Longitudinal Changes in IGF-I and IGFBP-3, and Mammographic Density among Postmenopausal Women

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Abstract
A relation between the breast cancer risk factors, insulin-like growth factor-I (IGF-I) and mammographic density, is biologically plausible, but results from cross-sectional epidemiologic studies have been mixed. Our objective was to examine the relation in a longitudinal manner, that is, between the change in circulating IGF-I concentrations and the change in mammographic measures over one year. Data from an exercise intervention trial conducted in 302 postmenopausal women ages 50 to 74 years were used. Blood drawn at baseline and postintervention was assessed for IGF-I and its binding protein (IGFBP-3) by direct chemiluminescent immunoassay. Area and volumetric measurements of mammographic dense fibroglandular and nondense fatty tissue were made. Statistical analyses were based on multiple linear regression. A one SD (20.2 ng/mL) change in IGF-I over one year was associated with small changes in percent dense area [mean: 0.8%; 95% confidence interval (CI), 0.1–1.4] and dense area (mean: 1.2 cm2; 95% CI, 0.2–2.1). Change in IGFBP-3 was also associated with percent and absolute dense area. Absolute and percent dense volume, and mammographic measures representing fatty tissue (nondense area and volume) were not associated with changes in IGF-I and IGFBP-3. Longitudinal associations may be more detectable than cross-sectional associations due to the absence of confounding by invariant personal factors. Absolute and percent dense area, measures that are related to breast cancer risk, may be affected by IGF-I. Confirmation should be sought in further longitudinal studies in which larger changes in the IGF system are evoked. Cancer Epidemiol Biomarkers Prev; 22(11); 2116–20. ©2013 AACR.

Introduction
Mammographic density is a risk factor for breast cancer. Having more than 75% of the breast area with radiologically dense tissue area puts women at four to six times higher risk than having less than 5% dense area (1). As this association may manifest because mammographic density reflects breast epithelial proliferation or mutagenesis (2), factors thought to affect breast cancer risk via similar mechanisms may also be related to mammographic density. Insulin-like growth factor-I (IGF-I), a circulating peptide, may be such a factor because its levels are associated with breast cancer risk (3) and it is mitogenic and inhibits apoptosis in breast epithelial cells (4). Despite this biologic plausibility, an association has not been observed between circulating IGF-I concentrations and percent dense area in most studies of postmenopausal women (5–16). As these studies were cross-sectional, how mammographic density changes as IGF-I levels change has not been examined. Furthermore, volumetric measures of mammographic density that may better reflect the true amounts of breast tissues have also not been examined with respect to IGF-I. Therefore, our objective was to determine, using longitudinal (within-woman) methods, the association of IGF-I and its binding protein, IGFBP-3, with both area and volumetric measures of mammographic density.

Materials and Methods
To address the present objective, data from a randomized controlled trial of a year-long aerobic exercise intervention on putative breast cancer intermediate endpoints (i.e., adiposity, sex hormones, growth factors, and mammographic density) conducted between 2003 and 2005 were used (17, 18). Included were women who were 50 to 74 years of age, were postmenopausal for at least 24...
months, had normal fasting blood glucose (<7 mmol/L), did not use hormone replacement therapy within the last 12 months, and had a body mass index (BMI) 22 to 40 kg/m². Excluded were women who were taking medications, herbs, vitamins, or supplements known to affect the study measurements, whose breasts were rated to be entirely fatty by the study radiologist (T. Terry) at baseline, or who had breast augmentation or reduction. The study was reviewed by the Research Ethics Boards at the University of Calgary, the University of Alberta (Alberta, Canada), and the Alberta Cancer Board. All participants were informed about the nature of the study and signed a consent form.

A self-administered Baseline Health Questionnaire collected information including demographics and reproductive and medical history. Exercise physiologists measured weight, height, and waist circumference at baseline and 12 months. BMI was calculated as weight in kilograms divided by height in meter squared.

Fasting blood was collected at baseline (60 mL) and 12 months (40 mL). Participants were asked not to exercise for 24 hours beforehand. All blood samples were processed and stored within 12 hours of collection at −86°C. IGF-I and IGFBP-3 were measured by direct chemiluminescent immunoassay using an Immulite analyzer (Siemens Healthcare Diagnostics) at the Reproductive Endocrine Research Laboratory at the University of Southern California (Los Angeles, CA; F.Z. Stanczyk). Each participant’s samples from all time points were included in a single batch in random order. Laboratory personnel were blinded to the identity of the samples. For IGF-I and IGFBP-3, intra-assay coefficient of variations (CV) were 2% and 7%, and inter-assay CVs were 4% and 7%.

Film screen craniocaudal mammograms done within 6 months [median: 70 days; interquartile range (IQR): 48–115 days] of baseline and at 12 months were digitized with a Lumisys 85 laser film scanner. Area measurements made on the digitized mammograms were done using Cumulus computer-assisted thresholding software. To allow volumetric measurements, the mammography machines had been calibrated throughout the study with a tissue-equivalent phantom and were equipped with a thin aluminum step wedge that was non-obtrusively imaged with each film to compensate for variations in exposures and film processing (19). Thus the optical density at each pixel on the film could be related to the proportion of nondense fatty to dense fibroglandular tissue. The dense volume, nondense volume, breast volume, and percent dense volume were calculated.

We used linear regression models to estimate the association within women, that is, the association between change in concentrations of IGF-I and IGFBP-3 from baseline to 12 months with change in mammographic measures from baseline to 12 months. Associations were adjusted for study site (Calgary, Edmonton), change in BMI, and change in waist circumference. Other covariates (i.e., age, baseline BMI, ethnicity, years since menopause, past use of postmenopausal hormones, benign breast disease, alcohol consumption, intervention group, and time between baseline mammogram and blood draw) did not change the regression parameters of interest by more than 10% and were, therefore, not included in the models.

The significance of the interaction terms representing the product of intervention group (aerobic exercise, control) and the IGF variables was used to test for effect modification. As interactions were not significant, the intervention groups were analyzed together. Effect modification by BMI (<30, ≥30 kg/m²) and time since menopause (<10, ≥10 years) were also tested. For purposes of comparison with cross-sectional results, we also used linear regression to estimate the associations between the mean of the baseline and 12-month concentrations of IGF-I and IGFBP-3 against the mean of the baseline and 12-month mammographic measurements while controlling for age, site, benign breast disease, BMI, and waist circumference. All analyses were done using SAS, version 9.2 (SAS Institute).

Results

Included were 302 women in the analysis of area mammographic measurements and 282 women in the analysis of volumetric mammographic measurements. Participant characteristics are shown in Table 1. The median (IQR) age was 60.4 (56.5–64.4) years, and BMI was 28.3 (25.5–31.2) kg/m². Most women (90.4%) were Caucasian; the rest were Asian (6.6%), Aboriginal (1.3%), Latina (1.3%), or other (1.3%). We have previously reported that the aerobic exercise intervention did not have an effect on IGF-I and IGFBP-3 (17). Changes in BMI and waist circumference were associated with changes in circulating IGF-I (partial correlations adjusting for age and site were −0.13 and −0.14), but not with changes in IGFBP-3. Changes in IGF-I were moderately correlated with changes in IGFBP-3 (r = 0.31).

The associations between changes in IGF-I, IGFBP-3, and their ratio (IGF-I/IGFBP-3) and changes in mammographic measures adjusted for study site and changes in BMI and waist circumference are shown in Table 2. Correlations of these IGF measurements were not observed with nondense area or nondense volume from baseline to 12 months. With respect to mammographic measures of the amount of fibroglandular tissue in the breast, correlations with IGF-I and IGFBP-3 were observed with dense area (r = 0.14 and 0.20, respectively) and percent dense area (r = 0.13 and 0.18, respectively). Effect modification of these associations by BMI and time since menopause was not observed (P > 0.05; data not shown). Changes in dense volume were not correlated with changes in IGF-I, IGFBP-3, or their ratio. In analyses done for comparison with cross-sectional studies, no associations were observed between the mean of the baseline and 12-month concentrations of IGF-I and IGFBP-3 and the...
mean of the baseline and 12-month mammographic measurements (data not shown).

Discussion

In this analysis of data from 302 postmenopausal women, changes in IGF-I and IGFBP-3 over 1 year were associated with changes in mammographic dense area and percent dense area. In contrast, most cross-sectional studies of postmenopausal women have not observed an association after adjustment for covariates (5, 7–13, 15). The exception is a single study that observed a direct association with IGF-I and the IGF-I/IGFBP-3 ratio (14). Two other studies observed associations only among specific subgroups: a direct association with IGF-I specifically in women with BMI < 25 kg/m² (16) and an inverse association with the IGF-I/IGFBP-3 ratio only among former users of hormone replacement therapy (6). Moreover, no consistent association has been observed between mammographic density and either specific polymorphisms in or variation across the IGF1 and IGFBP3 genes (20, 21), which could better reflect differences in IGF-I exposure across the lifespan.

Associations within women in longitudinal studies may be more easily detected than associations between women in cross-sectional studies. In longitudinal studies, personal factors that do not change over time will be completely controlled, and the inclusion of each woman’s samples in the same laboratory batch will eliminate interbatch variation in analyte concentrations. Furthermore, the time span over which the changes were examined in the current study had only minor variability among women; adjustment for time between the baseline mammogram and blood draw did not alter the observed associations, reducing the possibility that changes in age confounded the results. On the other hand, changes in

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Table 1. Characteristics of 302 women with mammographic area measurements

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clemson site</td>
<td>152 (50.3)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>273 (90.4)</td>
</tr>
<tr>
<td>Past use of postmenopausal hormones</td>
<td>142 (47.0)</td>
</tr>
<tr>
<td>Benign breast disease</td>
<td>75 (24.9)</td>
</tr>
</tbody>
</table>

Baseline percent dense volume 14.6 (5.8)
Baseline IGF-I (ng/mL) 117 (97–141)
Baseline IGFBP-3 (µg/mL) 3.9 (3.5–4.4)
Baseline IGF-I/IGFBP-3 (ng/µg) 29.7 (26.1–35.3)

Change in IGF-I (ng/mL) –2 (–3–9)
Change in IGFBP-3 (µg/mL) –0.1 (–0.3–0.2)
Change in IGF-I/IGFBP-3 (ng/µg) –0.2 (–3.2–2.3)

Baseline percent dense area 14.6 (5.8–27.3)
Baseline percent dense volume 1.2 (0.4–3.7)

Change in percent dense area 0.0 (–2.9–2.8)
Change in percent dense volume –0.1 (–5.2–0.8)

aAll other participants were from the city of Edmonton.
bTo convert to a molar ratio, multiply by 0.00361.

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Table 2. Longitudinal associations between IGF-I, IGFBP-3, and mammographic measures

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mammographic measure</th>
<th>Association with area mammographic measures</th>
<th>Association with volume mammographic measures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Nondense</td>
<td>–0.01</td>
<td>–0.3</td>
</tr>
<tr>
<td></td>
<td>Dense</td>
<td>0.14</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Percent dense</td>
<td>0.13</td>
<td>0.8</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Nondense</td>
<td>–0.11</td>
<td>–2.5</td>
</tr>
<tr>
<td></td>
<td>Dense</td>
<td>0.20</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Percent dense</td>
<td>0.18</td>
<td>1.1</td>
</tr>
<tr>
<td>IGF-I/IGFBP-3</td>
<td>Nondense</td>
<td>0.06</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Dense</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Percent dense</td>
<td>0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

aPearson correlations between the difference of the 12-month and baseline IGF measurements and the difference of the 12-month and baseline mammographic measurements. They are adjusted for site (Calgary, Edmonton), change in BMI, and change in waist circumference.
bThe regression parameters represent the estimated mean increase in the mammographic variable per SD of the factor. The SDs are: IGF-I (20.2 ng/mL), IGFBP-3 (0.41 µg/mL), and IGF-I/IGFBP-3 (5.1 ng/µg).
IGF-I concentration in women over a single year are of lesser magnitude than differences between women. Additional information about the effect of IGF-I on mammographic density may come from clinical trials of agents targeting the growth hormone/IGF axis for breast cancer chemoprevention or treatment.

We detected an association between change in levels of IGF-I and change in dense area, but not dense volume. Volumetric mammographic measurements are theoretically more relevant to breast cancer development, as they better represent the volume of fibro glandular tissue in which breast tumors arise. In epidemiologic studies, however, the volumetric measurements used here do not add information about breast cancer risk beyond what has already explained by area measurements (19). One reason that area measurements may be more strongly related to breast cancer and other risk factors is that information about breast thickness is not factored into area measures, meaning that the variation in dense area may be attributable to both the amount of fibro glandular tissue and breast thickness. How much a breast can be deformed or compressed for a mammogram could incorporate information about the fiber density and tensile strength of the collagen in the breast, which is another aspect that could be etiologically relevant (22). Alternatively, error introduced to the volumetric measurements by inaccuracy in breast thickness estimation (derived from the mammography machines in the current study) would make it less likely that associations would be observed than with area measurements. It will be of interest to examine in future studies the association between circulating IGF-I and other novel mammographic measures that have been strongly associated with breast cancer risk (23).

The associations between the changes in circulating IGF-I and IGFBP-3 and absolute and percent dense area observed in the present study are what would be hypothesized on the basis of biologic mechanisms. Both circulating IGF-I and mammographic density are associated with breast cancer risk (1, 3). IGF-I stimulates mitosis and inhibits apoptosis of breast epithelial cells (4), and mammographic density is directly associated with epithelial and glandular area (24). As much of the amount of radiologically dense breast tissue is due to collagen and fibrosis (24), it is also pertinent that normal breast stromal fibroblasts express the receptor to IGF-I (IGF-IR). Normal breast fibroblasts show the changes in gene expression upon stimulation with IGF-I that are highly correlated with signatures identified in wound healing and breast cancer-associated fibroblasts (25). Also, levels of IGF-I have been correlated with lobule types that indicate a higher degree of age-related mammary involution, which in turn are associated with lower mammographic density (16). The age-related decrease in IGF-I (3) may contribute to the ongoing decrease in mammographic density after menopause.

Our study had limitations in addition to the ones aforementioned, such as a moderate sample size, no ability to consider other components of the growth hormone/IGF axis, and some variability in the timing of the baseline mammogram that may have attenuated the results. It also had several strengths including; fasting blood samples taken early morning in all study participants, two blood samples and mammograms per woman so that associations could be examined within women over time, and volumetric as well as area mammographic measures.

With reasonably strong biologic plausibility and the observation that changes in IGF-I and IGFBP-3 over 1 year are associated with changes in mammographic dense area and percent dense area, breast composition may be affected by IGF-I. However, because associations have not been observed in most cross-sectional studies or with volumetric measures of mammographic density in the present study, confirmation should be sought in longitudinal studies in which larger changes in the IGF system are evoked.
References


