Insulin-Like Growth Factor-I, IGF-Binding Protein-3, and Mammographic Breast Density

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Abstract

Some studies have suggested that insulin-like growth factor (IGF) pathway is related to premenopausal breast density, one of the strongest known breast cancer risk factors. This study was designed specifically to test the hypothesis that higher levels of IGF-I and lower levels of IGF-binding protein (IGFBP)-3 are associated with high mammographic breast density among premenopausal but not among postmenopausal women. A total of 783 premenopausal and 791 postmenopausal healthy women were recruited during screening mammography examinations. Blood samples were collected at the time of mammography, and plasma IGF-I and IGFBP-3 levels were measured by ELISA. Mammographic breast density was estimated using a computer-assisted method. Spearman's partial correlation coefficients (r_s) were used to evaluate the associations. Adjusted mean breast density was assessed by joint levels of IGF-I and IGFBP-3 using generalized linear models. Among premenopausal women, high levels of IGF-I and low levels of IGFBP-3 were independently correlated with high breast density (r_s = 0.083; P = 0.021 and r_s = −0.124; P = 0.0005, respectively). Correlation of IGF-I with breast density was stronger among women in the lowest tertile of IGFBP-3 than among those in the highest tertile of IGFBP-3 (r_s = 0.138; P = 0.027 and r_s = −0.039; P = 0.530, respectively). In contrast, the correlation of IGFBP-3 with breast density was stronger among women in the highest tertile of IGF-I than among those in the lowest tertile of IGF-I (r_s = −0.150; P = 0.016 and r_s = −0.008; P = 0.904, respectively). Women in the combined top tertile of IGF-I and bottom tertile of IGFBP-3 had higher mean breast density than those in the combined bottom tertile of IGF-I and top tertile of IGFBP-3 (53.8% versus 40.9%; P = 0.014). No significant association was observed among postmenopausal women. Our findings confirm that IGF-I and IGFBP-3 are associated with breast density among premenopausal women. They provide additional support for the idea that, among premenopausal women, these growth factors may affect breast cancer risk, at least in part, through their influence on breast tissue morphology as reflected on mammogram. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1065–73)

Introduction

Insulin-like growth factor (IGF)-I is a well-established mitogen for breast tissue (1). In the bloodstream, IGF-I is bound to one of several IGF-binding proteins (IGFBP). Among these, IGFBP-3 carries >95% of circulating IGF-I (2). In addition to prolonging IGF-I half-life and modulating its biological activities in serum, tissue IGFBP-3 can promote apoptosis independently of IGF-I (3, 4).

There is growing evidence that IGF-I may contribute to the progression of several human cancers (5, 6), including breast cancer (7), whereas IGFBP-3 has been proposed as an anticancer protein (8). Women with acromegaly have clinically higher levels of IGF-I (9) and have an increased incidence of breast cancer compared with the general population (10-12). Moreover, high circulating levels of IGF-I were consistently found to be positively associated with breast cancer risk in premenopausal women (13-21), with few exceptions (22-25). Among postmenopausal women, some studies observed an IGF-I to breast cancer association (18, 24, 26) but most did not (14-17, 19-21, 23, 25, 27-29). Relationship between levels of IGFBP-3 and breast cancer risk is less clear. In studies conducted in premenopausal women, some observed that higher circulating levels of IGFBP-3 were associated with low breast cancer risk (13, 14), whereas positive (16, 17, 19-21) or null associations (15, 22-24) were found by others. Only two (20, 21) of several studies (14-17, 19, 23, 24, 28, 29) showed a positive association of IGFBP-3 with breast cancer risk in postmenopausal women. Finally, Bohlke et al. (13) examined the joint effect of IGF-I and IGFBP-3 on incidence of ductal carcinoma *in situ*. Their data suggest that premenopausal women with a combination of high levels of IGF-I and low levels of IGFBP-3 had an elevated risk of ductal carcinoma *in situ* of the breast compared with those with a combination of low levels of IGF-I and high levels of IGFBP-3.

Mammographic breast density is one of the strongest risk factors for breast cancer (30). Data from three small cross-sectional studies suggest that the extent of mammographic breast density, among premenopausal women, may be associated with high levels of IGF-I and low levels of IGFBP-3 (31-33). No association has been observed among postmenopausal women (31, 32, 34). Thus, the growth factor-breast density associations seem to mirror the growth factor-breast cancer relations.

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This cross-sectional study was designed specifically to determine whether plasma levels of IGF-I, IGFBP-3, and the molar ratio IGF-I/IGFBP-3 (an indicator of bioavailability of IGF-I) were separately related to mammographic breast density among premenopausal and postmenopausal women. Data also allowed examination of the combined relation of IGF-I and IGFBP-3 with breast density.

Materials and Methods

Study Population and Recruitment Procedures. The study subjects were women who received a screening mammogram between February 2001 and March 2002 at two private radiology clinics. Women were considered to be having a screening mammogram if they were referred for (*a*) a mammography within the Quebec organized breast cancer screening program (Programme québécois de dépistage du cancer du sein), (*b*) a routine periodic mammography in the absence of any breast problem (such as family history of breast cancer) even if outside of the Programme québécois de dépistage du cancer du sein, or (*c*) a routine periodic mammography for follow-up of a known and stable benign breast condition.

To be eligible for the present study, women were either premenopausal if they had at least one natural menstrual cycle within 12 months or were younger than 48 years (if a nonsmoker) or 46 years (if a smoker) after hysterectomy without bilateral oophorectomy or use of hormonal derivatives (35). They were considered postmenopausal if they reported complete cessation of menses for at least 12 months, radiation-induced menopause, or bilateral oophorectomy or were at least ages 56 years (if a nonsmoker) or 54 years (if a smoker) after hysterectomy without bilateral oophorectomy or use of hormonal derivatives (35). Finally, eligibility was restricted to women not taking hormone medication, including oral contraceptives or postmenopausal hormones, within 3 months of the mammography, never having used tamoxifen or raloxifene, not pregnant, without a history of cancer at any site, without breast reduction or implants, and without diabetes mellitus, dwarfism/acromegaly, or thyroid, adrenal, or hepatic disease. No restriction criteria on age were applied.

Eligible women who accepted to participate provided written consent, including authorization for blood sampling and banking of samples, to provide information on breast cancer risk factors, to borrow, digitize, evaluate, and keep a digitized copy of their mammogram, and to review medical records to obtain the results of the mammographic examination, including pathologic findings. Women with known cognitive deficit of any cause were excluded because of impaired ability to provide informed consent.

Of the 9,559 women who received a screening mammogram and were approached, 1,021 refused to participate in our study. In the remaining 8,538 women, 6,924 were ineligible because they were using hormonal derivatives (n = 4,987) or did not meet other eligibility criteria (n = 1,937). A total of 800 premenopausal and 814 postmenopausal women were identified as potentially eligible for the study and provided informed consent. Among these women, 7 women (n = 1 premenopausal and n = 6 postmenopausal) were found ineligible during the interview because they had had a breast reduction (n = 1 postmenopausal), they used hormone replacement therapy within the last 3 months (n = 1 premenopausal and n = 3 postmenopausal), they used raloxifene (n = 1 postmenopausal), or they had uncertain menopausal status (n = 1 postmenopausal). After the review of the reports provided by the radiologists, 9 women (n = 8 premenopausal and n = 1 postmenopausal) were excluded because they did not meet our definition of screening mammogram and 7 women (n = 4 premenopausal and n = 3 postmenopausal) were excluded because the investigation recommended by the radiologists following their screening mammogram led to a diagnosis of breast cancer. In the remaining 787 premenopausal and 804 postmenopausal women, a blood sample could not be obtained for 3 postmenopausal women and film mammograms were not available for 3 women (n = 2 premenopausal and n = 1 postmenopausal). Finally, 10 women (n = 2 premenopausal and n = 8 postmenopausal) declined to be interviewed and 1 postmenopausal woman revoked her participation. Therefore, a total of 783 premenopausal and 791 postmenopausal women were eligible for the present analysis. Of those, 99.5% were recruited at the Clinique Radiologique Audet (n = 1,566) and 8 were recruited at the Clinique de radiologie Saint-Pascal.

Data Collection

Anthropometric Measures and Blood Sampling at Time of Mammography. Women wearing light clothing without shoes were weighed (kg), and height (cm) was measured by a trained research nurse. Waist circumference was measured using a soft tape midway between the lowest rib margin and the iliac crest in the standing position, and hip circumference was measured over the widest of the gluteal region. From these measurements, the body mass index (BMI; kg/m^2) and waist-to-hip ratio (WHR; an indicator of central body fat distribution) were obtained. For each woman, blood (20 mL) was drawn and fasting status was recorded as the number of hours since last meal. Anthropometric measures and blood sampling occurred at time of mammography for 95.4% of the subjects (n = 1,501), with an average ± SD of 0.4 ± 1.9 day between the time of the mammogram and when the blood was drawn. For premenopausal women, the first day of the last menstrual cycle was documented. In addition, a calendar was distributed to indicate the first day of the menstrual cycle after their mammogram and to transmit this information during the phone interview. Age (years) at time of the mammogram was recorded for all women. Finally, each woman received a validated (36) and self-administered semiquantitative food frequency questionnaire (97GP copyrighted at Harvard University) and was requested to return it by mail once completed. Intake of foods obtained through the questionnaire was translated into nutrient intake, including energy intake (kcal/d), at the Channing Laboratory of Harvard University (Boston, MA). This semiquantitative questionnaire was answered by 99.3% of women (n = 1,563).

Information during Telephone Interview. Data on potential breast cancer risk factors were collected by trained interviewers using a questionnaire designed for this study. Risk factors for breast cancer included reproductive history, family history of breast cancer, history of breast biopsies, past use of hormonal derivatives, smoking status, alcohol intake, education, and physical activity. For the latter, the level of physical activity in metabolic equivalents-hour/wk was assessed using the Nurses' Health Study II Activity and Inactivity Questionnaire (37) and the classification by Ainsworth et al. (38) for the metabolic equivalent. Phone interviews took place on average ± SD of 27 ± 13 days after the mammogram; 72.7% of the subjects had their interview within 1 month of their screening mammogram.

Digitization of Mammograms and Assessment of Mammographic Features. All mammograms were digitized using a Kodak Lumiscan85 digitizer at 260 μm per pixel (0.067 mm^2 per pixel), which creates a 12-bit gray scale image that is linear in the absorbance range 0 to 4.0. Calibration of the scanner was verified before each utilization. All mammograms were reviewed by one of the authors (C.D.). This reviewer was trained in the assessment of breast density using a set of mammographic images (n = 110) previously read by one of the authors (C.B.) who has experience in the assessment

of breast density by computer-assisted method (31, 39-41). After the training period, proficiency in assessment of breast density was evaluated comparing C.D.'s readings with those of C.B.'s based on an additional 220 mammograms. The intraclass correlation coefficients of the mammographic features, including breast density and total and dense regions, between these two readers were 0.97, 0.98, and 0.96, respectively.

Assessment of mammographic features was done, without any knowledge of the participants status or medical history, using a computer-assisted method developed by one of us (M.Y.) and described elsewhere (42-44). Breast density was measured for one craniocaudal view for each woman, the right or left view being chosen randomly. The mammograms were read in batches of at least 100 images. A typical batch included one craniocaudal view of 80 women (n = 40 premenopausal and n = 40 postmenopausal). The batch also included 10 images chosen at random among the initial group of 80 images allowing assessment of intrabatch variability. Moreover, in all batches, the same group of 10 images was inserted to assess the interbatch variability. The 100 images of each batch were randomly ordered. For two batches, craniocaudal views of both breasts were included to assess variability of density between left and right breasts. For the mammographic breast density measurements in the present study, the within-batch intraclass correlation coefficient was 0.98 and the between-batch coefficient of variation was 4%. These measures of variability were similar for premenopausal and postmenopausal women. In addition, the mean difference in breast density between the right and the left breasts was 0.56% and the intraclass correlation coefficient between both sides was 0.95. All 21 batches were read within 1 month.

Laboratory Measures of IGF-I and IGFBP-3. At the time of mammography, blood specimens collected were kept on ice until they were submitted for centrifugation. Blood constituents were then aliquoted and stored at −80°C until analysis. Time between blood donation and blood constituents storage, including plasma, was <3 hours for 99.4% of the subjects for an average ± SD of 123 ± 37 minutes. Aliquots of frozen plasma were sent on dry ice in batches of 39 samples for laboratory analyses without any information on women. Blinded split samples were randomly included in each batch (four samples per batch) to allow assessment of intraassay and interassay variabilities of laboratory measurements. Under the supervision of one of us (M.P.), IGF-I and IGFBP-3 were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX). For the present study, the intrabatch coefficients of variation were 10.5% and 13.2% and the between-batch coefficients of variation were 7.9% and 10.5% for IGF-I and IGFBP-3, respectively.

Statistical Methods. Univariate and multivariate associations between continuous levels of growth factors (IGF-I, IGFBP-3, or IGF-I/IGFBP-3 molar ratio) and continuous measures of breast density were evaluated with the Spearman correlation coefficient (r_s). The molar ratio IGF-I/IGFBP-3 was calculated as: [0.130 × level of IGF-I (ng/mL)] / [0.036 × level of IGFBP-3 (ng/mL)], which has been suggested to reflect availability of IGF-I in tissue (45). Multivariate-adjusted mean breast density by category of growth factors was calculated using generalized linear model sum of squares error estimates. The same approach was used to obtain multivariate-adjusted mean level of growth factors by category of breast density. Statistical significance was based on two-sided Ps.

In the present analysis, factors included as confounders in multivariate models were age (years), BMI (kg/m^2), and IGF-I (ng/mL) or IGFBP-3 (ng/mL) among premenopausal women. Among postmenopausal women, parity (yes/no) was also included in models. Further adjustment for factors potentially associated with breast density and/or levels of growth factors

Table 1. Characteristics of the study population

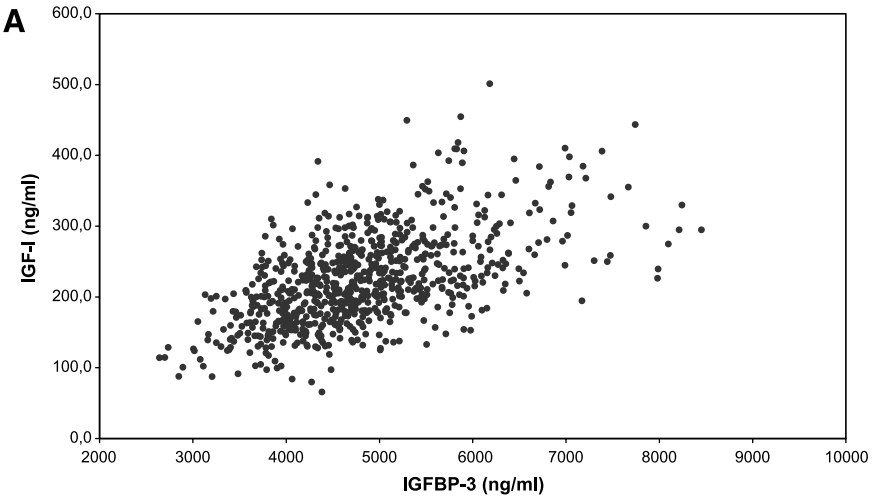
	Premenopausal women (n = 783)*	Postmenopausal women (n = 791)†
Age (y), mean (SD)	46.8 (4.6)	61.4 (6.8)
Age at menarche (y), mean (SD)	12.8 (1.6)	12.7 (1.6)
Age at first full-term pregnancy (y),‡ mean (SD)	26.3 (4.2)	25.2 (4.1)
No. full-term pregnancies, mean (SD)	1.6 (1.1)	2.1 (1.8)
BMI (kg/m^2), mean (SD)	25.2 (4.6)	27.1 (4.7)
WHR, mean (SD)	0.78 (0.06)	0.81 (0.06)
Weight (kg), mean (SD)	65.0 (12.1)	67.3 (11.9)
Height (cm), mean (SD)	160.5 (5.8)	157.6 (5.6)
Waist circumference (cm), mean (SD)	79.7 (10.8)	85.2 (11.3)
Hip circumference (cm), mean (SD)	101.6 (8.9)	104.8 (9.4)
Physical activity (metabolic equivalents-hour/wk), mean (SD)	26.9 (22.2)	25.7 (23.4)
Energy intake (kcal/d), mean (SD)	1,912 (521)	1,978 (669)
Alcohol intake (drinks/wk), mean (SD)	3.4 (3.8)	2.5 (4.4)
Parity (parous), %	75.4	74.8
Lactation (yes),‡ %	62.2	28.8
Use of hormonal derivatives (ever),§ %	91.8	70.3
Family history of breast cancer in first-degree relative (yes), %	37.1	30.7
History of breast biopsies (yes), %	14.4	16.1
Smoking status (never), %	45.5	59.0
Education (college or university diploma), %	62.1	39.2
Breast density (%), median (range)	41.2 (0.1-92.9)	13.6 (0-82)
Dense region (cm^2), median (range)	43.6 (0.1-163.7)	18.1 (0-180.8)
Nondense region (cm^2), median (range)	64.0 (5.3-360.1)	116.6 (11.0-453.8)
Total region (cm^2), median (range)	114.4 (34.2-389.2)	138.8 (34.5-456.5)
IGF-I (ng/mL), median (range)	218.0 (65.6-501.1)	184.3 (42.2-511.7)
IGFBP-3 (ng/mL), median (range)	4,696 (2,643-8,451)	4,806 (2,126-9,581)
IGF-I/IGFBP-3 molar ratio, median (range)	0.17 (0.05-0.33)	0.14 (0.05-0.33)

*Missing values for age at menarche (n = 19), physical activity (n = 1), energy intake (n = 6), alcohol intake (n = 4), family history of breast cancer in first-degree relative (n = 7), and education (n = 1).

†Missing values for age at menarche (n = 21), WHR (n = 4), waist (n = 3), hip (n = 4), physical activity (n = 2), energy intake (n = 5), alcohol intake (n = 3), lactation (n = 2), family history of breast cancer in first-degree relative (n = 8), and education (n = 1).

‡Among parous women.

§Contraceptives and/or replacement therapy.



IGFBP-3 (ng/ml) / IGF-I (ng/ml)	> 2000; ≤ 3000	> 3000; ≤ 4000	> 4000; ≤ 5000	> 5000; ≤ 6000	> 6000; ≤ 7000	> 7000; ≤ 8000	> 8000; ≤ 9000	> 9000; ≤ 10000	Total
> 500; ≤ 600	-	-	-	-	0.1	-	-	-	0.1
> 400; ≤ 500	-	-	-	0.9	0.1	0.3	-	-	1.3
> 300; ≤ 400	-	0.3	2.6	3.5	2.3	1.2	0.1	-	10.0
> 200; ≤ 300	-	4.7	24.1	16.9	4.7	0.8	0.4	-	51.6
> 100; ≤ 200	0.5	12.0	18.7	4.5	0.3	0.1	-	-	36.1
> 0; ≤ 100	0.1	0.5	0.5	-	-	-	-	-	1.1
Total	0.6	17.5	45.9	25.8	7.5	2.4	0.5	-	100.2

Figure 1. Scatter plots of IGF-I and IGFBP-3 levels among (**A**) premenopausal (●) and (**B**) postmenopausal (▲) women and the percentage of premenopausal (**A**) and postmenopausal (**B**) women by joint levels of IGF-I and IGFBP-3.

(age at menarche, number of full-term pregnancies, age at first full-term pregnancy, lactation, WHR, family history of breast cancer, history of breast biopsies, smoking, alcohol intake, education, past use of oral contraceptive, past use of hormone-replacement therapy, physical activity, and energy intake) did not materially alter the results. Therefore, they were not added in the models. All statistical analyses were carried out using the SAS (SAS Institute, Inc., Cary, NC) software system.

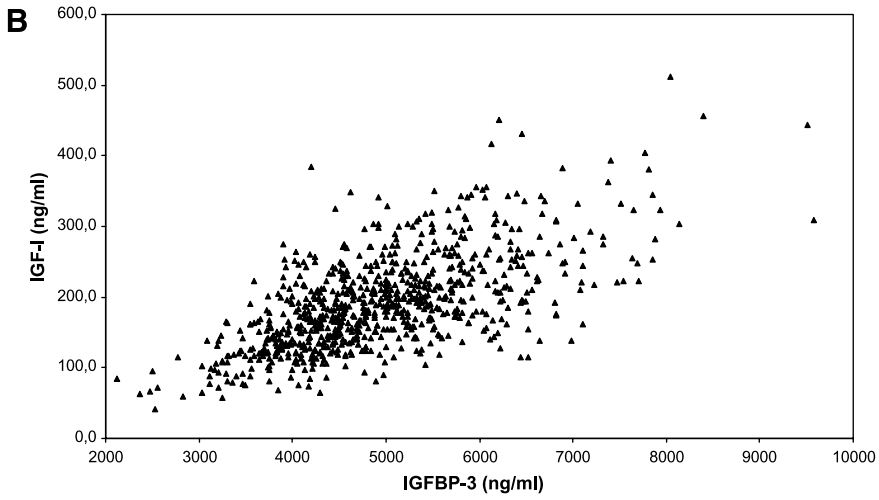
Results

The characteristics of the study population of 783 premenopausal and 791 postmenopausal women are described in Table 1. In summary, the mean age was 46.8 years for premenopausal women and 61.4 years for postmenopausal women. Postmenopausal women had greater mean anthropometric measurements than premenopausal women, with the exception of height. Premenopausal women reported more frequent previous use of hormonal derivatives (91.8% versus 70.3%) and a family history of breast cancer (37.1% versus 30.7%) than postmenopausal women. Premenopausal women had higher median breast density (41.2% versus 13.6%) than postmenopausal women.

IGF-I and IGFBP-3 levels varied by menopausal status (Table 1; Fig. 1). Median level of IGF-I was higher in premenopausal compared with postmenopausal women (218.0 versus 184.3 ng/mL; Table 1). In addition, 63.0% of premenopausal women had levels of IGF-I >200 ng/mL compared with 39.9% of postmenopausal women (Fig. 1A and B). In contrast, premenopausal women had lower median level of IGFBP-3 compared with postmenopausal women (4,696 versus 4,806 ng/mL; Table 1). Percentage of women with low levels of IGFBP-3 (≤5,000 ng/mL) was higher in premenopausal than in postmenopausal women (64.0% versus 56.9%; Fig. 1A and B). The joint distribution of IGF-I and IGFBP-3 also varied by menopausal status (Fig. 1A and B). For instance, the correlation of IGF-I levels with IGFBP-3 levels was weaker in premenopausal women ($r_s = 0.552$; $P < 0.0001$) than in postmenopausal women ($r_s = 0.628$; $P < 0.0001$). The percentage of premenopausal women with a combination of higher levels of IGF-I (>200 ng/mL) and lower levels of IGFBP-3 (≤5,000 ng/mL) was more than twice the percentage seen among postmenopausal women (31.7% versus 12.7%; Fig. 1A and B). In contrast, the percentage of women with lower IGF-I (≤200 ng/mL) and higher IGFBP-3 (>5,000 ng/mL) was substantially lower in premenopausal compared with postmenopausal women (4.9% versus 15.9%; Fig. 1A and B).

Table 2 shows that, among premenopausal women, levels of IGF-I were positively correlated with breast density after adjustment for confounding factors ($r_s = 0.083$; $P = 0.021$). Multivariate-adjusted negative correlation between IGFBP-3 levels and breast density was also significant in premenopausal women ($r_s = -0.124$; $P = 0.0005$). Breast density was positively correlated with the molar ratio in premenopausal women before and after adjustment for confounding factors ($r_s = 0.162$; $P < 0.0001$ and $r_s = 0.069$; $P = 0.056$, respectively). No association was observed among postmenopausal women after adjustment for confounding factors.

Figure 2 shows mean breast density by joint levels of IGF-I and IGFBP-3 after adjustment for age and BMI among premenopausal women. The multivariate-adjusted mean breast density was 12.9% higher in the combined top tertile of IGF-I and bottom tertile of IGFBP-3 than the combined bottom tertile of IGF-I and top tertile of IGFBP-3 (53.8% versus 40.9%; $P = 0.014$). In the lowest tertile of IGFBP-3, adjusted mean breast density was higher by ascending levels of IGF-I (42.7%, 47.1%, and 53.8%, respectively), but this relation was not seen in the highest tertile of IGFBP-3 (40.9%, 38.7%, and 39.5%, respectively). Stratified analysis (Table 3) showed that the multivariate-adjusted correlation between IGF-I and breast density was stronger in the lowest tertile of IGFBP-3 ($r_s = 0.138$;



IGFBP-3 (ng/ml) / IGF-I (ng/ml)	> 2000; ≤ 3000	> 3000; ≤ 4000	> 4000; ≤ 5000	> 5000; ≤ 6000	> 6000; ≤ 7000	> 7000; ≤ 8000	> 8000; ≤ 9000	> 9000; ≤ 10000	Total
> 500; ≤ 600	-	-	-	-	-	-	0.1	-	0.1
> 400; ≤ 500	-	-	-	-	0.4	0.1	0.1	0.1	0.7
> 300; ≤ 400	-	-	0.8	2.3	2.0	1.0	0.1	0.1	6.3
> 200; ≤ 300	-	1.1	10.8	13.3	5.6	2.0	-	-	32.8
> 100; ≤ 200	0.1	12.0	27.1	12.4	3.4	0.1	-	-	55.1
> 0; ≤ 100	0.9	2.8	1.3	-	-	-	-	-	5.0
Total	1.0	15.9	40.0	28.0	11.4	3.2	0.3	0.2	100.0

Figure 1. Continued.

$P = 0.027$) compared with the third tertile of IGFBP-3 ($r_s = -0.039$; $P = 0.530$). Similarly, within the highest tertile of IGF-I, the adjusted mean breast density was lower with ascending levels of IGFBP-3 (53.8%, 41.4%, and 39.5%). From Table 3, multivariate-adjusted correlation of IGFBP-3 with breast density was stronger in the highest tertile of IGF-I ($r_s = -0.150$; $P = 0.016$) compared with the lowest tertile of IGF-I ($r_s = -0.008$; $P = 0.904$).

Among premenopausal women, multivariate-adjusted correlation of growth factors with breast density varied according to some anthropometric measures (Table 4). Magnitude of the correlation of IGF-I with breast density was stronger in the top tertile of height and in the bottom tertile of other anthropometric measurements. In contrast, IGFBP-3 and breast density was negatively and significantly correlated in the second tertile of weight, BMI, waist and hip circumferences, and WHR and in the top tertile of height.

In stratified analysis using the WHO cutoff for BMI (<25 kg/m^2, normal and thin), stronger correlations among IGF-I, IGFBP-3, and the molar ratio with breast density were observed in premenopausal women with BMI of <25 kg/m^2 ($r_s = 0.129$; $P = 0.007$, $r_s = -0.124$; $P = 0.010$, and $r_s = 0.119$; $P = 0.013$, respectively, for $n = 437$) compared with premenopausal women with BMI of ≥25 kg/m^2 ($r_s = 0.013$; $P = 0.813$, $r_s = -0.090$; $P = 0.095$, and $r_s = 0.0003$; $P = 0.996$, respectively, for $n = 346$).

Among premenopausal women, 37.1% reported a family history of breast cancer and 91.8% had ever used hormonal derivatives. Correlation of IGF-I, IGFBP-3, and molar ratio with breast density were similar among women without a family history of breast cancer ($r_s = 0.093$; $P = 0.041$, $r_s = -0.133$; $P = 0.003$, and $r_s = 0.084$; $P = 0.065$, respectively, for $n = 488$) and among those with such a history ($r_s = 0.065$; $P = 0.271$, $r_s = -0.121$; $P = 0.041$, and $r_s = 0.059$; $P = 0.321$, respectively, for $n = 288$). In contrast, we observed that breast density was more strongly correlated with IGF-I, IGFBP-3, and molar ratio among women that had never used hormonal derivatives ($r_s = 0.230$; $P = 0.075$, $r_s = -0.286$; $P = 0.026$, and $r_s = 0.232$; $P = 0.070$, respectively, for $n = 64$) compared with women who had ever used hormonal derivatives ($r_s = 0.066$; $P = 0.076$, $r_s = -0.105$; $P = 0.005$, and $r_s = 0.051$; $P = 0.175$, respectively, for $n = 719$).

Among premenopausal women, correlation of growth factors with breast density were similar among women with regular menstrual cycle (21-35 days) to those with an irregular menstrual cycle or who had an hysterectomy (data not shown). Among women with regular cycles, the magnitude of the correlation between growth factors and breast density was not materially altered by further adjustment of the phase of menstrual cycle at the time of the mammogram (data not shown).

No association of growth factors with breast density was observed within strata of any breast cancer risk factor among postmenopausal women (data not shown).

Eligibility to the present study was restricted to women not taking hormonal derivatives within 3 months of the mammography. Results were essentially unchanged after exclusion of those who used hormonal derivatives within the past 6 or 12 months of the mammography. For instance, exclusion of women using hormonal derivatives within the past 12 months of the mammography had little or no effect on the correlation of IGF-I, IGFBP-3, and molar ratio with breast density in either premenopausal women ($r_s = 0.088$; $P = 0.017$, $r_s = -0.125$; $P = 0.0006$, and $r_s = 0.071$; $P = 0.052$, respectively, for $n = 754$) or postmenopausal women ($r_s = 0.031$; $P = 0.409$, $r_s = -0.019$; $P = 0.605$, and $r_s = 0.030$; $P = 0.432$, respectively, for $n = 713$).

Discussion

Our data confirm that higher IGF-I and lower IGFBP-3 levels are independently related to high mammographic breast density in premenopausal but not in postmenopausal women. In addition, the strength of the association of IGF-I with breast

Table 2. Relations of IGFs and breast density

	Premenopausal women (n = 783)				Postmenopausal women (n = 791)			
	n	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I/IGFBP-3 molar ratio	n	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I/IGFBP-3 molar ratio
Breast density (%)		Mean values of IGFs*				Mean values of IGFs*		
<5.0	48	212.9	5,061	0.160	196	185.0	5,023	0.135
5.0-24.9	169	225.7	4,889	0.169	369	193.0	4,962	0.141
25.0-44.9	217	221.5	4,847	0.167	158	197.8	4,803	0.144
45.0-64.9	193	223.9	4,732	0.169	53	188.5	5,009	0.136
≥65.0	156	229.9	4,694	0.175	15	218.2	4,513	0.160
Type of adjustment		$r_s^{\dagger}$ (P)				$r_s^{\dagger}$ (P)		
Crude		0.046 (0.194)	−0.152 (<0.0001)	0.162 (<0.0001)		0.123 (0.0005)	0.030 (0.399)	0.132 (0.0002)
IGF-I or IGFBP-3 (if applicable)		0.158 (<0.0001)	−0.213 (<0.0001)			0.134 (0.0002)	−0.061 (0.086)	
Confounding factors*		0.083 (0.021)	−0.124 (0.0005)	0.069 (0.056)		0.032 (0.365)	−0.013 (0.724)	0.029 (0.410)

*Means and correlations are adjusted for age (years), BMI (kg/m^2), IGF-I (ng/mL), or IGFBP-3 (ng/mL) if applicable.
†Spearman correlation between continuous variables. Adjusted correlations are partial Spearman coefficients.

density appeared stronger at low levels of IGFBP-3, whereas the strength of the association of IGFBP-3 with breast density seemed stronger at high levels of IGF-I. These results support the idea that premenopausal women with high levels of IGF-I and low levels of IGFBP-3 have higher mammographic breast density and may have an increased risk of breast cancer.

Among postmenopausal women, all studies to date, including our own, have shown little or no association of IGF-I or IGFBP-3 with breast density (31, 32, 34). Among premenopausal women, results have been less consistent (31-34). One study failed to show an IGF-I to breast density association (34). Among the three studies (31-33) finding that higher IGF-I levels were associated with high breast density, the strength of observed associations varied. Compared with our data, the strength of the correlation was greater in the Nurses' Health Study (ref. 31; r_s = 0.36; P = 0.007) but was similar in the study conducted by Maskarinec et al. (ref. 33; r_s = 0.11; P = 0.06). Boyd et al. (32) used the coefficient of determination of the unadjusted association (R^2 = 0.05) to evaluate this relation impairing such comparison. Two of the studies (31, 33) found that high levels of IGFBP-3 were related to lower breast density. Compared with our findings, the magnitude of the correlation was stronger in the Nurses' Health Study (r_s = −0.24; P = 0.07) but not in the study of Maskarinec et al. (r_s = −0.15; P = 0.02). Finally, the only histologic study we know of observed that amounts of IGF-I in breast tissue were higher in women with high mammographic breast density compared with amounts in those with low breast density, and this association was stronger for women ages <50 years (46). The presence of associations between growth factors and breast density in premenopausal but not in postmenopausal women might be due, at least in part, to differences in the distribution of IGF-I and IGFBP-3 among these two groups of women. All of the above studies, including ours, observed higher mean/median levels of IGF-I and lower mean/median levels of IGFBP-3 in premenopausal women compared with postmenopausal women (31, 32, 34). In the Nurses' Health Study, correlation of IGF-I and IGFBP-3 levels was also stronger among postmenopausal (r_s = 0.59) than in premenopausal (r_s = 0.43) women.

Among premenopausal women, we found that the association of IGF-I with mammographic breast density was stronger

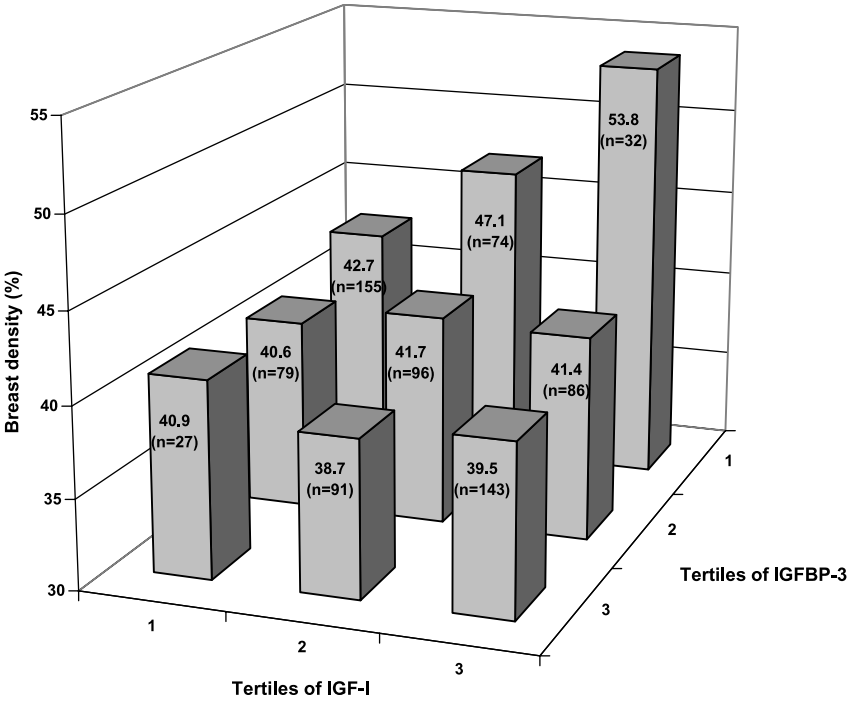


Figure 2. Multivariate-adjusted breast density means for joint relation of IGF-I with IGFBP-3 in premenopausal women. The subjects were cross-classified according to both tertiles of IGF-I (≤193.941, 193.942-246.058, and >246.058 ng/mL) and tertiles of IGFBP-3 (≤4,353.9, 4,354.0-5,036.7, and >5,036.7 ng/mL). Mean breast density for each combined category of growth factors adjusted for age and BMI is given with the number of subjects in parentheses for each column.

Table 3. Correlations of IGFs with breast density by tertiles of IGFs among premenopausal women

	n	r_s* (*P*)		
		IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I/IGFBP-3 molar ratio
IGF-I (ng/mL)				
≤193.941	261	—	−0.008 (0.904)	—
193.942-246.058	261	—	−0.178 (0.004)	—
>246.058	261	—	−0.150 (0.016)	—
IGFBP-3 (ng/mL)				
≤4,353.9	261	0.138 (0.027)	—	0.139 (0.025)
4,354.0-5,036.7	261	0.096 (0.124)	—	0.091 (0.146)
>5,036.7	261	−0.039 (0.530)	—	−0.024 (0.706)

*Spearman correlation between continuous variables. Adjusted correlations are partial Spearman coefficients. Adjusting for age (years), BMI (kg/m^2), IGF-I (ng/mL), or IGFBP-3 (ng/mL) if applicable.

at low compared with high levels of IGFBP-3. Similarly, the association of IGFBP-3 with breast density was stronger at high compared with low IGF-I levels. Thus, the highest breast density was observed for women with the combination of high IGF-I and low IGFBP-3. To our knowledge, combined IGF-I and IGFBP-3 levels have not been investigated in relation with breast density. However, the combination of high IGF-I with low IGFBP-3 levels is related to an increased risk of ductal carcinoma *in situ* of the breast among premenopausal women compared with those with a combination of low IGF-I and high IGFBP-3 (13). Prospective data from the Physicians' Health Study on advanced-stage prostate cancer risk (47) and colorectal cancer risk (48) also suggest that patients with a combination of high IGF-I and low IGFBP-3 levels incur the greatest risk.

The strength of association of growth factors with breast density may vary substantially according to some characteristics of women. Among premenopausal women, stronger association of IGF-I and IGFBP-3 levels with breast density was observed among taller women. In addition, a stronger association was observed between IGF-I and breast density in leaner women. These results are consistent with the only previous study that reported a potential modifying effect of BMI, using the WHO cutoff, on the association of IGF-I levels and the molar ratio with breast density, but statistical significance was reached only for the molar ratio (33). Therefore, the variability in the strength of association of growth factors with breast density among premenopausal women observed across studies might be explained, at least in part, by variations in the characteristics of women in those studies. On the other hand, studies that examined the modifying effect of BMI on the association of growth factors with breast cancer risk found inconsistent results (17, 20). For instance, Yu et al. observed a stronger positive association of IGF-I and IGFBP-3 levels with breast cancer risk in a population of premenopausal and postmenopausal women with high BMI or high WHR (20). Muti et al. observed no effect modification of BMI on these associations in premenopausal women but found a stronger positive association between IGF-I levels and breast cancer risk among postmenopausal women with high BMI (17).

This study has several strengths. Firstly, the quality of the mammographic images was maximized. Almost all mammograms were done in the same clinic with the same equipment (mammography units, LORAD M4) that was accredited by the Canadian Association of Radiology in addition to satisfying the high-quality standards of the Quebec breast cancer screening program. This clinic rigorously follows the quality control protocol recommended by the Canadian Association of Radiology, including the development of high-contrast mammographic films. Secondly, quantitative measures of breast density were obtained without any information on women, using a computer-assisted method, in a short period of time, by one reader whose reliability of reading was shown to be high. Although the density of only one breast was measured, the concordance of the measures between right and left breasts in this study was high. Thus, the misclassification of breast

Table 4. Correlations of IGFs with breast density by tertiles of anthropometric measures among premenopausal women

	n	r_s* (*P*)		
		IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I/IGFBP-3 molar ratio
Height (cm)				
≤157	254	−0.009 (0.888)	0.016 (0.801)	−0.033 (0.600)
158-162	261	0.093 (0.137)	−0.147 (0.018)	0.107 (0.086)
>162	268	0.140 (0.022)	−0.216 (0.0004)	0.120 (0.050)
Weight (kg)				
≤58.6	260	0.126 (0.044)	−0.089 (0.156)	0.118 (0.059)
58.7-67.4	258	0.072 (0.253)	−0.216 (0.0005)	0.080 (0.203)
>67.4	265	0.031 (0.616)	−0.057 (0.356)	0.005 (0.940)
BMI (kg/m^2)				
≤22.876	260	0.108 (0.083)	−0.089 (0.155)	0.097 (0.121)
22.877-26.094	261	0.080 (0.199)	−0.211 (0.0007)	0.088 (0.157)
>26.094	262	0.043 (0.491)	−0.035 (0.578)	0.016 (0.794)
Waist circumference (cm)				
≤73	249	0.107 (0.095)	−0.068 (0.288)	0.089 (0.161)
74-82	278	0.079 (0.193)	−0.190 (0.002)	0.084 (0.163)
>82	256	0.047 (0.458)	−0.045 (0.479)	0.017 (0.785)
Hip circumference (cm)				
≤97	271	0.116 (0.058)	−0.098 (0.111)	0.106 (0.082)
98-103	240	0.077 (0.236)	−0.258 (<0.0001)	0.072 (0.266)
>103	272	0.016 (0.800)	−0.008 (0.897)	0.024 (0.670)
WHR				
≤0.7526	260	0.098 (0.117)	−0.054 (0.387)	0.083 (0.181)
0.7527-0.8034	261	0.089 (0.156)	−0.185 (0.003)	0.055 (0.375)
>0.8034	262	0.065 (0.298)	−0.102 (0.101)	0.052 (0.401)

*Spearman correlation between continuous variables. Adjusted correlations are partial Spearman coefficient. Adjusting for age (years), BMI (kg/m^2), IGF-I (ng/mL), or IGFBP-3 (ng/mL) if applicable.

density should be relatively small, most likely be random, and therefore should not have biased our results. Thirdly, circulating levels of IGF-I and IGFBP-3 were each measured within 1 month using the same type of reagents for all assays. The laboratory analyses were done without any information on women, and the reliability of these measures was also shown to be high. Thus, our findings are unlikely to be explained by random misclassification of the measurements of the analytes. Fourth, for 95.4% of women, the blood was drawn on the same day as the mammogram, eliminating the potential problem of timing of density and growth factor measurements. Fifth, several factors potentially related to breast density and/or growth factors were documented and their confounding effects were assessed and taken into account when necessary. Finally, the effective sample size is relatively large.

This study has some limitations. Women in the present study reported a family history of breast cancer more frequently than those in other studies on the same topic (31-34). However, associations of growth factors with breast density appeared as strong in women with a family history than in those without such a history. Residual effect by past exogenous hormones use could be possible because eligibility in our study was restricted to women not taking hormonal derivatives within 3 months of the mammography. However, our results were essentially unchanged after exclusion of those who used hormonal derivatives within the past 6 or 12 months of the mammography. Blood collection (and mammography) was not timed with a specific phase of the menstrual cycle among premenopausal women. In our data, phase of the menstrual cycle was associated with levels of IGF-I but not with levels of IGFBP-3 or with breast density. Moreover, additional adjustment for phase of the menstrual cycle at time of the mammogram had essentially no confounding effect in these data. Finally, blood was not drawn after a period of fasting. However, no association was observed between the number of hours since last meal with neither IGF-I, IGFBP-3, nor breast density. Moreover, further adjustment for time since last meal did not materially alter our results.

Mammographic breast density is an estimate of the extent of fibroglandular tissue (including stromal and epithelial cells) in relation to fat. Laboratory studies proposed that IGF-I is able to stimulate both stromal and epithelial human breast cell growth (49, 50). Likewise, IGFBP-3 may have an IGF-independent inhibitory effect on epithelial human breast cell growth (49) but an IGF-dependent inhibitory effect on stromal human breast cell growth (50). Because mammographic breast density is strongly associated with breast cancer risk (30), our results provide additional support for the idea that IGF-I and IGFBP-3 may act on breast cancer development through their influence on the morphogenesis of breast tissue at least among premenopausal women.

The temporality of the relation between growth factors and breast density cannot be determined due to the cross-sectional design. If causality is nonetheless confirmed by prospective data, it will suggest that mammographic breast density should be evaluated as an intermediate marker in studies aimed at developing or evaluating interventions that are thought to act, at least in part, by affecting the IGF-breast cancer pathway.

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References

1. 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.
2. Le Roith D. Insulin-like growth factors. N Engl J Med 1997;336:633–40.
3. Baxter R. Signalling pathways involved in antiproliferative effects of IGFBP-3: a review. Mol Pathol 2001;54:145–8.
4. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
5. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev 2000;21:215–44.
6. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505–18.
7. Sachdev D, Yee D. The IGF system and breast cancer. Endocr Relat Cancer 2001;8:197–209.
8. 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.
9. van der Lely AJ, de Herder WW, Janssen JA, Lamberts SW. Acromegaly: the significance of serum total and free IGF-I and IGF-binding protein-3 in diagnosis. J Endocrinol 1997;155 Suppl 1:S9–13; discussion S15–6.
10. Baris D, Gridley G, Ron E, et al. Acromegaly and cancer risk: a cohort study in Sweden and Denmark. Cancer Causes Control 2002;13:395–400.
11. Cheung NW, Boyages SC. Increased incidence of neoplasia in females with acromegaly. Clin Endocrinol (Oxf) 1997;47:323–7.
12. Popovic V, Damjanovic S, Micic D, et al. Increased incidence of neoplasia in patients with pituitary adenomas. The Pituitary Study Group. Clin Endocrinol (Oxf) 1998;49:441–5.
13. Bohlke K, Cramer DW, Trichopoulos D, Mantzoros CS. Insulin-like growth factor-I in relation to premenopausal ductal carcinoma *in situ* of the breast. Epidemiology 1998;9:570–3.
14. Bruning PF, Van Doorn J, Bonfrer JM, et al. Insulin-like growth-factor-binding protein 3 is decreased in early-stage operable pre-menopausal breast cancer. Int J Cancer 1995;62:266–70.
15. 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.
16. Krajcik RA, Borofsky ND, Massardo S, Orentreich N. Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1566–73.
17. 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.
18. Peyrat JP, Bonneterre J, Hecquet B, et al. Plasma insulin-like growth factor-I (IGF-I) concentrations in human breast cancer. Eur J Cancer 1993;29A:492–7.
19. Toniolo P, Bruning PF, Akhmedkhanov A, et al. Serum insulin-like growth factor-I and breast cancer. Int J Cancer 2000;88:828–32.
20. Yu H, Jin F, Shu XO, et al. Insulin-like growth factors and breast cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev 2002;11:705–12.
21. Yu H, Shu XO, Li BD, et al. Joint effect of insulin-like growth factors and sex steroids on breast cancer risk. Cancer Epidemiol Biomarkers Prev 2003;12:1067–73.
22. 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.
23. Hirose K, Toyama T, Iwata H, Takezaki T, Hamajima N, Tajima K. Insulin, insulin-like growth factor-I and breast cancer risk in Japanese women. Asian Pac J Cancer Prev 2003;4:239–46.
24. Kaaks R, Lundin E, Manjer J, 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.
25. Petridou E, Papadiamantis Y, Markopoulos C, Spanos E, Dessypris N, Trichopoulos D. Leptin and insulin growth factor I in relation to breast cancer (Greece). Cancer Causes Control 2000;11:383–8.
26. Agurs-Collins T, Adams-Campbell LL, Kim KS, Cullen KJ. Insulin-like growth factor-1 and breast cancer risk in postmenopausal African-American women. Cancer Detect Prev 2000;24:199–206.
27. Jernstrom H, Barrett-Connor E. Obesity, weight change, fasting insulin, proinsulin, C-peptide, and insulin-like growth factor-1 levels in women with and without breast cancer: the Rancho Bernardo Study. J Womens Health Gend Based Med 1999;8:1265–72.
28. 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.
29. Schairer C, Hill D, Sturgeon SR, et al. Serum concentrations of IGF-I, IGFBP-3 and c-peptide and risk of hyperplasia and cancer of the breast in postmenopausal women. Int J Cancer 2004;108:773–9.
30. Harvey JA, Bovbjerg VE. Quantitative assessment of mammographic breast density: relationship with breast cancer risk. Radiology 2004;230:29–41.
31. 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.
32. Boyd NF, Stone J, Martin LJ, et al. The association of breast mitogens with mammographic densities. Br J Cancer 2002;87:876–82.
33. 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.
34. Lai JH, Vesprini D, Zhang W, Yaffe MJ, Pollak M, Narod SA. 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.
35. London SJ, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Speizer FE. Prospective study of relative weight, height, and risk of breast cancer. J Am Med Assoc 1989;262:2853–8.

36. Caan BJ, Slattery ML, Potter J, Quesenberry CP, Coates AO, Schaffer DM. Comparison of the Block and the Willett self-administered semiquantitative food frequency questionnaires with an interviewer-administered dietary history. Am J Epidemiol 1998;148:1137–47.
37. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. Int J Epidemiol 1994;23:991–9.
38. Ainsworth BE, Haskell WL, Leon AS, et al. Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993;25:71–80.
39. Byrne C, Hankinson SE, Pollak M, Willett WC, Colditz GA, Speizer FE. Insulin-like growth factors and mammographic density. Growth Horm IGF Res 2000;10 Suppl A:S24–5.
40. Byrne C, Schairer C, Brinton LA, et al. Effects of mammographic density and benign breast disease on breast cancer risk (United States). Cancer Causes Control 2001;12:103–10.
41. Haiman C, Hankinson S, De VI, et al. Polymorphisms in steroid hormone pathway genes and mammographic density. Breast Cancer Res Treat 2003; 77:27–36.
42. 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.
43. 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.
44. Byng JW, Yaffe MJ, Lockwood GA, Little LE, Trichtler DL, Boyd NF. Automated analysis of mammographic densities and breast carcinoma risk. Cancer 1997;80:66–74.
45. Juul A, Dalgaard P, Blum WF, et al. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-1, IGFBP-2, age, sex, body mass index, and pubertal maturation. J Clin Endocrinol Metab 1995;80: 2534–42.
46. 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.
47. Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst 2002;94:1099–106.
48. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999;91:620–5.
49. Strange K, Wilkinson D, Emerman J. Mitogenic properties of insulin-like growth factors I and II, insulin-like growth factor binding protein-3 and epidermal growth factor on human breast epithelial cells in primary culture. Breast Cancer Res Treat 2002;75:203–12.
50. Strange KS, Wilkinson D, Edin G, Emerman JT. Mitogenic properties of insulin-like growth factors I and II, insulin-like growth factor binding protein-3 and epidermal growth factor on human breast stromal cells in primary culture. Breast Cancer Res Treat 2004;84:77–84.

Insulin-Like Growth Factor-I, IGF-Binding Protein-3, and Mammographic Breast Density

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