Premenopausal Insulin-Like Growth Factor-I Serum Levels and Changes in Breast Density over Menopause

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Abstract

Background: **A high proportion of glandular and stromal tissue in the breast (percentage breast density) is a strong risk factor for breast cancer development. Insulin-like growth factor-I (IGF-I) is hypothesized to influence breast cancer risk by increasing breast density.**
Objectives: **We studied the relation between premenopausal circulating IGF-I levels and premenopausal and postmenopausal, absolute nondense and dense area, and percentage breast density as well as changes in these measures over menopause.**
Design and Methods: **Mammograms and blood samples of 684 premenopausal participants of the Prospect-European Prospective Investigation into Cancer and Nutrition cohort were collected at baseline. A second mammogram of these women was collected after they became postmenopausal. Premenopausal IGF-I levels were measured in serum. Premenopausal and postmenopausal breast measures were assessed using a computer-assisted method. Mean values of breast measures were calculated for quartiles of serum IGF-I using linear regression analysis.**
Results: **Women with higher premenopausal IGF-I levels showed a slightly smaller decrease in dense area over menopause (-12.2 cm^2 in the highest versus -12.9 cm^2 in the lowest quartile; P trend = 0.58) and, at the same time, a smaller increase in the nondense (fat) area (P trend = 0.09). Due to the changes over menopause, high premenopausal IGF-I serum levels were associated with lower nondense area (P trend = 0.05), somewhat higher dense area (P trend = 0.66), and consequently higher percentage breast density (P trend = 0.02) after menopause.**
Conclusion and Discussion: **Women with higher premenopausal IGF-I levels have a smaller increase in nondense area and also a slightly smaller decrease in absolute dense area during menopause, resulting in higher breast density after menopause.** (Cancer Epidemiol Biomarkers Prev 2007;16(3):451–7)

Introduction

From all types of cancer affecting women in the Western world, breast cancer has the highest incidence, and in many countries, breast cancer screening programs are implemented using mammography. Connective (stromal) and epithelial tissues of the female breast are radiologically dense and appear light on a mammogram, whereas the radiologically lucent fat tissue has a dark appearance. High mammographic density has been associated with a 3- to 6-fold increase in risk of both premenopausal and postmenopausal breast cancer and is therefore one of the strongest known risk factors for developing breast cancer (1-4).

Insulin-like growth factor-I (IGF-I) has mitogenic properties and is involved in the development of normal breast tissue (5, 6). *In vitro* studies showed that IGF-I is also a potent mitogen for several breast cancer cell lines and that it inhibits apoptosis (7, 8). Furthermore, in xenograft models, tumor growth was decreased in IGF-I–deficient animals (6). Prospective studies on the association between circulating levels of IGF-I and subsequent breast cancer risk showed contradictive results. High levels of IGF-I were associated with increased risk of premenopausal breast cancer in most studies (9-14) but not all (15-17). Most studies with postmenopausal women did not show a clear association between IGF-I levels and breast cancer risk (9-15, 18, 19), with few exceptions. Muti et al. (12) reported decreased cancer risk in women with high IGF-I levels. Rinaldi et al. (16) found the opposite effect in women >50 years of age.

Several studies have been published on the association between circulating levels of IGF-I and breast density (20-25). IGF-I was positively associated with breast density among premenopausal women in most studies (20, 21, 23, 25), with the exception of two (22, 24). Only one of five studies (25) found an association between IGF-I circulating levels and breast density in postmenopausal women (20, 22-25), which was, however, no longer statistically significant after adjusting for age and waist circumference (25). Guo et al. (26) studied IGF-I staining in paraffin blocks of tissues surrounding benign legions. IGF-I staining was higher in blocks from highly dense breasts but only among women <50 years.

It has been shown that involution of the breast is stronger during the menopausal transition phase compared with involution before menopause (27). It is known that IGF-I levels gradually decrease with age (28), and it is possible that the involution of dense breast tissue during menopause, and consequently also postmenopausal breast density, is largely determined before menopause when IGF-I levels are still high. Results from a recently published study by Rollison et al. (29), reporting higher premenopausal IGF-I levels to be associated with increased postmenopausal breast cancer risk, support this. In this longitudinal study, we investigated whether premenopausal circulating levels of IGF-I were associated with premenopausal mammographic density and also how these levels affected changes in mammographic density during menopause.

Materials and Methods

The Study Population. This study included women participating in Prospect-European Prospective Investigation into

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Cancer and Nutrition (EPIC), one of two Dutch cohorts participating in the EPIC. EPIC is a multicenter cohort study with 10 participating European countries. Rationale and design of both EPIC and Prospect-EPIC are described in detail elsewhere (30-32). The Prospect-EPIC cohort consists of 17,357 women ages 49 to 69 years. Women were recruited between 1993 and 1997 through a regional program for breast cancer screening and reside in Utrecht or its vicinity. The regional program is part of the national screening program, which covers the entire Dutch population. As part of the population-based screening program, a mediolateral oblique mammogram is taken every 2 years, starting at age 50 until age 75, and stored in archives.

At time of study enrollment, pulse rate, blood pressure, and anthropometric measurements were taken. Furthermore, a blood sample was drawn and stored at −196°C under liquid nitrogen, and participants were asked to fill out two questionnaires. A general questionnaire was used to gather information on demographic, reproductive, and lifestyle factors and past and current morbidity. To determine regular dietary intake, an extensive self-administered food frequency questionnaire was used containing 178 food items, which was validated and described in more detail by Ocke et al. (33, 34).

Approximately 5 years after recruitment, participating women were asked to fill out a follow-up questionnaire containing questions about demographic, reproductive, and lifestyle factors and past and current morbidity.

All participants signed an informed consent and the study was approved by The Institutional Review Board of the University Medical Center Utrecht.

Blood Collection and IGF-I Measurements in Serum. A 30-mL nonfasting blood sample was donated by each participant using three safety monovettes: one dry monovette for serum and two citrated monovettes for plasma. Within 24 h, samples of 4 mL serum, 9 mL citrate plasma, and 2 mL WBCs were fractionated into 0.5 mL aliquots and stored in heat-sealed plastic straws under liquid nitrogen at −196°C.

IGF-I in serum was measured by Labor Benrath (Dusseldorf, Germany) using Immulite 2000 technology with reagents from Diagnostic Products Corp. (Frankfurt, Germany). The samples were analyzed in 11 batches, each of which contained the same two control samples. The interbatch coefficients of variation for the two control samples were 3.73% and 2.62%, respectively.

Study Subjects and Mammogram Selection. From the 16,917 subjects that had donated a blood sample, 4,055 were premenopausal at baseline. Women were considered premenopausal if they had menses at least once during the last 12 months before blood collection. Current users of oral contraceptives or postmenopausal hormone therapy were excluded, as were former users who quit using oral contraceptives or postmenopausal hormone therapy <2 years before blood donation. Women having had ovariectomy on both ovaries were also excluded. Three women had had a hysterectomy. However, all three still had both their ovaries and met all other selection criteria. Therefore, they were not excluded. At the start of mammogram collection, our study group comprised 2,325 women. Of these, 264 women were excluded because their mammograms could not be retrieved from the archives. Another 14 were excluded for having silicone prostheses. Twenty-two women had breasts too large to fit on a single mammogram. The latter group had therefore more than one mammogram, which could not be used because the overlap between these mammograms could not be measured properly. A premenopausal mammogram was available for 2,025 women.

A follow-up questionnaire was filled out ~5 years after baseline by 1,607 of the 2,025 women with a premenopausal mammogram, and 1,015 women had become postmenopausal at the time that they filled out the follow-up questionnaire. Because no other information about menopausal status of study participants in the time span between baseline and filling out the follow-up questionnaire was available, answers to the follow-up questionnaire were used to determine postmenopausal status. Women were considered postmenopausal when they had not had any menses during the 12 months before filling out the follow-up questionnaire. The first mammogram after the time women filled out the follow-up questionnaire was collected for these women and could be retrieved from the archives for 695 women. None of these women were current users of oral contraceptives or postmenopausal hormone therapy or had used these compounds <2 years before filling out the follow-up questionnaire. One woman was excluded because she had silicone protheses. None of the participants had breasts too large to fit a single mammogram.

Of the 694 women with both a premenopausal and a postmenopausal mammogram available, 684 had a serum sample available to be used for IGF-I measurements.

Mammographic Density Analysis. Mammographic density was assessed using the mediolateral oblique mammogram, which is the routine view for breast cancer screening in the Netherlands. It has been observed that the proportions of mammographic density on craniocaudal views and mediolateral oblique views and on left and right views show very strong correlation and that representative information on mammographic density is provided in a single view (35). For each study subject, mammographic density was assessed on the left view.

After digitizing the films using a laser film scanner (Lumiscan 50, Lumisys, Eastman Kodak Co., Rochester, NY), mammographic density was quantified using a computer-assisted method based on gray levels of pixels in the digitized mammogram. This computer-assisted method to determine mammographic density has proved to be very reliable, and the method is described elsewhere in detail (36). Briefly, for each image, the reader first sets a threshold to determine the outside edge of the breast to separate the image of the breast from the dark background surrounding it. A second threshold is set to determine the area of dense tissue within the breast, which is the lightest tissue visible on the mammogram. The program then determines the amount of pixels within the total breast area and within the dense area.

To compute percentage breast density, the dense area of a breast is divided by the total breast area and multiplied by 100. Percentage breast density is used in most publications on breast density. However, a small-sized breast and a larger-sized breast could have the same percentage breast density, whereas the absolute amount of connective and epithelial tissue, which is regarded as the target tissue for breast cancer (37, 38), is higher in the larger breast (39). Hence, we will present results on both relative and absolute measures of breast density.

All mammograms were assessed by one observer in sets composed of randomly ordered films. Both the premenopausal and postmenopausal images of the same woman were always read in the same set, which contained 36 images. The order in which the mammograms within a set were presented to the reader was also randomized. To assess the reliability of the reader, a library set was created, which consisted of 36 randomly chosen films from our study subjects. This library set was read before the first set and at five time points between sets, which were blinded for the reader. The images in the library set were randomly ordered every time they were read to prevent the observer from recognizing this set. In this study, an average intraclass correlation coefficient of 0.99 (range, 0.99-1.00) for total breast area, 0.81 (range, 0.75-0.86) for absolute dense area, and 0.90 (range, 0.88-0.92) for percentage breast density was reached between repeated readings.

Statistical Analyses. Breast measures that were not normally distributed were square root transformed. Breast measures that were transformed included premenopausal

Table 1. General characteristics of the study population

	Total (N = 684)	Quartiles of premenopausal serum IGF-I (cutoff points in ng/mL)			
		Q1 (<94.6)	Q2 (94.6-111.3)	Q3 (111.4-132.0)	Q4 (≥132.0)
Age at baseline, y (SD)	51.4 (2.1)	51.4 (2.1)	51.7 (2.2)	51.4 (2.1)	51.1 (2.0)
Age at follow-up, y (SD)	55.8 (2.3)	55.9 (2.3)	56.1 (2.4)	55.7 (2.3)	55.4 (2.2)
BMI at baseline, kg/m^2 (SD)	25.2 (3.8)	25.2 (4.2)	25.1 (3.9)	24.9 (3.5)	25.4 (3.5)
BMI at follow-up,* kg/m^2 (SD)	25.7 (4.4)	26.0 (5.4)	25.7 (4.7)	25.5 (3.7)	25.6 (3.5)
Time between mammogram measurements,† y (SD)	5.5 (1.2)	5.7 (1.3)	5.4 (1.2)	5.4 (1.3)	5.5 (1.1)
Reproductive factors					
Age at menarche, y (SD)	13.2 (1.4)	13.2 (1.4)	13.2 (1.5)	13.2 (1.4)	13.3 (1.5)
Age at menopause, y (SD)	52.0 (2.8)	52.0 (2.5)	52.3 (2.7)	51.7 (2.9)	52.0 (2.9)
Nulliparous, n (%)	70 (11.4)	20 (11.8)	17 (10.7)	25 (14.7)	16 (9.1)
Age at first childbirth, y (SD)	25.1 (3.9)	25.4 (4.2)	25.4 (3.8)	24.9 (3.8)	24.7 (3.7)
Ever breast-feeding,‡ n (%)	458 (75.6)	114 (76.0)	111 (73.0)	109 (75.2)	124 (78.0)
Lifestyle factors, n (%)					
Ever used OC	490 (71.6)	116 (68.2)	124 (73.4)	116 (68.2)	134 (76.6)
Ever used HT	36 (5.3)	3 (1.8)	13 (7.7)	10 (5.9)	10 (5.8)
Current smoking	152 (22.2)	48 (28.2)	42 (24.9)	37 (21.8)	25 (14.3)
Ever smoked	417 (61.0)	102 (60.0)	108 (63.9)	106 (62.4)	101 (57.7)

Abbreviations: OC, oral contraceptive; HT, postmenopausal hormone therapy.
*Calculated using body weight at follow-up and body height at baseline.
†Time between the measurement of the premenopausal and the postmenopausal mammogram.
‡Among parous women only.

nondense area and dense area as well as postmenopausal nondense area, dense area, and percentage breast density. These transformed values were used in regression analyses and when calculating correlation coefficients. For ease of interpretation, presented means and 95% confidence intervals (95% CI) have been transformed to the original scale. Changes in breast measures over menopause were all normally distributed.

Means and 95% CIs of breast measures (total breast area, nondense breast area, dense breast area, and percentage breast density) by quartile level of serum IGF-I were estimated with linear regression models using the Statistical Analysis System ''General Linear Modeling'' procedure. The changes of breast measures over menopause were calculated as the absolute difference between postmenopausal and premenopausal measures. To test for linear trends over the quartiles, median values within quartiles were calculated and evaluated as a continuous variable using linear regression analysis. Regression coefficients and corresponding P values were calculated for levels of IGF-I on a continuous scale, in linear models, also using the Statistical Analysis System General Linear Modeling procedure. Models used to calculate postmenopausal means, 95% CIs, and β-estimates as well as means, 95% CIs, and β-estimates for menopausal changes in breast measures also included the premenopausal breast measure of interest as covariate. Potential confounding of various factors was assessed by adding those variables to the crude models. The following characteristics were evaluated for confounding using continuous variables: age, body mass index (BMI), change in BMI over menopause (postmenopausal models only), waist circumference, age at menarche, age at menopause (postmenopausal models only), total physical activity, time span between first and second mammography, and dietary intake of total energy, proteins, carbohydrates, fat, and alcohol. Dichotomous variables were used to evaluate ever use of oral contraceptives, ever use of postmenopausal hormone therapy, and ever breast-feeding. Parity and age at first childbirth were evaluated using a combined variable with a category for nulliparous women and three categories of parous women combined with tertiles of age at birth of the first child. Smoking was evaluated using a variable with three categories for current smokers, past smokers, and never smokers. Analytic batches of IGF-I measurements were added to the regression models using a categorical variable, as were the analytic batches of breast measurements. Postmenopausal age, BMI, oral contraceptive ever use, postmenopausal hormone therapy ever use, and smoking habits were used in the postmenopausal models. Only variables that changed crude mean breast measures with at least 1% were added to the regression models. Models used to calculate mean values, 95% CIs, and β-estimates of menopausal changes in breast measurements were adjusting for the same variables included in the postmenopausal models. Breast measurements were measured in a total of 81 batches, and adding the analytic batch of measurement only changed crude mean values of breast measures marginally. To increase the number of degrees of freedom, this variable was not included in the final models.

To evaluate potential confounding of the associations between IGF-I and breast measures by BMI, Pearson's correlation coefficients were calculated between BMI and breast measures on the one hand and serum IGF-I on the other hand.

All P values are two sided, and when <0.05, results were considered statistically significant. All analyses were conducted using the Statistical Analysis System software package, release 9.1 (SAS Institute, Cary, NC).

Results

All study participants were premenopausal at baseline, when questionnaire data were collected, a blood sample was drawn and the first mammogram was taken. Additional data were collected on average 4.4 years after baseline (interquartile range, 3.8-5.0 years) when a follow-up questionnaire was filled out. The postmenopausal mammogram was taken on average 1.1 years after this follow-up questionnaire (interquartile range, 0.6-1.4 years); thus, the average time between the dates of the premenopausal and the postmenopausal mammograms was 5.5 years.

Baseline characteristics of the study population are presented in Table 1. At baseline, mean age was 51.4 years and mean BMI was 25.2 kg/m^2. BMI increased slightly over menopause to an average 25.7 kg/m^2 at time of follow-up when all women were postmenopausal. Age at baseline was

Table 2. Premenopausal and postmenopausal breast measures and changes in breast measures over menopause

		Premenopause (baseline)	Postmenopause (follow-up)	Absolute change over menopause
Breast area (cm^2)	Median	105.4	95.0	−9.6
	Interquartile range	84.4-133.8	68.6-120.0	−25.5 to 2.2
Nondense area (cm^2)	Median	57.4	63.8	0.5
	Interquartile range	36.9-89.2	34.5-94.3	−13.5 to 14.7
Dense area (cm^2)	Median	42.7	26.8	−11.8
	Interquartile range	30.1-57.3	14.3-37.7	−23.5 to −3.9
% Breast density	Median	44.4	34.3	−6.7
	Interquartile range	27.4-59.2	16.5-52.0	−15.0 to 0.7

comparable between quartiles of premenopausal serum IGF-I, as were BMI at baseline, BMI at follow-up, age at menarche, age at menopause, being nulliparous, smoking in the past, and time span between mammogram measurements. Women with high premenopausal IGF-I levels were somewhat younger at first childbirth, and the number of women who reported to have breast-fed was highest in the upper quartile of IGF-I serum levels. There was no clear association between past smoking and IGF-I level; however, most current smokers had low IGF-I levels. Because we excluded current postmenopausal hormone therapy users from the study population, only few women reported a history of postmenopausal hormone therapy use.

Median values of total breast area, nondense area, dense area, and percentage breast density are presented in Table 2. The median of the dense area was 11.8 cm^2 smaller after menopause compared with the median dense area before menopause, likely explained by the involution of glandular tissue during menopause. Both total breast area and percentage breast density were also smaller after menopause. The nondense area (i.e., the amount of adipose tissue) slightly increased over menopause.

Total breast size as well as nondense breast area was strongly positively correlated with BMI before menopause (Table 3). These correlations were somewhat less strong after menopause. Absolute density was inversely correlated with BMI, both before and after menopause, as was percentage breast density. Circulating levels of IGF-I showed no clear linear or nonlinear relationship with BMI.

In Table 4, associations between premenopausal IGF-I levels in serum and premenopausal breast measures are presented. There was no clear linear association between premenopausal IGF-I level and total breast size, nondense area, absolute dense area, or percentage breast density, although women in the highest quartile of serum IGF-I had a slightly higher total breast area, nondense area, and dense area. As shown in Table 5, the absolute amount of breast density decreased during the menopausal transition phase. This decrease was slightly smaller in women with high premenopausal IGF-I levels than in those with low IGF-I levels, although the association was not linear (P trend = 0.58). In contrast, the nondense area increased slightly during menopause, and this increase was strongest in women with low levels of premenopausal IGF-I (P trend = 0.09). This inverse relation between IGF-I and increase in nondense area remained after corrections for confounding factors. As a consequence of these opposing effects on the absolute size of the dense tissue and the nondense tissue, the decrease in percentage breast density over menopause was lowest in women with high levels of premenopausal IGF-I (P trend = 0.06). Table 6 shows associations between premenopausal IGF-I and postmenopausal breast measures. Higher levels of premenopausal IGF-I were associated with significantly higher percentage breast density after menopause (P trend = 0.02) and with lower total breast size and nondense area (P trend = 0.21 and 0.05 for breast size and nondense area, respectively). Women with high premenopausal IGF-I levels had slightly higher absolute density (28.6 cm^2 in the highest versus 27.8 cm^2 in the lowest quartile); however, there was no evidence for a linear relationship (P trend = 0.66).

Table 3. Pearson's coefficients of correlation between breast measures, IGF-I serum levels, and BMI

	Premenopausal BMI (baseline)*	Postmenopausal BMI (follow-up)†
Breast area	0.62 (0.57-0.66)	0.46 (0.40-0.52)
Nondense area	0.61 (0.56-0.66)	0.51 (0.45-0.56)
Dense area	−0.12 (−0.19 to −0.04)	−0.15 (−0.22 to −00.7)
% Breast density	−0.46 (−0.52 to −0.40)	−0.40 (−0.46 to −0.33)
IGF-I	0.02 (−0.06 to 0.09)	−0.02 (−0.10 to 0.05)

*Correlations of premenopausal breast measurements and IGF-I serum levels with premenopausal BMI.

† Correlations of postmenopausal breast measurements and premenopausal IGF-I serum levels with postmenopausal BMI. Postmenopausal BMI was calculated with premenopausal body height and postmenopausal body weight.

Discussion

In this study, we did not find serum IGF-I levels to be associated with breast size, nondense (fat) breast tissue, or breast density before menopause. However, high levels of IGF-I before menopause were associated with lower total breast size and nondense breast tissue and with higher breast density after menopause. The association with percentage breast density after menopause was mostly explained by a smaller increase in nondense breast tissue over menopause in women with higher levels of premenopausal IGF-I.

A major strength of our study is its longitudinal design. IGF-I levels in serum as well as premenopausal breast measures were measured on average 5.5 years before the postmenopausal mammogram was taken, which enabled us to look at the effect of premenopausal circulating levels of IGF-I on the menopausal involution process of the breast. To our knowledge, the present study is the first study presenting results of premenopausal IGF-I levels and both premenopausal and postmenopausal breast measures of the same women. Another advantage of the present study is its size. With 684 participants, our study is one of the largest studies conducted thus far on circulating IGF-I levels and breast density.

All women in the present study had at least one menstrual period in the last 12 months before their first mammographic measurement and were therefore classified to be premenopausal. Part of these women were, however, probably already perimenopausal because all participants were ≥49 years of age at study entry. Furthermore, the exact date of the last menstrual period was unknown. Hence, the time to menopause (for the premenopausal mammogram) and the time from menopause (for the postmenopausal mammogram) could not be determined and may have influenced all of the measures used. Nevertheless, the time between the first and the second mammogram (5.5 years on average) will still have included the main part of the menopausal transition phase

Table 4. Cross-sectional analyses of premenopausal serum IGF-I concentrations and premenopausal breast measures

	Total	Quartiles of premenopausal serum IGF-I (cutoff points in ng/mL)				*P* trend	β-estimate *P*
		Q1 (<94.6)	Q2 (94.6-111.3)	Q3 (111.4-132.0)	Q4 (≥132.0)		
Total breast size* (cm^2)							
Mean	111.4	111.9	109.7	111.7	112.8		0.013
95% CI	105.9-117.0	105.1-118.7	103.2-116.3	105.0-118.4	106.1-119.5	0.61	0.74
Nondense area† (cm^2)							
Mean	62.1	62.0	60.1	62.5	63.9		0.001
95% CI	56.3-68.3	54.9-69.6	53.2-67.4	55.5-69.9	56.9-71.4	0.63	0.73
Dense area‡ (cm^2)							
Mean	37.6	37.7	36.8	37.5	38.6		0.002
95% CI	33.5-42.0	32.7-43.0	32.0-41.9	32.6-42.7	33.6-43.9	0.68	0.35
% Breast density§							
Mean	41.8	41.9	42.3	41.4	41.5		0.006
95% CI	38.1-45.5	37.4-46.4	37.9-46.7	37.1-45.8	37.1-45.9	0.70	0.81

NOTE: Means and 95% CIs were calculated using linear regression analyses with quartiles of premenopausal serum IGF-I. β-Estimates and corresponding *P* values were calculated for premenopausal IGF-I levels on a continuous scale using linear regression analyses. To normalize distributions, square root–transformed variables of nondense area and dense area were used in the regression models. For ease of interpretation, means and 95% CIs have been transformed to the original scale.
*Means, 95% CIs, and β-estimate were adjusted for BMI, former postmenopausal hormone therapy use, and breast-feeding.
†Means and 95% CIs were adjusted for age, BMI, former postmenopausal hormone therapy use, breast-feeding, age at menarche, age at first childbirth, parity, and analytic batch of IGF-I measurements.
‡Means and 95% CIs were adjusted for former oral contraceptive use, former postmenopausal hormone therapy use, smoking status, age at first childbirth, parity, and analytic batch of IGF-I measurements.
§Means and 95% CIs were adjusted for age, BMI, former oral contraceptive use, former postmenopausal hormone therapy use, breast-feeding, age at first childbirth, parity, and analytic batch of IGF-I measurements.

when stronger breast involution occurs compared with breast involution before menopause (27). Restricting the analyses to a selection of the women who had had at least six menstrual periods in the 12 months before their first mammogram measurement did not change the associations markedly.

A very large proportion of IGF-I in the circulation is bound to IGF-binding protein-3 (IGFBP-3), and blood levels of IGFBP-3 are therefore an important codeterminant of the fraction of IGF-I that can diffuse to target tissues. We did not have any measurement of circulating IGFBP-3 levels and thus could not adjust our analyses. It is hard to speculate if the present results would have been influenced by IGFBP-3 adjustments. IGF-I and IGFBP-3 are positively correlated, which was also shown in the larger EPIC cohort (16). IGFBP-3 may influence breast density in an IGF-I–independent manner by inducing apoptosis and inhibiting cell growth (40). Hence, IGFBP-3 is a potential confounder for the association between IGF-I levels and breast density. The positive relation between IGF-I and IGFBP-3 and the inverse effect of IGFBP-3 on breast density may have attenuated a positive association between premenopausal IGF-I levels and premenopausal breast density, both as absolute measure and as percentage of the whole breast, in the present study. Indeed, in previous studies on premenopausal women, the direct association of IGF-I with percentage breast density generally became stronger after adjustment for IGFBP-3 (20, 23, 25).

Another explanation for the absence of association between IGF-I levels and premenopausal breast density may be the

Table 5. Premenopausal serum IGF-I concentrations and changes in breast measures over menopause

	Total	Quartiles of premenopausal serum IGF-I (cutoff points in ng/mL)				*P* trend	β-estimate *P*
		Q1 (<94.6)	Q2 (94.6-111.3)	Q3 (111.4-132.0)	Q4 (≥132.0)		
Total breast size* (cm^2)							
Mean	−11.5	−8.1	−11.4	−12.7	−12.8		−0.061
95% CI	−17.0 to −5.9	−15.2 to −1.1	−18.1 to −4.7	−19.5 to −5.8	−19.5 to −6.0	0.21	0.14
Nondense area† (cm^2)							
Mean	3.7	7.3	3.6	2.6	2.1		−0.070
95% CI	−3.3 to 10.7	−0.9 to 15.4	−4.3 to 11.4	−5.3 to 10.5	−5.8 to 10.0	0.09	0.06
Dense area‡ (cm^2)							
Mean	−13.0	−12.9	−13.4	−13.2	−12.2		0.017
95% CI	−16.9 to −9.1	−17.7 to −8.2	−17.9 to −8.9	−17.8 to −8.7	−16.8 to −7.6	0.58	0.43
% Breast density§							
Mean	−8.7	−11.3	−8.6	−8.2	−7.9		0.043
95% CI	−12.3 to −5.2	−15.4 to −7.2	−12.6 to −4.7	−12.1 to −4.2	−11.9 to −3.9	0.06	0.02

NOTE: Changes over menopause were calculated as postmenopausal value − premenopausal value. Means and 95% CIs were calculated using linear regression analyses with quartiles of premenopausal serum IGF-I. β-Estimates and corresponding *P* values were calculated for premenopausal IGF-I levels on a continuous scale using linear regression analyses. To normalize distributions, square root–transformed variables of premenopausal nondense area and dense area and postmenopausal nondense area, dense area, and percentage breast density were used in the regression models. For ease of interpretation, means and 95% CIs have been transformed to the original scale.
*Means and 95% CIs were adjusted for premenopausal total breast size, BMI, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.
†Means and 95% CIs were adjusted for premenopausal nondense area, BMI, change in BMI over menopause, age at menopause, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.
‡Means and 95% CIs were adjusted for premenopausal dense area, BMI, change in BMI over menopause, smoking status, age at menopause, former postmenopausal hormone therapy use, age at first childbirth, and parity.
§Means and 95% CIs were adjusted for premenopausal percentage breast density, BMI, change in BMI over menopause, age at menopause, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.

Table 6. Premenopausal serum IGF-I concentrations and postmenopausal breast measures

	Total	Quartiles of premenopausal serum IGF-I (cutoff points in ng/mL)				*P* trend	β-estimate *P*
		Q1 (<94.6)	Q2 (94.6-111.3)	Q3 (111.4-132.0)	Q4 (≥132.0)		
Total breast size* (cm^2)							
Mean	100.0	103.4	100.1	98.9	98.7		−0.061
95% CI	94.5-105.6	96.3-110.5	93.5-106.8	92.0-105.7	92.0-105.5	0.21	0.14
Nondense area† (cm^2)							
Mean	65.4	70.5	65.9	63.6	63.6		−0.006
95% CI	57.5-73.7	61.1-80.6	57.1-75.2	55.0-72.9	54.9-72.8	0.05	0.03
Dense area‡ (cm^2)							
Mean	27.8	27.8	27.8	27.1	28.6		0.001
95% CI	23.9-31.9	23.4-32.6	23.6-32.4	22.9-31.7	24.2-33.4	0.66	0.52
% Breast density§							
Mean	30.1	27.8	29.8	30.4	31.4		0.004
95% CI	26.7-33.6	24.2-31.8	26.1-33.7	26.7-34.4	27.6-35.5	0.02	0.01

NOTE: Means and 95% CIs were calculated using linear regression analyses with quartiles of premenopausal serum IGF-I. β-Estimates and corresponding *P* values were calculated for premenopausal IGF-I levels on a continuous scale using linear regression analyses. To normalize distributions, square root–transformed variables of premenopausal nondense area and dense area were used in the regression models.

*Means and 95% CIs were adjusted for premenopausal total breast size, BMI, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.

†Means and 95% CIs were adjusted for premenopausal nondense area, BMI, change in BMI over menopause, age at menopause, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.

‡Means and 95% CIs were adjusted for premenopausal dense area, BMI, change in BMI over menopause, smoking status, age at menopause, former postmenopausal hormone therapy use, age at first childbirth, and parity.

§Means and 95% CIs were adjusted for premenopausal percentage breast density, BMI, change in BMI over menopause, age at menopause, former oral contraceptive use, former postmenopausal hormone therapy use, and analytic batch of IGF-I measurements.

relative old age of our study population at baseline (51.4 years) compared with the average age of premenopausal women in studies that did find higher IGF-I levels to be associated with higher breast density (range, 42.9-48.3 years; refs. 20, 21, 23, 25). Maybe a premenopausal association between IGF-I levels and breast density decreases on menopause. However, two other studies on this association, which were in the same age range (43.3 and 46.5 years), also did not find IGF-I levels and breast density to be associated (22, 24).

Our hypothesis was that IGF-I, with its mitogenic and antiapoptotic properties, increases the amount of dense tissue, which is regarded as the target tissue for breast cancer (37, 38). Results of epidemiologic studies showing IGF-I levels to be associated with increased breast density and also with increased breast cancer risk support this hypothesis, although the associations were only seen in premenopausal women (9-25). Given these effects and the fact that IGF-I gradually decreases with age (28), we have hypothesized that postmenopausal risk could be mainly determined before menopause when IGF-I levels are still high. Our finding of the association between high premenopausal IGF-I levels and increased postmenopausal breast density supports this hypothesis and is in line with results of a study recently published by Rollison et al. (29), showing increased postmenopausal breast cancer risk in women with high premenopausal IGF-I levels. However, we only found IGF-I levels to be associated with the relative measure of breast density and not with the absolute breast density measure, although women in the highest quartile of IGF-I levels had a slightly higher dense area. The association between IGF-I and relative breast density (percentage breast density) was mainly driven by the inverse association of IGF-I with the nondense area (fat tissue), which is part of the denominator of the algorithm to calculate relative breast density. These findings are not in line with the idea that IGF-I increases the number of glandular cells (dense tissue) and, in this regard, do not support the hypothesis that IGF-I can increase breast cancer risk through an increase in dense tissue. Most studies on IGF-I and breast density do not report results of the absolute dense area. Only two studies reporting increased percentage breast density with high IGF-I levels also reported associations with the absolute dense area. Maskarinec et al. (21) found comparable associations for the relative and absolute measures, but Boyd et al. (25) showed a somewhat stronger effect for relative breast density compared with absolute density. A third study did not show any association with either the relative or the absolute measure of breast density (24). Nondense area was only reported by two studies. The first found a small, nonsignificant inverse correlation (21), and the second reported a borderline significant association but only among postmenopausal women, which disappeared after correction for confounding factors (24).

The lack of association between premenopausal IGF-I levels and premenopausal breast density mirrors the lack of association between IGF-I circulating levels and breast cancer risk in the larger EPIC study reported by Rinaldi et al. (16). The present study is not a subset of that study, although there is a small overlap (n = 21). IGF-I measurements were done independently using a different method. Therefore, the lack of association could be more than coincidence. Genetic variation in the IGF-I gene was, however, associated with increased cancer risk within the EPIC cohort, especially among women aged ≤55 years (41). More research is therefore needed to elucidate the exact role of IGF-I in breast density and breast cancer development.

In the present study, IGF-I levels were borderline significantly associated with smaller increase of the nondense area during the menopausal transition phase and, consequently, with smaller nondense area after menopause. IGF-I is known to reflect levels of growth hormone, which provides the key stimulus for IGF-I synthesis in the liver and in many other tissues. Growth hormone has strong lipolytic actions and will tend to reduce adipose tissue mass (42-46). Furthermore, a study by Mulhall et al. (47) showed two polymorphisms in the growth hormone gene to be associated with increased growth hormone levels and decreased nondense area. This may explain the borderline significant trend for menopausal change in nondense area (fat) and postmenopausal nondense area over the quartiles of IGF-I observed in the present study.

An artifact in measurement of breast density may give another explanation for the lack of association between circulating levels of IGF-I and the absolute measure of breast density we observed. Possibly the absolute measurement of breast density and changes therein are more prone to

measurement error than the relative measure caused by the influence of the level of compression of the breast when making a two-dimensional image. If the number of glandular cells really is associated with breast cancer risk, this implies that the absolute measure of breast density is preferred over the relative measure. Hopefully in the near future, the culprit of the compression of the breast during mammography will be solved using a volumetric method. This method, using digital mammography, adjusted for compression and the amount of radiation used, will enable us to measure absolute density with much more precision (48).

In conclusion, high levels of circulating IGF-I before menopause were associated with a slightly smaller decrease of absolute dense tissue over menopause. Consequently, absolute density after menopause was larger in women with higher premenopausal levels of IGF-I, although differences between extreme quartiles of absolute breast density were small and the data did not support a linear relationship. Hence, these findings only to some extend support the hypothesis that premenopausal IGF-I levels increase postmenopausal breast density. The significant association between premenopausal IGF-I levels and postmenopausal percentage breast density was mainly explained by the smaller increase in nondense area during the menopausal transition phase in women with high levels of IGF-I.

References

1. Boyd NF, Lockwood GA, Martin LJ, et al. Mammographic density as a marker of susceptibility to breast cancer: a hypothesis. IARC Sci Publ 2001; 154:163–9.
2. 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.
3. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995;87:670–5.
4. Van Gils CH, Hendriks JH, Holland R, et al. Changes in mammographic breast density and concomitant changes in breast cancer risk. Eur J Cancer Prev 1999;8:509–15.
5. Kleinberg DL. Role of IGF-I in normal mammary development. Breast Cancer Res Treat 1998;47:201–8.
6. Pollak MN. Endocrine effects of IGF-I on normal and transformed breast epithelial cells: potential relevance to strategies for breast cancer treatment and prevention. Breast Cancer Res Treat 1998;47:209–17.
7. Lee AV, Yee D. Insulin-like growth factors and breast cancer. Biomed Pharmacother 1995;49:415–21.
8. Sachdev D, Yee D. The IGF system and breast cancer. Endocr Relat Cancer 2001;8:197–209.
9. 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.
10. Toniolo P, Bruning PF, Akhmedkhanov A, et al. Serum insulin-like growth factor-I and breast cancer. Int J Cancer 2000;88:828–32.
11. Krajcik RA, Borofsky ND, Massardo S, et al. Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1566–73.
12. Muti P, Quattrin T, Grant BJ, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2002;11: 1361–8.
13. Allen NE, Roddam AW, Allen DS, et al. A prospective study of serum insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3, and breast cancer risk. Br J Cancer 2005;92:1283–7.
14. Schernhammer ES, Holly JM, Pollak MN, et al. Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:699–704.
15. 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.
16. Rinaldi S, Peeters PH, Berrino F, et al. IGF-I, IGFBP-3, and breast cancer risk in women: The European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer 2006;13:593–605.
17. Schernhammer ES, Holly JM, Hunter DJ, et al. Insulin-like growth factor-I, its binding proteins (IGFBP-1 and IGFBP-3), and growth hormone and breast cancer risk in The Nurses Health Study II. Endocr Relat Cancer 2006; 13:583–92.
18. Keinan-Boker L, 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 2003;106:90–5.
19. Gronbaek H, Flyvbjerg A, Mellemkjaer L, et al. Serum insulin-like growth factors, insulin-like growth factor binding proteins, and breast cancer risk in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:1759–64.
20. Byrne C, Colditz GA, Willett WC, et al. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000;60:3744–8.
21. Maskarinec G, Williams AE, Kaaks R. A cross-sectional investigation of breast density and insulin-like growth factor I. Int J Cancer 2003;107:991–6.
22. Lai JH, Vesprini D, Zhang W, et al. A polymorphic locus in the promoter region of the IGFBP3 gene is related to mammographic breast density. Cancer Epidemiol Biomarkers Prev 2004;13:573–82.
23. Diorio C, Pollak M, Byrne C, et al. Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005;14:1065–73.
24. dos Santos Silva I, Johnson N, De Stavola B, et al. The insulin-like growth factor system and mammographic features in premenopausal and post-menopausal women. Cancer Epidemiol Biomarkers Prev 2006;15:449–55.
25. Boyd NF, Stone J, Martin LJ, et al. The association of breast mitogens with mammographic densities. Br J Cancer 2002;87:876–82.
26. 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.
27. Boyd N, Martin L, Stone J, et al. A longitudinal study of the effects of menopause on mammographic features. Cancer Epidemiol Biomarkers Prev 2002;11:1048–53.
28. 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.
29. Rollison DE, Newschaffer CJ, Tao Y, et al. Premenopausal levels of circulating insulin-like growth factor I and the risk of postmenopausal breast cancer. Int J Cancer 2006;118:1279–84.
30. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 Suppl 1:S6–14.
31. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
32. Boker LK, Van Noord PA, van der Schouw YT, et al. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. European Prospective Investigation into Cancer and Nutrition. Eur J Epidemiol 2001; 17:1047–53.
33. Ocke MC, Bueno-de-Mesquita HB, Pols MA, et al. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26 Suppl 1:S49–58.
34. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26 Suppl 1:S37–48.
35. Byng JW, Boyd NF, Little L, et al. Symmetry of projection in the quantitative analysis of mammographic images. Eur J Cancer Prev 1996;5:319–27.
36. Byng JW, Boyd NF, Fishell E, et al. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629–38.
37. Trichopoulos D, Lipman RD. Mammary gland mass and breast cancer risk. Epidemiology 1992;3:523–6.
38. Albanes D, Winick M. Are cell number and cell proliferation risk factors for cancer? J Natl Cancer Inst 1988;80:772–4.
39. Haars G, Van Noord PA, Van Gils CH, et al. Measurements of breast density: no ratio for a ratio. Cancer Epidemiol Biomarkers Prev 2005;14:2634–40.
40. Ali O, Cohen P, Lee KW. Epidemiology and biology of insulin-like growth factor binding protein-3 (IGFBP-3) as an anti-cancer molecule. Horm Metab Res 2003;35:726–33.
41. Canzian F, McKay JD, Cleveland RJ, et al. Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. Br J Cancer 2006;94:299–307.
42. Djurhuus CB, Gravholt CH, Nielsen S, et al. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. Am J Physiol Endocrinol Metab 2004;286:E488–94.
43. Louveau I, Gondret F. Regulation of development and metabolism of adipose tissue by growth hormone and the insulin-like growth factor system. Domest Anim Endocrinol 2004;27:241–55.
44. Flint DJ, Binart N, Kopchick J, et al. Effects of growth hormone and prolactin on adipose tissue development and function. Pituitary 2003;6:97–102.
45. Hansen TK, Gravholt CH, ORskov H, et al. Dose dependency of the pharmacokinetics and acute lipolytic actions of growth hormone. J Clin Endocrinol Metab 2002;87:4691–8.
46. Moller N, Gjedsted J, Gormsen L, et al. Effects of growth hormone on lipid metabolism in humans. Growth Horm IGF Res 2003;13 Suppl A:S18–21.
47. Mulhall C, Hegele RA, Cao H, et al. Pituitary growth hormone and growth hormone-releasing hormone receptor genes and associations with mammographic measures and serum growth hormone. Cancer Epidemiol Biomarkers Prev 2005;14:2648–54.
48. van Engeland S, Snoeren PR, Huisman H, et al. Volumetric breast density estimation from full-field digital mammograms. IEEE Trans Med Imaging 2006;25:273–82.

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