancer comparing the fourth with the first quartiles was 1.20 [95% confidence interval (95% CI), 0.87-1.65] for IGF-I and 1.09 (95% CI, 0.78-1.53) for IGFBP-3. Both associations varied with age: for IGF-I, the HRs for breast cancer comparing the fourth with the first quartiles were 0.60 (95% CI, 0.25-1.45) before age 50 and 1.61 (95% CI, 1.04-2.51) after age 60 (test for the log-linear trend of HR according to age, $P = 0.05$); for IGFBP-3, the HRs were 0.79 (95% CI, 0.34-1.83) before age 50 and 1.62 (95% CI, 1.03-2.55) after age 60 (test for log-linear trend, $P = 0.08$). IGF-I and IGFBP-3 were positively associated with breast cancer risk in older women but not in younger women. More prospective studies are needed to clarify the age dependence of the association between IGF-I and IGFBP-3 and breast cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(4):763–8)

Introduction

Insulin-like growth factor I (IGF-I) is a peptide hormone involved in regulating human growth and development by stimulating cell proliferation and inhibiting apoptosis, with a recognized effect on tumor growth (1). In the circulation, IGF-I binds mainly to IGF-binding protein-3 (IGFBP-3; refs. 2, 3), a protein with specific binding affinities to IGFs, which not only regulates the mitogenic action of IGFs and inhibits their antiapoptotic effect, but also has an IGF-independent inhibitory effect on cell growth (1).

The literature on the relationship between breast cancer risk and circulating concentrations of IGF-I and IGFBP-3 had indicated an increased risk for premenopausal women with increasing levels of IGF-I and IGFBP-3, but no association with risk for postmenopausal women (3-6). Recently, the European Prospective Investigation into Cancer and Nutrition (EPIC) analyzed data from 1,081 cases and 2,098 matched controls, approximately the same number of incident cases as all previous prospective studies combined, and reported that women with the highest circulating levels of total IGF-I or IGFBP-3 had a 40% increased risk for breast cancer diagnosed after age 50, but no evidence of increased risk before this age (7). Similarly, they found an association when they restricted the analysis to postmenopausal women at the time of blood collection, but observed no association in women who were premenopausal. Another recent report from the prospective Nurses' Health Study II showed that IGF-I and IGFBP-3 were not associated with breast cancer risk in a large group of primarily premenopausal women (8). These findings reopen the debate about the age dependence of the associations between IGF-I and IGFBP-3 and breast cancer risk.

We investigated IGF-I and IGFBP-3 and breast cancer risk in the women of the Melbourne Collaborative Cohort Study with the specific aim of determining how the associations might vary with age.

Materials and Methods

Subjects and Case-Cohort Design. The Melbourne Collaborative Cohort Study is a prospective cohort study of 41,528 people (24,479 women) ages between 27 and 75 years at baseline (99.3% of whom were ages 40-69 years). Recruitment occurred between 1990 and 1994 in the Melbourne metropolitan area. The details of the study have been published elsewhere (9). The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol. Subjects gave written consent to participate and for the investigators to obtain access to their medical records.

All women who had a confirmed diagnosis of breast cancer before baseline (2%) were excluded, as were women who did not provide a blood sample (3%) and those taking hormone replacement therapy at baseline (17%), leaving 19,347 women eligible for the case-cohort study. All women first diagnosed with breast cancer between baseline and June 30, 2002 were included in the study sample, as did a random sample (hereafter called the subcohort) of 2,031 women from the cohort.

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Case Ascertainment. Addresses and vital status of the subjects were determined by record linkage to the Electoral Rolls, the Victorian death records, the National Death Index, and from electronic phone books, and from responses to mailed questionnaires and newsletters. Between baseline attendance and June 30, 2002, among those eligible, 20 women had left Australia and 676 had died. Cases had adenocarcinoma of the breast (International Classification of Disease 9th revision rubric 174.0-174.9, or 10th revision rubric C50.0-C50.9). Women with *in situ* breast cancer were not included as cases. When this study was done, cases were ascertained by record linkage to the population-based Victorian Cancer Registry, which covers the state in which the cohort resides. Subsequently, we linked the cohort with the National Cancer Statistics Clearing House, which holds cancer incidence data from all Australian states and three more cases were identified (one was in the subcohort). A total of 440 women were diagnosed with breast cancer over an average of 9.1 person-years of follow-up. Forty-one of these cases were members of the subcohort.

Assessment of Circulating Levels of IGF-I and IGFBP-3. IGF-I and IGFBP-3 measurements were not made for 144 women, including 17 cases; 138 had insufficient plasma, 1 sample was contaminated, and 5 cases (including the two diagnosed outside Victoria) were identified after the measurements were done. Therefore, measurements were made for 2,286 women: 1,903 members of the subcohort (94%) and 423 case subjects (96%; 40 were members of the subcohort). There was little difference in age at baseline, reproductive history (age at menarche, parity, duration of lactation, oral contraceptive use, menopausal status, and hormone replacement therapy use), or demographic and lifestyle variables [ethnicity, education, body mass index (BMI), physical activity, energy from diet, alcohol intake, and smoking] between women who had their hormone measured and those who did not. Two women from the subcohort who had missing information on menopausal status at baseline were excluded from all the analyses, leaving 1,901 women in the subcohort and 423 cases. Among these women, IGFBP-3 measurements were missing for 53 women (11 cases). Information about confounders was not available for 268 women (52 cases) who were excluded from all adjusted analyses.

Plasma samples were retrieved from storage, aliquotted into 450 μL amounts, and shipped on dry ice in batches of about 80 samples each to the laboratory of one of the authors (H. Morris), where IGF-I and IGFBP-3 were measured. Assignment to batches was random, and the proportions of cases and subcohort members were approximately equal across batches. Ten percent of the samples in each batch were aliquots from pooled plasma that had been stored with the samples from the participants. The laboratory was blind to the status of the samples. One scientist did all the measurements.

Samples were thawed in a warm water bath, vortexed rapidly for a few seconds, and centrifuged at 2,000 rpm ($210 \times g$) for 10 min. IGF-I was measured by ELISA (DSL-10-5600; Diagnostics System Laboratories, Webster, TX) with an inter-assay coefficient of variation of 11.1% at 16.3 nmol/L. IGFBP-3 was measured by ELISA (DSL-10-6600; Diagnostics System Laboratories) with a coefficient of variation at 110 nmol/L of 9.5%.

A reliability study was done before the study commencement. Plasma samples from 71 women who had given blood twice ~1 year apart were each divided into two aliquots. The two aliquots were measured in separate batches 1 week apart. As a measure of reliability, we used the intraclass correlation (ICC), which is the proportion of the total variance due to variation between persons, in which the total variance included components due to between-person, between-sampling occasions, and between-laboratory runs.

Statistical Analyses. In order to adjust for variation in circulating levels of IGF-I and IGFBP-3 among laboratory batches and by age and menopausal status, quartiles were assigned following a two-step procedure. First, in a linear regression model in the subcohort, log-transformed values of IGF-I and IGFBP-3 were regressed according to batch, age, and menopausal status at blood collection; second, the predicted values of these regressions were calculated for all women and the residuals, centered on the grand means, were categorized into quartiles according to the distribution of the values for the subcohort. To further adjust IGF-I for IGFBP-3, the residuals were calculated from a model which also included the logarithm of IGFBP-3 among the regressors.

Cox regression, with age as the time axis (10), was used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI). We used the Prentice method to take the case-cohort sampling into account and the robust method was used to calculate the variance-covariance matrix (11, 12). Follow-up for a subcohort member began at baseline and ended at diagnosis of breast cancer or cancer of unknown primary site, death, the date last known to be in Australia, or June 30, 2002, whichever came first.

Analyses were adjusted for country of birth, age at menarche, parity, duration of lactation, oral contraceptive use, menopausal status at baseline, past hormone replacement therapy use, physical activity, alcohol consumption, energy from diet, smoking, and level of education and were stratified according to BMI categories because for this variable, the hazards were not proportional (see Table 1 for description of all the confounders).

Tests for linear trend were based on pseudo-continuous variables under the assumption that all subjects within each quartile had the same concentration, equal to the within-quartile median. The pseudo-continuous variables were log 2–transformed before inclusion in the models so that the HR would represent the relative difference in risk associated with a doubling of the concentration.

We estimated the HRs for IGF-I and IGFBP-3 overall, according to menopausal status at baseline, within three follow-up age bands (<50, 50-59, and 60+) and within two categories of time since blood collection (<2 and ≥2 years). For the latter two analyses, we split the record of each subject into multiple records, with each record containing the follow-up on the subject through one age band (or time since blood collection band) and fitted Cox models with the interaction of IGF-I and IGFBP-3 with age band (or time since blood collection band). To study the dependence of the HRs on age continuously, we fitted models in which the coefficients of IGF-I and IGFBP-3 varied linearly with the analysis time (13).

Statistical analyses were done using Stata/SE 8.2 (Stata Corporation, College Station, TX). Because the robust method was used to calculate the variance-covariance matrix, the Wald test, not the likelihood ratio test, was used to test hypotheses. All P values were two-sided, and $P < 0.05$ was considered as statistically significant.

Results

Table 1 summarizes the baseline characteristics of the cases and the subcohort. Seventy-six percent of the subcohort were born in Australia, New Zealand, or the United Kingdom, and 24% in Italy or Greece, and 60% were postmenopausal at baseline. For the 423 cases, the mean age at diagnosis was 61 years (range, 41-79 years), 29% were diagnosed before age 55 and 20% within the first 2 years of follow-up.

Overall, we did not observe a significant increase in breast cancer risk associated with higher levels of IGF-I or IGFBP-3 (Table 2). The HRs for the highest versus the lowest quartiles from the model adjusted for confounders were 1.20 (95% CI,

Table 1. Characteristics and hormone levels of subjects at baseline (cases and subcohort)

	Breast cancer cases* (N = 423)	Subcohort (N = 1,901)
Age at baseline, years; mean ± SD (range)	56 ± 9 (39-70)	55 ± 9 (36-70)
Country of birth, *n* (%)		
Australia/New Zealand	324 (76.6)	1,318 (69.3)
United Kingdom	14 (3.3)	127 (6.7)
Italy	47 (11.1)	257 (13.5)
Greece	38 (9.0)	199 (10.5)
Age at menarche (years), *n* (%)		
<12	73 (17.3)	309 (16.3)
12	68 (16.1)	386 (20.3)
13	112 (26.5)	507 (26.7)
14+	168 (39.7)	695 (36.6)
Parity (age at first pregnancy and number of full-term pregnancies), *n* (%)		
Nulliparous	74 (17.5)	261 (13.7)
<25 and 1	10 (2.4)	46 (2.4)
<25 and >1	166 (39.2)	829 (43.6)
≥25 and 1	26 (6.1)	131 (6.9)
≥25 and >1	146 (34.5)	632 (33.2)
Duration of lactation, *n* (%)		
Never	145 (34.3)	543 (28.6)
Up to 6 mo	74 (17.5)	385 (20.3)
7-12 mo	64 (15.1)	311 (16.4)
13-24 mo	79 (18.7)	346 (18.2)
>24 mo	52 (12.3)	291 (15.3)
Oral contraceptive use, *n* (%)		
Never	195 (46.1)	806 (42.4)
Past or current	227 (53.7)	1,093 (57.5)
Menopausal status, *n* (%)		
Premenopausal	166 (39.2)	769 (40.5)
Postmenopausal	257 (60.8)	1,132 (59.5)
Hormone replacement therapy use, *n* (%)		
Never	376 (88.9)	1,690 (88.9)
Past	41 (9.7)	191 (10.0)
Physical activity, *n* (%)		
None	82 (19.4)	425 (22.4)
Low	99 (23.4)	394 (20.7)
Medium	161 (38.1)	676 (35.6)
High	81 (19.1)	406 (21.4)
BMI, kg/m^2; *n* (%)		
<25	159 (37.6)	767 (40.4)
25-29	160 (37.8)	707 (37.2)
30+	104 (24.6)	426 (22.4)
Alcohol consumption, *n* (%)		
Abstainers	163 (38.5)	766 (40.3)
Ex-drinkers	14 (3.3)	77 (4.1)
1-19 g/d	205 (48.5)	858 (45.1)
20-39 g/d	26 (6.1)	157 (8.3)
≥40 g/d	15 (3.5)	42 (2.2)
Energy from diet, kJ/d; mean (SD)	8,463 (2,851)	8,510 (2,789)
Smoking, *n* (%)		
Never	316 (74.7)	1,321 (69.5)
Past	77 (18.2)	406 (21.4)
Current	30 (7.1)	174 (9.2)
Education, *n* (%)		
Primary school	79 (18.7)	400 (21.0)
Some high school	183 (43.3)	797 (41.9)
Completed high school	84 (19.9)	348 (18.3)
Degree/diploma	77 (18.2)	356 (18.7)
IGF-I, nmol/L; geometric mean (95% CI)	21.1 (20.4-21.9)	21.0 (20.6-21.3)
IGFBP-3, nmol/L; geometric mean (95% CI)	111.6 (109.2-114.0)	110.1 (109.0-111.3)

NOTE: Percentages do not always sum up to 100% because of missing values.
*Forty breast cancer cases were also included in the subcohort.

0.87-1.65) for IGF-I and 1.09 (95% CI, 0.78-1.53) for IGFBP-3. No association between IGF-I and breast cancer risk was found when further adjusting for IGFBP-3 (Table 2).

The associations between IGF-I and IGFBP-3 and breast cancer risk varied according to menopausal status at baseline. For both IGF-I and IGFBP-3, no significant association was observed for women who were premenopausal at the time of blood collection, but positive associations were observed for postmenopausal women (Table 3). The HRs associated with a doubling of hormone concentrations were 0.76 (95% CI, 0.46-1.26) and 1.47 (95% CI, 1.00-2.14) for premenopausal and postmenopausal women, respectively, for IGF-I (test for interaction, P = 0.04); and 0.57 (95% CI, 0.24-1.34) and 1.88 (95% CI, 0.96-3.67) for premenopausal and postmenopausal women, respectively, for IGFBP-3 (test for interaction, P = 0.03). Similarly, associations between breast cancer risk and IGF-I and IGFBP-3 were observed in women ages 50 or more at the time of blood collection, but no associations were observed in younger women (data not shown).

Consistent with the results according to menopausal status, the associations of IGF-I and IGFBP-3 with breast cancer risk was heterogeneous according to attained age during follow-up (Table 4). For IGF-I, the HRs for the highest versus the lowest quartiles were 0.60 (95% CI, 0.25-1.45) before age 50 and 1.61

Table 2. HRs (95% CI) of breast cancer for IGF-I and IGFBP-3 levels

	Quartiles, HR (95% CI)*				Doubling hormone concentration†	P_{trend}‡
	Q1	Q2	Q3	Q4		
IGF-I						
Cases/person-years§	107/4,342	105/4,291	92/4,315	119/4,296		
Crude	Reference	1.02 (0.76-1.37)	0.89 (0.65-1.20)	1.13 (0.85-1.51)	1.08 (0.82-1.43)	0.57
Adjusted, model 1‖	Reference	1.08 (0.78-1.51)	0.96 (0.68-1.34)	1.20 (0.87-1.65)	1.14 (0.85-1.55)	0.38
Adjusted, model 2¶	Reference	0.83 (0.60-1.16)	0.87 (0.63-1.22)	0.95 (0.69-1.32)	0.95 (0.65-1.40)	0.80
IGFBP-3						
Cases/person-years**	105/4,240	95/4,189	102/4,226	110/4,185		
Crude	Reference	0.93 (0.69-1.26)	0.99 (0.73-1.34)	1.05 (0.78-1.42)	1.10 (0.69-1.73)	0.69
Adjusted, model 1‖	Reference	0.86 (0.61-1.22)	0.99 (0.70-1.39)	1.09 (0.78-1.53)	1.20 (0.71-2.02)	0.50

*Quartiles were adjusted for variations between batches and by age and menopausal status at the time of blood collection, according to the procedure described in Materials and Methods. Estimates from the Cox regression model were based on the following number of women (cases): IGF-I, crude, 2,284 (423); adjusted, model 1, 2,016 (371); adjusted, model 2, 1,969 (360); IGFBP-3, crude, 2,231 (412); adjusted, model 1, 1,969 (360).
† Estimates from the model including the pseudo-continuous variable log 2–transformed.
‡ Test for linear trend using the pseudo-continuous variable log 2–transformed.
§Breast cancer cases and person-years calculated from the 2,284 women with IGF-I measured.
‖HRs from the Cox model adjusted for country of birth, age at menarche, parity, duration of lactation, oral contraceptive use, menopausal status at baseline, hormone replacement therapy use, physical activity, alcohol consumption, energy from diet, smoking, and level of education, and stratified for BMI categories.
¶HRs from the Cox model adjusted for all the confounders and for IGFBP-3, using the residual procedure described in Materials and Methods.
**Breast cancer cases and person-years calculated from the 2,231 women with IGFBP-3 measured.

(95% CI, 1.04-2.51) after age 60 (test for interaction between pseudo-continuous IGF-I and age grouping, $P = 0.06$); for IGFBP-3, the HRs were 0.79 (95% CI, 0.34-1.83) before age 50 and 1.62 (95% CI, 1.03-2.55) after age 60 (test for interaction, $P = 0.02$).

Figure 1 shows the log-linear dependence on age of the HRs for IGF-I (test for log-linear trend, $P = 0.05$ for IGF-I). The age at which the effect of doubling hormone concentrations shifted from decreasing to increasing risk was 57 years. The curve was similar for IGFBP-3 (test for log-linear trend, $P = 0.08$; age at which the effect shifted from decreasing to increasing risk, 57 years).

There was little heterogeneity in breast cancer risks for IGF-I and IGFBP-3 according to time since blood collection: the HRs for the top versus the bottom quartile of IGF-I for duration of follow-up of less than 2 years and more than 2 years were 0.97 (95% CI, 0.36-2.61) and 0.81 (95% CI, 0.48-1.38), respectively, for cancer diagnosed at ages less than 55 and 1.35 (95% CI, 0.57-3.23) and 1.59 (95% CI, 0.88-2.87) for cancer diagnosed at older ages; the HRs for the top versus the bottom quartile of IGFBP-3 for duration of follow-up of less than 2 years and more than 2 years were 0.55 (95% CI, 0.21-1.47) and 0.68 (95% CI, 0.39-1.78), respectively, for cancer diagnosed at ages less than 55 and 1.62 (95% CI, 0.68-3.88) and 2.02 (95% CI, 1.12-3.63) for cancer diagnosed at older ages.

There was also little heterogeneity in breast cancer risks for IGF-I and IGFBP-3 according to BMI for cancers diagnosed at ages less than 55 years and 55 years or older (data not shown).

Reliability and Quality Control. From the reliability study, the ICC for IGF-I was 0.42 (95% CI, 0.27-0.57) and 0.67 (95% CI, 0.57-0.77) for IGFBP-3. For the pooled plasma samples, the overall coefficient of variation was 12% for IGF-I (9% within batches and 7% between batches) and 9% for IGFBP-3 (8% and 3%). The Spearman correlation coefficient between IGF-I and IGFBP-3 in the subcohort was 0.49.

Discussion

We found age-dependent associations between prediagnostic circulating concentrations of IGF-I and IGFBP-3 and breast cancer risk: women with hormone concentrations higher relative to their age and menopausal status had an increased risk of breast cancer after, but not before, the age of 60 years.

Table 3. HR (95% CI) of breast cancer for IGF-I and IGFBP-3 levels according to menopausal status at baseline

	Quartiles,* cases/person-years† and HR (95% CI)‡				Doubling hormone concentration§	P_{trend}‖	P¶
	Q1	Q2	Q3	Q4			
IGF-I							
Premenopausal	46/1,444	39/1,730	33/1,787	33/1,391			
	Reference	0.78 (0.48-1.29)	0.62 (0.38-1.03)	0.83 (0.49-1.38)	0.76 (0.46-1.26)	0.29	
Postmenopausal	42/2,303	54/2,104	52/2,052	72/2,426			
	Reference	1.42 (0.90-2.23)	1.35 (0.86-2.13)	1.59 (1.03-2.44)	1.47 (1.00-2.14)	0.05	0.04
IGFBP-3							
Premenopausal	44/1,487	40/1,705	33/1,677	28/1,316			
	Reference	0.74 (0.45-1.22)	0.68 (0.41-1.14)	0.73 (0.42-1.26)	0.57 (0.24-1.34)	0.20	
Postmenopausal	45/2,213	44/2,081	55/2,120	71/2,291			
	Reference	0.97 (0.61-1.55)	1.30 (0.83-2.05)	1.42 (0.92-2.19)	1.88 (0.96-3.67)	0.06	0.03

*Quartiles were adjusted for variations between batches and by age and menopausal status at time of blood collection, according to procedures described in Materials and Methods.
† Breast cancer cases and person-years.
‡ Estimates from the Cox regression model adjusted for country of birth, age at menarche, parity, duration of lactation, oral contraceptive use, hormone replacement therapy use, physical activity, alcohol consumption, energy from diet, smoking, and level of education, and stratified for BMI categories. Estimates were based on the following number of women (cases): IGF-I, 2,016 (371); IGFBP-3, 1,969 (360).
§Estimates from the model including the pseudo-continuous variable log 2–transformed.
‖Test for linear trend using the pseudo-continuous variable log 2–transformed.
¶Test for the interaction between hormone concentration (pseudo-continuous log 2–transformed) and menopausal status.

The main strengths of our study include its prospective design, large sample size, duration, and completeness of follow-up, and the availability of accurate information on potential confounders (14). The principal advantage of using a prospective rather than a retrospective study design is the ability to measure hormone concentrations before diagnosis, as circulating levels of IGF-I and IGFBP-3 after diagnosis may reflect tumor activity rather than a causal association (3). In our study, the association between hormone concentrations and breast cancer risk did not change with duration of follow-up, suggesting that the presence of incipient breast cancers at the time of blood collection did not affect the associations. Another strength is the quality of the measurement of IGFBP-3 as evidenced by high ICCs and low coefficients of variation for the pooled plasma samples. The reliability of the IGF-I measurements was lower as evidenced by the ICC. Other studies have generally reported good IGF-I and IGFBP-3 reproducibility when comparing either two values (15, 16) or multiple values over time (17, 18). In a reliability study conducted to investigate the utility of IGF-I as a biomarker in epidemiologic studies, the ICC for females was 0.69 for samples taken 1 year apart and 0.71 for samples taken 5 years apart (17); in the New York University Women's Health Study, the ICC was 0.67 for IGF-I and 0.86 for IGFBP-3 for measurement over an average time of 14 months (18). However, other researchers have shown that the intraindividual variability of IGF-I measurements can be high, reducing the utility of a single measure of IGF-I for association studies (19). It has also been suggested that circulating IGF-I and IGFBP-3 concentrations might vary with the menstrual cycle, although not all agree (20-23). Without information about the menstrual phase at the time of blood collection for premenopausal women, we were unable to adjust for this possible source of variability in hormone concentration that might have contributed to the attenuation of risk estimates. The effect of a random measurement error is usually to bias a relative risk toward the null association, and to reduce the precision of the estimates (24, 25). Thus, the variability of IGF-I measurements was likely to have reduced the true association with breast cancer risk at older ages and decreased our ability to detect any inverse association at younger ages.

Because some information about menopausal status during follow-up was missing, we analyzed the data according to attained age and found that the age at which high hormone concentrations start to be associated with increased breast cancer risk was close to the age of menopausal transition for both IGF-I and IGFBP-3.

Our findings were not consistent with the conclusions of four systematic reviews and meta-analyses of prospective and case-control studies which indicated an increased risk for premenopausal breast cancer with increasing IGF-I and a similar but less consistent trend for IGFBP-3 (3-6). Our findings were consistent, instead, with the Nurses Health Study II study, which found no important association between IGF-I and IGFBP-3 with breast cancer risk in premenopausal women (8), and with a recent report from the EPIC study of a statistically significant increase of breast cancer risk for women with high IGF-I and IGFBP-3 concentrations for tumors diagnosed after, but not before, the age of 50 years (7). We found little evidence for heterogeneity of the relation between hormone concentrations and breast cancer risk according to duration of follow-up and BMI, as reported by the EPIC study (7). The short follow-up duration was postulated as a possible cause of inconsistency between the EPIC study and previous reports (7), but it is not an issue in our study, in which the average length of 9.1 years of follow-up was one of the longest among the published studies (26-29). Differences in the assay methods used for peptide measurements may have had a role in the between-study heterogeneity and this would be more likely for IGFBP-3 due to different specificities of different assays to measure intact forms of the protein present in the blood (30). Publication bias cannot be excluded as a possible explanation of the inconsistencies among the three most recent reports, including the present study (7, 8), and previous literature (3-6, 26-29).

It has been argued that IGF-I concentrations may be particularly relevant to the risk of breast cancer for premenopausal women because estradiol enhances the action of IGF-I in breast cells, whereas in postmenopausal women, the lower concentrations of both hormones are not able to affect tumorigenesis (3, 31). Our finding of an increased risk of breast cancer according to age, with increasing IGF-I and IGFBP-3 concentrations, could be explained by the hypothesis

Table 4. HR (95% CI) of breast cancer for IGF-I and IGFBP-3 levels according to attained age during follow-up

	Quartiles* cases/person-years† and HR (95% CI)‡				Doubling hormone concentration§	P_{trend}‖	P¶
	Q1	Q2	Q3	Q4			
IGF-I							
<50	14/685	19/895	10/957	9/736			
	Reference	0.98 (0.46-2.07)	0.50 (0.21-1.18)	0.60 (0.25-1.45)	0.52 (0.24-1.16)	0.11	
50-59	34/1,182	24/1,265	30/1,175	28/1,127			
	Reference	0.70 (0.39-1.24)	0.85 (0.49-1.46)	0.96 (0.55-1.67)	0.97 (0.56-1.68)	0.92	
60+	40/1,879	50/1,675	45/1,708	68/1,954			
	Reference	1.43 (0.90-2.28)	1.24 (0.77-1.99)	1.61 (1.04-2.51)	1.47 (0.99-2.19)	0.06	0.06
IGFBP-3							
<50	15/752	16/888	9/875	10/654			
	Reference	0.86 (0.41-1.82)	0.52 (0.22-1.23)	0.79 (0.34-1.83)	0.52 (0.13-2.03)	0.35	
50-59	35/1,125	27/1,177	31/1,248	21/1,119			
	Reference	0.69 (0.39-1.22)	0.81 (0.47-1.40)	0.61 (0.33-1.11)	0.52 (0.21-1.25)	0.14	
60+	39/1,823	41/1,721	48/1,674	68/1,834			
	Reference	1.01 (0.62-1.64)	1.34 (0.83-2.18)	1.62 (1.03-2.55)	2.32 (1.14-4.71)	0.02	0.02

*Quartiles were adjusted for variations between batches and by age and menopausal status at time of blood collection, according to procedures described in Materials and Methods.

† Breast cancer cases and person-years.

‡ Estimates from the Cox regression model adjusted for country of birth, age at menarche, parity, duration of lactation, oral contraceptive use, menopausal status at baseline, hormone replacement therapy use, physical activity, alcohol consumption, energy from diet, smoking, and level of education and stratified according to BMI category. Estimates were based on the following number of women (cases): IGF-I, 2,016 (371); IGFBP-3, 1,969 (360).

§Estimates from the model including the pseudo-continuous variable log 2–transformed.

‖Test for log-linear trend using the pseudo-continuous variable log 2–transformed.

¶Test for homogeneity in the HRs for hormone concentration (pseudo-continuous log 2–transformed) in the three age groups.

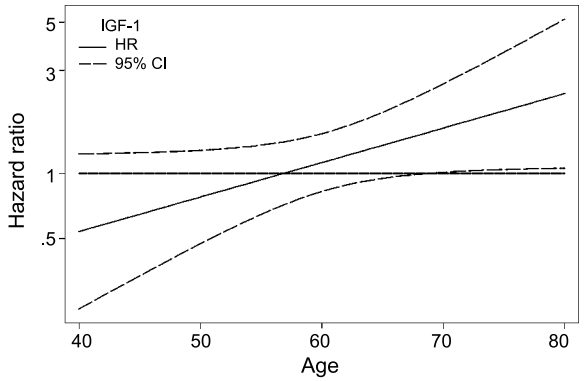

Figure 1. HR and 95% CI of breast cancer for doubling IGF-I concentrations in women with the same age and menopausal status according to attained age during follow-up.

that these hormones increase the accumulation of genetic damage in breast tissue (32). Both hormonal and nonhormonal agents such as tamoxifen, raloxifene, and synthetic retinoid fenretinide have been shown to decrease breast cancer incidence (33-35) and to lower circulating IGF-I and IGFBP-3 levels (36-38). A better understanding of the association between IGF-I and IGFBP-3 and breast cancer according to age and menopausal status would be important before considering targeting them for chemoprevention.

Our study, the Nurses Health Study II, and the EPIC study found no association between IGF-I and IGFBP-3 and breast cancer risk in premenopausal women; our study and the EPIC study have also found the same age-dependent associations between breast cancer risk and circulating IGF-I and IGFBP-3 concentrations using a number of incident cases approximately as large as that for all previous prospective studies combined. Given that these findings are in direct contrast with the previously held consensus, they rekindle discussion about the role played by IGF-I and IGFBP-3 in breast carcinogenesis.

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