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Serum insulin-like growth factor-I and insulin-like growth factor binding protein-3 in relation to terminal duct lobular unit involution of the normal breast in Caucasian and African American women: the Susan G. Komen Tissue Bank

Hannah Oh[1,2,3], **Ruth M. Pfeiffer**[1], **Roni T. Falk**[1], **Hisani N. Horne**[1,4], **Jackie Xiang**[1], **Michael Pollak**[5], **Louise A. Brinton**[1], **Anna Maria V. Storniolo**[6], **Mark E. Sherman**[1,7], **Gretchen L. Gierach**[*,1], and **Jonine D. Figueroa**[*,1,8]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA

[2]Section of Population Science, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

[3]School of Health Policy & Management, College of Public Health Science; Department of Public Health Science, Graduate School, Korea University, Seoul, Republic of Korea

[4]U.S. Food and Drug Administration, Silver Spring, MD, USA

[5]McGill University, Montreal, Québec, Canada

[6]Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, IN, USA

[7]Mayo Clinic, Jacksonville, FL, USA

[8]Usher Institute of Population Health Sciences and Informatics, Institute of Genomics and Molecular Medicine, Edinburgh Cancer Research Centre, University of Edinburgh, UK

Abstract

Lesser degrees of terminal duct lobular unit (TDLU) involution, as reflected by higher numbers of TDLUs and acini/TDLU, are associated with elevated breast cancer risk. In rodent models, the insulin-like growth factor (IGF) system regulates involution of the mammary gland. We examined associations of circulating IGF measures with TDLU involution in normal breast tissues among women without precancerous lesions. Among 715 Caucasian and 283 African American (AA) women who donated normal breast tissue samples to the Komen Tissue Bank between 2009 to 2012 (75% premenopausal), serum concentrations of IGF-I and binding protein (IGFBP)-3 were quantified using enzyme-linked immunosorbent assay. Hematoxilyn & eosin-stained tissue sections were assessed for numbers of TDLUs ("TDLU count"). Zero-inflated Poisson regression models with a robust variance estimator were used to estimate relative risks (RRs) for association of IGF measures (tertiles) with TDLU count by race and menopausal status, adjusting for potential confounders. AA (vs. Caucasian) women had higher age-adjusted mean levels of serum IGF-I

Corresponding author: Hannah Oh, 145 Anam-Ro, Hana Science Building, Room 358, Seongbuk-Gu, Seoul, 136-713, Republic of Korea, Tel: +82-2-3290-5678, hannahoh@korea.ac.kr.

*These authors contributed equally to this work.

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(137 vs. 131 ng/mL, p=0.07) and lower levels of IGFBP-3 (4165 vs. 4684 ng/mL, p<0.0001). Postmenopausal IGFBP-3 was inversely associated with TDLU count among AA ($RR_{T3vs.T1}$=0.49, 95% CI=0.28-0.84, p-trend=0.04) and Caucasian ($RR_{T3vs.T1}$=0.64, 95% CI=0.42-0.98, p-trend=0.04) women. In premenopausal women, higher IGF-I:IGFBP-3 ratios were associated with higher TDLU count in Caucasian ($RR_{T3vs.T1}$=1.33, 95% CI=1.02-1.75, p-trend=0.04), but not in AA ($RR_{T3vs.T1}$=0.65, 95% CI=0.42-1.00, p-trend=0.05), women. Our data suggest a role of the IGF system, particularly IGFBP-3, in TDLU involution of the normal breast, a breast cancer risk factor, among Caucasian and AA women.

Keywords

insulin-like somatomedin peptide I; somatomedin C; IGF-I; IGFBP-3; TDLU; normal breast tissue; lobular involution

Introduction

Terminal duct lobular units (TDLUs) are the anatomical structures of the breast from which most breast cancers arise, and acini within TDLUs are the epithelial milk-producing substructures.[1] With physiological aging and completion of child bearing, TDLUs involute, reflected in lower acini count/TDLU and total TDLU count per standard unit of tissue area. However, levels of involution vary among women of similar ages and multiple studies have shown that having lesser degrees of age-related TDLU involution is a risk factor for subsequent breast cancer.[2, 3] Hence, evaluation of factors associated with TDLU involution may reveal underlying biological pathways related to breast cancer risk.

The insulin-like growth factor (IGF)-I signaling plays an important role in stimulating cell proliferation and inhibiting apoptosis.[4, 5] Circulating IGF-I binds one of multiple IGF binding proteins (IGFBPs),[6] with IGFBP-3 being the most abundant (80%) type that regulates bioavailable levels of IGF-I.[7] Studies suggest IGFBP-3 also has functional activity to influence apoptosis, independent of IGF-I bioavailability.[8] Epidemiologic evidence supports the associations of higher circulating IGF-I and IGF-I:IGFBP-3 molar ratio with an increased risk of various cancer types [9, 10] including breast cancer.[11] The most convincing evidence comes from a pooled analysis of 17 prospective studies (4,790 cases and 9,428 controls) that showed circulating IGF-I was associated with a 25% increased risk of breast cancer when comparing women in the highest vs. the lowest quintiles and that the association did not vary by menopausal status.[11] However, little is known about whether the IGF system acts upon cancer risk through influencing histologic characteristics of normal glandular tissue.

In rodent models, the IGF system has been shown to regulate growth, development, and involution of the mammary gland.[5, 12-16] For example, mice with genetically-deleted IGF present with reduced ductal branching in the mammary gland.[5, 15, 16] IGF signaling contributes to mammary epithelial stem cell maintenance and renewal, as well as progenitor cell expansion.[13] Dysregulated IGF signaling is likely to inhibit programmed cell death, including the atrophy of epithelial cells during involution.[13, 14, 17] Hence, we hypothesized that one mechanism by which IGF might influence breast cancer risk is through reduced

involution. To date, the role of IGF system in TDLU involution in the human breast has been examined in two independent studies of Caucasian women with benign breast disease (BBD).[18, 19] In these studies, higher levels of IGF-I and IGF-I:IGFBP-3 molar ratio were consistently associated with lower levels of TDLU involution.[18, 19] However, it is unknown whether similar relationships are observed in women without BBD or if associations differ by race. Given documented racial differences in circulating levels of IGF-I and IGFBP-3 [20-23] and potential heterogeneity in IGF-I and IGFBP-3 associations with risk by breast cancer subtype,[11] it is hypothesized that the IGF system may help understand the biological underpinnings of racial disparities in breast cancer (e.g., the observed higher age-specific incidence of triple-negative breast cancers in younger African American [AA] women [24, 25]). In this study, using the diverse population of the Komen Tissue Bank (KTB) and standardized, quantitative measures of TDLU involution, we examined the relationships of serum IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio with standardized measures of TDLU involution in healthy women of European or African descent.

Methods

Study population

The KTB is an annotated biobank, which has recruited healthy volunteer women, aged 18-91 years, since 2007. From the entire KTB population, the current analysis targeted participants (n=2,321) who were recruited from January 10, 2009 to September 14, 2012. Participants provided demographic, lifestyle, reproductive history, and cancer-related information via self-administered questionnaire and donated blood and/or normal breast tissue samples on the same day. Details of the KTB have been described elsewhere (http://komentissuebank.iu.edu) [26, 27]. A woman was considered postmenopausal if menstrual periods had stopped more than 12 months prior, she had undergone bilateral oophorectomy, or she had undergone a hysterectomy and was 55 years or older at the time of biospecimen collection. All participants provided informed consent and the study was approved by the Indiana University Institutional Review Board and the NIH Office of Human Subjects Research.

Of the 2,321 participants, we excluded women who were previously diagnosed with any cancer (n=185), currently pregnant (n=19), currently taking oral contraceptives (n=204) or menopausal hormones (n=81), missing menopausal status (n=28), not of European or African descent (n=201), and missing BMI (n=4). We also excluded women aged <18 or >75 years (n=19), women who had ever had a prior breast biopsy (n=233), women who were missing TDLU data (n=13), and women without sufficient serum samples (n=33). Repeated donations from the same women (n=113), identified through either self-report or genotype data, were excluded from our analysis, resulting in a total of 1,188 women (905 Caucasian and 283 AA women) in the study base.

Among the 1,188 women in the study base, we selected 998 women (544 Caucasian and 203 AA premenopausal women; 171 Caucasian and 80 AA postmenopausal women) for the final analytic population as follows. To optimize power to assess associations by race, in addition to cost considerations, we included all AA women regardless of TDLU status (n=193 with ≥1 observed TDLUs and 90 with zero observed TDLUs) and all Caucasian women with ≥1

observed TDLUs (n=590). We also randomly selected a sample of 125 Caucasian women with zero observed TDLUs, frequency matched by age (10-year categories) and BMI (<25, 25-29, ≥30 kg/m^2) [1:1 for ages 30-49 years and 2:1 for other age groups] to the 90 AA women with zero observed TDLUs, for a total of 998 women.

Laboratory assay

Serum concentrations (ng/mL) of IGF-I and IGFBP-3 were measured in duplicate at McGill University by enzyme-linked immunosorbent assay (ELISA) using the reagents from Diagnostic Systems Laboratory (Webster, TX, USA) as described previously.[28] For each woman, the average of duplicate measurements was used as a summary measure in the analysis. Six quality control samples (1 follicular phase, 2 luteal phase, and 3 postmenopausal samples) were included in duplicate within and across 28 batches in a masked fashion. Coefficients of variation and intra-class correlation coefficients from the masked quality control samples were 7.4% and 0.97 for IGF-I, and 4.3% and 0.98 for IGFBP-3. The Spearman correlations between IGF-I and IGFBP-3 were 0.55 in premenopausal women and 0.62 in postmenopausal women. To approximate the circulating bioactive levels of IGF-I, the molar ratio of IGF-I to IGFBP-3 was estimated as previously described.[29, 30] Because IGF-I binds to IGFBP-3 in a 1:1 molar ratio, higher levels of IGF-I:IGFBP-3 molar ratio are likely to indicate higher circulating levels of unbound, bioactive IGF-I.

TDLU measurements

We evaluated two highly reproducible standardized measures of TDLU involution, the number of TDLUs and acini per TDLU, both of which have been described previously.[27, 31, 32] In brief, digitized images of hematoxylin and eosin (H & E)-stained tissue sections from core biopsies obtained using a standard 10-gauge needle were used to visually assess the number of TDLUs ("TDLU count") and percentage of fat on the slide (0-25%, 26-50%, 51-75%, 76-100%).[27] For up to 10 TDLUs per woman, the number of acini per TDLU ("acini count/TDLU") was quantified using the TDLU analyzer software [31, 32] and the median value was used as a single summary measure for each woman. To estimate the cumulative epithelial content in the H&E slide, a product of the TDLU count and the median acini count/TDLU was calculated. Higher TDLU count, higher acini count/TDLU, and higher product of the two measures indicate lesser degrees of TDLU involution and have previously been associated with higher breast cancer risk.[3]

Statistical analysis

To identify potential confounders, we first assessed correlates of IGF measures. After log-transformation of the data to better approximate normal distributions, age-adjusted geometric means (GMs) and 95% confidence intervals (CIs) of each IGF measure were estimated using weighted linear regression models. Inverse probability of sampling weights was used to weigh the sampled Caucasian women back to the base population and allows a population level interpretation of associations. In the multivariable-adjusted models, we included all the risk factors that were associated with IGF measures in the age-adjusted models. For categorical variables, we tested for difference across risk factor categories using

a global F test. We also performed a test for trend by including risk factors in the models as continuous variables.

Separately in Caucasians and AA, we evaluated the associations between IGF measures and TDLU involution using menopausal status- and race-specific tertiles (T1, T2, T3) of IGF measures. Similar results were found using common tertile cutpoints for the two groups. Because TDLU measures vary greatly by menopausal status,[27] associations were estimated for all women combined and separately in pre- and postmenopausal women. Zero-inflated Poisson regression (ZIP) [33] models, with a sandwich robust variance estimator,[34, 35] were fit to accommodate the count data with excess zeros (zero TDLU count) and to estimate relative risks (RRs) and 95% CIs for the relationships between IGF measures and TDLU count, adjusting for sampling factors (age, BMI), parity/age at first birth, and percent fat on the H & E slide. Adjustment for other potential confounders did not change the estimates, thus we did not include them in the final models. For analyses with the product of TDLU count and acini count/TDLU, ordinal logistic regression models were used to estimate odds ratios (ORs) and 95% CIs after categorizing the outcome into tertiles. We also assessed the associations of IGF measures with acini count/TDLU alone using ordinal logistic regression models, restricted to women with at least one observed TDLU (n=594 premenopausal, 189 postmenopausal). To assess the robustness of results, in sensitivity analyses we additionally fit inverse probability weighted linear regression models with log-transformed IGF measures as the independent and TDLU measures as the dependent variables, and found similar results. We also tested for interactions by race and BMI using likelihood ratio tests.

All statistical tests were two-sided with 5% type I error. The ZIP models with the robust variance were estimated using R software, version 3.2.4, and all other analyses were conducted with SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

Results

Participant characteristics

Study participants were largely premenopausal (75%) and non-Hispanic white (72%). The mean age was 36.1 years in premenopausal women and 56.8 years in postmenopausal women. Demographic characteristics stratified by race and menopausal status are shown in Table 1. AA women tended to have higher BMI ($p<0.0001$ premenopausal; $p=0.15$ postmenopausal), higher percentage of fat on the H & E slide ($p=0.0004$ premenopausal; $p=0.26$ postmenopausal), and younger age at first birth ($p<0.01$ pre- and postmenopausal) and were less likely to breastfeed ($p<0.05$ pre- and postmenopausal) than Caucasian women (Table 1). As expected, among both Caucaisan and AA women, median TDLU count, acini count/TDLU, and the product of the two measures were higher in premenopausal women than in postmenopausal women.

Correlates of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio in all women and stratified by race

Associations for risk factors and IGF measures for all women combined are shown in Table 2. Older women had lower adjusted means of serum IGF-I, IGFBP-3, and IGF-I:IGFBP-3

molar ratio (all p-trend<0.0001). Although postmenopausal women had lower levels of all three IGF measures than premenopausal women (all p<0.0001), the differences did not persist after adjustment for age (all p≥0.07) (data not shown). BMI (≥30 vs. <25 kg/m^2) was inversely associated with IGF-I (GM=124 vs. 142 ng/mL) and IGF-I:IGFBP-3 molar ratio (GM=0.101 vs. 0.111) (all p-trend<0.0001). Similar patterns of associations were observed with finer BMI categories (<22.5, 22.5-24.9, 25.0-27.4, 27.5-29.9, 30.0-34.9, ≥35 kg/m^2) (data not shown). Compared with nulliparous women, parous women who had their first birth at age ≥25 years had higher levels of IGF-I (GM=140 vs. 129, p=0.001). Age at menarche (≥14 vs. ≤12 years: GM=4753 vs. 4474 ng/mL) and current alcohol consumption (≥7 vs. 0 drinks/wk: GM=4970 vs. 4456 ng/mL) were positively associated with IGFBP-3 (all p-trend≤0.002); however, the association with alcohol consumption did not persist after adjustment for other covariates. We did not observe any association of IGF measures with menstrual phase, height, breastfeeding, years since menopause, and family history of breast cancer.

Compared with Caucasian women, AA women had higher levels of IGF-I (GM=137 vs. 131, p=0.07) and lower levels of IGFBP-3 (GM=4165 vs. 4684 ng/mL, p<0.0001), resulting in higher levels of the ratio (GM=0.118 vs. 0.101, p<0.0001); the differences persisted after adjustment for covariates including BMI and parity/age at first birth. In stratified analyses, we observed consistent patterns of associations between Caucasian and AA women (Supplemental Table 1).

Associations of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio with standardized TDLU count

In all Caucasian women combined, serum levels of IGF-I ($RR_{T3vs.T1}$=1.35, 95% CI=1.03-1.76, p-trend=0.03) and IGF-I:IGFBP-3 ratio ($RR_{T3vs.T1}$=1.34, 95% CI=1.06-1.71, p-trend=0.01) were positively associated with TDLU count, adjusting for age, BMI, and menopausal status (Table 3). The positive association between IGF-I:IGBP-3 ratio and TDLU count persisted in premenopausal ($RR_{T3vs.T1}$=1.33, 95% CI=1.02-1.75, p-trend=0.04) but not in postmenopausal Caucasian women. In postmenopausal Caucasian women, IGFBP-3 was inversely associated with TDLU count ($RR_{T3vs.T1}$=0.64, 95% CI=0.42-0.98, p-trend=0.04). In AA women, no association was observed overall; however, as with Caucasian women, an inverse association was found between postmenopausal IGFBP-3 and TDLU count ($RR_{T3vs.T1}$=0.49, 95% CI=0.28-0.84, p-trend=0.04). After additional adjustment for parity/age at first birth and percentage of fat on the H & E slide, the association between postmenopausal IGFBP-3 and TDLU count persisted in AA women ($RR_{T3vs.T1}$=0.55, 95% CI=0.33-0.91, p-trend=0.05) but not in Caucasian women ($RR_{T3vs.T1}$=0.80, 95% CI=0.50-1.28, p-trend=0.37). The estimates in Caucasian women were substantially attenuated after adjustment for the percentage of fat on the H & E slide, possibly due to a positive correlation between postmenopausal IGFBP-3 and percentage of fat on the H&E slide that we observed in Caucasian women (Spearman r=0.25, p=0.001), but not in AA women (Spearman r= -0.11, p=0.34).

Similar patterns of associations were found with epithelial content as indicated by the product of TDLU count and acini count/TDLU, and the positive association with

premenopausal IGF-1:IGFBP-3 ratio in Caucasian women persisted after additional adjustments ($OR_{T3vs.T1}$=1.65, 95% CI=1.04-2.63, p-trend=0.03) (Supplemental Table 2). Acini count/TDLU alone was not associated with IGF measures among women with ≥1 TDLU (data not shown).

There were no statistically significant interactions by race or BMI (p-interaction≥0.10), although the associations for premenopausal IGF-I and IGF-I:IGFBP ratio appeared to be stronger among women with lower BMI (<25 vs. ≥30 kg/m^2) (data not shown).

Discussion

In this cross-sectional analysis of healthy women who donated normal breast tissue for research, we found evidence of associations of serum levels of IGF-I and IGFBP-3 with histologic measures of TDLU involution. Higher circulating levels of postmenopausal IGFBP-3 were associated with greater degrees of TDLU involution, indicated by lower TDLU count, in both Caucasian and AA women. In Caucasian women, we additionally found positive associations of premenopausal IGF-I:IGFBP-3 molar ratio with both TDLU count and the product of TDLU count and acini count/TDLU. Our data suggest the potential role of IGF system in TDLU involution of the normal breast among both Caucasian and AA women.

Consistent with findings from previous studies,[11, 20, 21, 36-39] our data demonstrated that several breast cancer risk factors may be associated with circulating levels of IGF-I and IGFBP-3. As expected,[37-41] age was inversely associated with circulating levels of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio, likely due to the lower levels of growth hormone (GH) in older women,[42] as GH regulates and stimulates secretion of IGF-I and IGFBP-3.[43] While some studies have reported an upside down U-shaped relationship [11, 20, 21, 39] between BMI and IGF-I, the current study found a linear inverse association, possibly due to differences in range of BMI, body fat distribution, and insulin profile. Insulin can increase GH-mediated synthesis of IGF-I from the liver by up-regulating GH receptors [44] and stimulating amino acid uptake;[45] however, too much insulin may lower IGF-I levels by enhancing negative feedback on GH secretion.[46, 47] After adjusting for age, BMI, and reproductive factors, our data agree with prior studies [20-23] that reported higher circulating levels of IGF-I and lower levels of IGFBP-3 in AA vs. Caucasian women. The racial differences in IGF-I levels are also present in children [23] and associated with lifestyle factors (e.g., diet [21, 36], physical activity [38], body fat distribution [29, 37, 40]). The overall agreement of our results with previous findings supports the validity of our IGF data.

To the best of our knowledge, this study is the first to examine the relationships of serum IGF-I and IGFBP-3 with TDLU involution in normal breast tissue from Caucasian and AA women without BBD. Previous studies have evaluated the relationships among Caucasian women with BBD only.[18, 19] A cross-sectional analysis of 472 women (84% premenopausal) with proliferative BBD from the Nurses' Health Study II used visual assessment of acini count/TDLU (i.e., lobule type) and found positive associations with circulating IGF-I and IGF-I:IGFBP-3 ratio.[18] Utilizing the same standardized, quantitative TDLU measures used in the current study, another cross-sectional analysis of 288 women with BBD found that

elevated circulating levels of postmenopausal IGF-I and pre- and postmenopausal IGF-I:IGFBP-3 ratio were associated with higher TDLU count.[19] The current analyses of women without BBD found an inverse association between circulating IGFBP-3 and TDLU count which was restricted to postmenopausal women but consistently found in both Caucasian and AA women; this inverse association agrees with the previous findings from postmenopausal Caucasian women with BBD.[19] The variation in associations by menopausal status may be due to the interaction of the IGF system with other endogenous hormones (e.g., estrogens [32, 48]) that are present at higher levels in premenopausal women as well as the differences in levels of both IGF measures and TDLU involution by age. In the current analysis, the inverse association between postmenopausal IGFBP-3 levels and TDLU count was attenuated after additional adjustment for percentage of fat on the H & E slide in Caucasian, but not in AA, women. The difference may be due, in part, to the positive correlation between IGFBP-3 and percentage of fat on the H & E slide in Caucasian women that was not observed in AA women. Other studies have also observed differential associations between IGF measures and breast cancer risk factors by race. For example, the Multiethnic Cohort Study found that IGF-1 levels were associated with BMI in AA, but not Caucasian, women.[49] Further, data from the Southern Community Cohort Study suggest that obesity during childhood or young adulthood may have a greater impact on IGF-1 levels among white women than in AA women.[20] Caucasian and AA women may differ with respect to their distributions of BMI (Table 1 and [50]), body composition,[51] breast density,[52] IGF levels (Table 2 and [20-23]), and other endogenous factors (e.g., estrogens,[53, 54] adipokines,[55, 56] inflammatory cytokines [57]); relationships between these factors are complex and may differentially influence the TDLU involution process. Future studies are needed to clarify IGF and TDLU involution relationships by race after accounting for these factors.

Although we did not find evidence of an association between IGF-I and TDLU involution in AA women, we observed a positive association among Caucasian women. The lack of a significant finding with IGF-I in AA women in particular may be due to limited power in AA women and differences in mammographic density between the two racial groups, as the positive association between IGF-I and TDLU count was previously found to be stronger among women with denser breasts.[19] Although the current study does not have mammographic density data, AA women had higher BMI, a strong inverse correlate of mammographic density.[58] The current study also showed suggestively stronger IGF-I and TDLU associations in Caucasian and AA women with lower BMI. Further, the discrepancy in results with previous studies may be partially explained by differences in normal breast tissue from women with vs. without BBD, and differences in study participant characteristics (e.g., age, range of IGF measures).

Prior studies of mammographic density, another strong predictor of breast cancer,[58] also support the role of the IGF system in TDLU involution of healthy women. Mammographic density reflects stromal and epithelial content and is closely correlated with TDLU count.[59, 60] Studies have linked higher circulating levels of IGF-I and lower levels of IGFBP-3 with dense breasts in healthy, premenopausal women.[28, 61, 62] Future studies evaluating interrelationships between IGF measures, mammographic density, and TDLU involution are warranted.

Altogether, the evidence suggests that the IGF system may influence breast cancer risk through modulating TDLU involution of the normal breast, possibly starting before the development of precancerous lesions. TDLU involution is the process by which the complexity and the content of breast epithelial tissue are gradually lost with aging of the mammary gland.[63] As most breast cancers arise from epithelial cells, reduction of epithelial tissue with involution may be a physiologically protective mechanism against breast cancer (i.e., removal of the progenitor population for tumor formation). While little is known about the signaling processes that regulate involution, evidence from rodent models suggests that IGF signaling inhibits the involution process of the mammary gland.[17] Our data from healthy women without precancerous lesions suggest that the IGF system influences the involution processes early in the disease process, shaping the molecular histology upon which other factors act.

Strengths of this study are the use of standardized, reproducible measures of TDLU involution, the racially diverse study population, and the unique resource of normal breast tissues from healthy volunteers. Limitations of the study are the use of IGF-I and IGFBP-3 measurements in a single serum sample. However, IGF-I and IGFBP-3 levels are relatively stable over 2-3 years within individuals,[64] thus a single measurement may be adequate. We also had a limited sample of AA women; larger studies of AA women are needed to replicate these findings.

In conclusion, our data suggest that the IGF system may influence TDLU involution of the normal breast in healthy women. By evaluating associations in healthy women, our findings provide additional insights into breast cancer etiology beyond what is known from animal models and women with precancerous breast lesions. Our findings also provide further support for the evaluation of normal glandular tissue in potentially clarifying etiologic pathways to various cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty & impact statement

Although insulin-like growth factor (IGF)-I signaling plays an important role in stimulating cell proliferation and inhibiting apoptosis, little is known about whether the IGF system acts upon breast cancer risk through influencing histologic characteristics of normal glandular tissue and whether relationships vary by race. In this study, we found an inverse association of IGF binding protein-3 with terminal duct lobular unit (TDLU) count, a breast cancer risk factor, in both Caucasian and African American women.

Table 1

Characteristics of study population in the Komen Tissue Bank, stratified by race and menopausal status, N=998

Characteristics		Premenopausal			Postmenopausal		
		Caucasians (N=544)	African Americans (N=203)	P-value	Caucasians (N=171)	African Americans (N=80)	P-value
		N (%)			N (%)		
Age							
	<30 years	164 (30.2)	45 (22.2)	0.13	0 (0.0)	0 (0.0)	0.58
	30-39 years	173 (31.8)	78 (38.4)		5 (2.9)	2 (2.5)	
	40-49 years	162 (30.0)	65 (32.0)		23 (13.5)	11 (13.8)	
	50-59 years	45 (8.3)	15 (7.4)		83 (48.5)	32 (40.0)	
	≥60 years	0 (0.0)	0 (0.0)		60 (35.1)	35 (43.8)	
Menstrual phase							
	Follicular	178 (32.7)	63 (31.0)	0.49	NA	NA	NA
	Peri-ovulatory	72 (13.2)	21 (10.3)		NA	NA	
	Luteal	142 (26.1)	52 (25.6)		NA	NA	
	Unknown	152 (27.9)	67 (33.0)		NA	NA	
Body mass index							
	<25 kg/m^2	197 (36.2)	36 (17.7)	<0.0001	47 (27.5)	13 (16.3)	0.15
	25.0-29.9 kg/m^2	131 (24.1)	51 (25.1)		46 (26.9)	25 (31.3)	
	≥30 kg/m^2	216 (39.7)	116 (57.1)		78 (45.6)	42 (52.5)	
Height							
	<160.0 cm	83 (15.3)	37 (18.2)	0.73	36 (21.1)	17 (21.3)	0.99
	160.0-164.9 cm	154 (28.3)	58 (28.6)		60 (35.1)	29 (36.3)	
	165.0-169.9 cm	156 (28.7)	52 (25.6)		44 (25.7)	19 (23.8)	
	≥170 cm	151 (27.8)	56 (27.6)		31 (18.1)	15 (18.8)	
Age at menarche							
	≤12 years	276 (50.7)	127 (62.6)	0.01	79 (46.2)	41 (51.3)	0.70
	13 years	150 (27.6)	40 (19.7)		46 (26.9)	21 (26.3)	
	≥14 years	118 (21.7)	36 (17.7)		46 (26.9)	18 (22.5)	
Parity/age at first birth							
	Nulliparous	272 (50.0)	83 (40.9)	0.0002	30 (17.5)	15 (18.8)	0.008

Characteristics	Premenopausal			Postmenopausal		
	Caucasians (N=544)	African Americans (N=203)	P-value	Caucasians (N=171)	African Americans (N=80)	P-value
Parous, <25 years	94 (17.3)	63 (31.0)		56 (32.8)	41 (51.3)	
Parous, ≥25 years	178 (32.7)	57 (28.1)		85 (49.7)	24 (30.0)	
Breastfeeding (among parous women)						
Never	56 (20.6)	36 (30.0)	0.04	45 (31.9)	31 (47.7)	0.03
Ever	216 (79.4)	84 (70.0)		96 (68.1)	34 (52.3)	
Years since menopause						
<5 years	NA	NA	NA	48 (28.1)	21 (26.3)	0.02
5-10 years	NA	NA		42 (24.6)	15 (18.8)	
11-15 years	NA	NA		50 (29.2)	21 (26.3)	
>15 years	NA	NA		18 (10.5)	21 (26.3)	
Unknown	NA	NA		13 (7.6)	2 (2.5)	
Use of hormone therapy (among postmenopausal women)						
Never	NA	NA	NA	107 (62.6)	58 (72.5)	0.12
Past	NA	NA		64 (37.4)	22 (27.5)	
Smoking status						
Never	380 (69.9)	186 (91.6)	<0.0001	112 (65.5)	44 (55.0)	0.23
Former	113 (20.8)	12 (5.9)		50 (29.2)	32 (40.0)	
Current	51 (9.4)	5 (2.5)		9 (5.3)	4 (5.0)	
Current alcohol consumption						
0 drink/wk	148 (27.2)	81 (39.9)	<0.0001	65 (38.0)	38 (47.5)	0.15 [a]
<7 drinks/wk	349 (64.2)	120 (59.1)		88 (51.5)	42 (52.5)	
≥7 drinks/wk	47 (8.6)	2 (1.0)		18 (10.5)	0 (0.0)	
First-degree relative with breast cancer						
No	439 (80.7)	179 (88.2)	0.02	130 (76.0)	60 (75.0)	0.86
Yes	105 (19.3)	24 (11.8)		41 (24.0)	20 (25.0)	
Percent fat on H&E slide						
≤75%	264 (48.5)	69 (34.0)	0.0004	57 (33.3)	21 (26.3)	0.26
>75%	280 (51.5)	134 (66.0)		114 (66.7)	59 (73.8)	
	Median (IQR)			**Median (IQR)**		
IGF measures						

Characteristics	Premenopausal			Postmenopausal		
	Caucasians (N=544)	African Americans (N=203)	P-value	Caucasians (N=171)	African Americans (N=80)	P-value
IGF-I	151 (118-190)	148 (118-184)		108 (88-125)	110 (95-140)	
IGFBP-3	4919 (4417-5459)	4306 (3787-4780)		4483 (3876-4962)	4080 (3676-4542)	
IGF-I:IGFBP-3 molar ratio	0.11 (0.09-0.14)	0.13 (0.10-0.15)		0.09 (0.08-0.10)	0.10 (0.09-0.12)	
Lobular involution measures among women with ≥1 observed TDLU						
Number of TDLU	8 (3-17)	7 (3-14)		5 (3-12)	5 (2-10)	
Number of acini/TDLU	13 (8-20)	16 (9-26)		7 (4-10)	8 (4-14)	
Product of TDLU count and acini count/TDLU	112 (35-252)	128 (39-280)		28 (12-76)	35 (12-88)	

Abbreviations: H & E=hematoxylin & eosin, IGF-I=insulin-like growth factor-I, IGFBP-3=insulin-like growth factor binding protein-3, IQR=interquartile range, NA=not applicable, TDLU=terminal duct lobular unit

Note: P-values were estimated using chi-square test.

[a]P-values were estimated for 0 drink/wk and >0 drink/wk categories

Table 2

Age-adjusted geometric means and 95% confidence intervals (CI) of IGF-I and IGFBP-3 concentration (ng/mL) and the IGF-I:IGFBP-3 molar ratio by population characteristics: The Komen Tissue Bank

Characteristics	N (weighted %[a])	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I:IGFBP-3 molar ratio
Age[b]				
<30 years	209 (21.6)	199 (184-214)	5219 (5114-5325)	0.137 (0.129-0.147)
30-39 years	258 (23.6)	145 (140-151)	4612 (4524-4702)	0.114 (0.110-0.118)
40-49 years	261 (26.2)	128 (123-134)	4487 (4350-4629)	0.103 (0.100-0.107)
50-59 years	175 (19.0)	109 (104-114)	4392 (4267-4522)	0.090 (0.086-0.093)
≥60 years	95 (9.6)	101 (96-107)	4118 (3850-4406)	0.089 (0.085-0.093)
p-trend[c]		**<0.0001**[e]	**<0.0001**[e]	**<0.0001**[e]
Race				
Caucasian	715 (76.2)	131 (128-135)	4684 (4600-4770)	0.101 (0.099-0.104)
African American	283 (23.8)	137 (132-141)	4165 (4072-4261)	0.118 (0.115-0.122)
p-value[d]		0.07[e]	**<0.0001**[e]	**<0.0001**[e]
Menstrual phase (among premenopausal women)				
Follicular	241 (30.9)	142 (136-148)	4695 (4566-4828)	0.109 (0.105-0.114)
Periovulatory	93 (11.9)	146 (137-156)	4646 (4467-4832)	0.114 (0.108-0.119)
Luteal	194 (27.1)	143 (133-154)	4644 (4525-4766)	0.112 (0.104-0.119)
Unknown	219 (30.1)	142 (136-148)	4681 (4546-4820)	0.109 (0.105-0.113)
p-value[d]		0.88	0.91	0.54
Body mass index				
<25 kg/m^2	293 (30.5)	142 (135-148)	4594 (4485-4706)	0.111 (0.107-0.116)
25-29.9 kg/m^2	253 (27.4)	136 (130-143)	4653 (4520-4790)	0.106 (0.101-0.111)
≥30 kg/m^2	452 (42.1)	124 (121-128)	4453 (4361-4548)	0.101 (0.098-0.103)
p-trend[c]		**<0.0001**[e]	**0.03**	**<0.0001**[e]
Height				
<160.0 cm	173 (17.9)	132 (126-138)	4534 (4383-4690)	0.105 (0.102-0.109)
160.0-164.9 cm	301 (27.8)	129 (125-134)	4463 (4354-4575)	0.105 (0.101-0.108)
165.0-169.9 cm	271 (27.7)	134 (129-139)	4545 (4414-4680)	0.106 (0.102-0.110)
≥170 cm	253 (26.6)	135 (128-143)	4680 (4551-4813)	0.104 (0.099-0.110)
p-trend[c]		0.30	0.06	0.91
Age at menarche				
≤12 years	523 (51.9)	130 (126-135)	4474 (4390-4559)	0.105 (0.102-0.108)
13 years	257 (25.1)	134 (128-140)	4512 (4403-4624)	0.107 (0.103-0.112)
≥14 years	218 (23.0)	136 (129-142)	4753 (4605-4905)	0.103 (0.099-0.108)
p-trend[c]		0.16	**0.001**[e]	0.61
Parity/age at first birth				
Nulliparous	400 (42.3)	129 (124-134)	4534 (4421-4651)	0.102 (0.099-0.106)
Parous, <25 years	254 (23.5)	129 (124-135)	4423 (4302-4549)	0.105 (0.102-0.109)

Characteristics	N (weighted %[a])	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I:IGFBP-3 molar ratio
Parous, ≥25 years	344 (34.1)	140 (135-144)	4665 (4539-4794)	0.108 (0.105-0.112)
p-value[d]		**0.001**[e]	**0.02**	0.06[e]
Breastfeeding (among parous women)				
Never	168 (27.6)	130 (124-137)	4431 (4277-4590)	0.106 (0.102-0.111)
Ever	430 (72.4)	137 (133-142)	4557 (4442-4675)	0.109 (0.105-0.112)
p-value[d]		0.09	0.20	0.36
Year since menopause (among postmenopausal women)				
<5 years	69 (25.7)	118 (106-133)	4313 (4037-4607)	0.099 (0.091-0.107)
5-10 years	57 (21.3)	114 (101-129)	4087 (3789-4407)	0.101 (0.093-0.109)
11-15 years	71 (30.5)	113 (99-128)	4305 (3968-4671)	0.095 (0.086-0.104)
>15 years	39 (17.1)	110 (96-126)	4043 (3607-4531)	0.098 (0.089-0.108)
Unknown	15 (5.4)	110 (93-130)	4267 (3761-4841)	0.093 (0.084-0.104)
p-trend[c]		0.25	0.35	0.56
Use of hormone therapy (among postmenopausal women)				
Never	165 (65.1)	116 (105-128)	4182 (3941-4438)	0.100 (0.094-0.107)
Past	86 (34.9)	113 (101-127)	4433 (4096-4798)	0.092 (0.085-0.100)
p-value[d]		0.55	0.12	**0.01**[e]
Smoking status				
Never	722 (72.5)	133 (129-136)	4529 (4440-4620)	0.106 (0.103-0.108)
Former	207 (20.8)	136 (130-142)	4642 (4495-4794)	0.106 (0.102-0.109)
Current	69 (6.7)	120 (110-130)	4465 (4272-4667)	0.097 (0.090-0.104)
p-value[d]		**0.03**	0.32	0.06
Current alcohol consumption				
0 drink/wk	332 (32.2)	131 (126-135)	4456 (4336-4579)	0.106 (0.103-0.109)
<7 drinks/wk	599 (60.8)	133 (129-138)	4561 (4477-4647)	0.106 (0.103-0.109)
≥7 drinks/wk	67 (7.0)	134 (124-145)	4970 (4693-5262)	0.098 (0.092-0.103)
p-trend[c]		0.38	**0.002**	0.12
First degree relative with breast cancer				
No	808 (80.2)	132 (129-136)	4551 (4475-4627)	0.105 (0.103-0.107)
Yes	190 (19.8)	133 (127-139)	4555 (4383-4734)	0.105 (0.101-0.110)
p-value[d]		0.89	0.96	0.91

Note: Geometric means and 95% confidence intervals were estimated using weighted linear regression models, adjusting for age (10-year categories). Age-adjusted p-values <0.05 are denoted in bold font. Multivariable-adjusted p-values <0.05 are denoted with a superscript e.

[a] Weighted % was estimated using inverse probability of sampling weights and refers to the overall Komen Tissue Bank study base.

[b] Not adjusted for age.

[c] p-trend was estimated using the Wald test for ordinal trend variables.

[d] p-value was estimated using the F test for categories.

[e] p<0.05 after multivariable adjustment. Multivariable adjusted models include age, BMI, and parity/age at first birth for IGF-I; age, race, BMI, age at menarche, parity/age at first birth, and alcohol consumption for IGFBP-3; age, race, BMI, and hormone therapy use for the IGF-I: IGFBP-3 molar ratio.

Abbreviations: CI=confidence interval, IGF-I=insulin-like growth factor-I, IGFBP-3=insulin-like growth factor binding protein-3

Table 3

Relative risks (RR) and 95% confidence intervals (CIs) for relationships of IGF-I, IGFBP-3, and the IGF-I:IGFBP-3 molar ratio with terminal duct lobular unit (TDLU) count, stratified by race and menopausal status: The Komen Tissue Bank

	Caucasians											
	All women				Premenopausal				Postmenopausal			
	Range (ng/mL)	N	Model 1[a] RR (95% CI)	Model 2[b] RR (95% CI)	Range (ng/mL)	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)	Range (ng/mL)	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)
IGF-I												
T1	<116	238	1.0 (ref)	1.0 (ref)	<127	182	1.0 (ref)	1.0 (ref)	<97	57	1.0 (ref)	1.0 (ref)
T2	116-162	239	1.06 (0.86-1.31)	1.00 (0.83-1.21)	127-175	181	1.10 (0.87-1.39)	1.02 (0.83-1.25)	97-116	57	0.80 (0.54-1.20)	0.74 (0.50-1.10)
T3	≥163	238	**1.35 (1.03-1.76)**	1.20 (0.95-1.51)	≥176	181	1.17 (0.89-1.53)	1.06 (0.83-1.35)	≥117	57	0.73 (0.50-1.08)	0.85 (0.60-1.21)
p-trend			**0.03**	0.13			0.25	0.65			0.12	0.35
IGFBP-3												
T1	<4490	238	1.0 (ref)	1.0 (ref)	<4603	181	1.0 (ref)	1.0 (ref)	<4020	57	1.0 (ref)	1.0 (ref)
T2	4490-5188	239	1.03 (0.85-1.25)	1.05 (0.88-1.25)	4603-5259	182	**1.26 (1.02-1.56)**	1.19 (0.98-1.44)	4020-4799	57	0.79 (0.55-1.13)	0.97 (0.69-1.37)
T3	≥5189	238	1.16 (0.94-1.43)	1.17 (0.96-1.42)	≥5260	181	1.20 (0.94-1.54)	1.21 (0.96-1.51)	≥4800	57	**0.64 (0.42-0.98)**	0.80 (0.50-1.28)
p-trend			0.16	0.12			0.12	0.11			**0.04**	0.37
IGF-I:IGFBP-3												
T1	<0.093	238	1.0 (ref)	1.0 (ref)	<0.099	181	1.0 (ref)	1.0 (ref)	<0.079	57	1.0 (ref)	1.0 (ref)
T2	0.093-0.118	239	1.14 (0.92-1.41)	1.06 (0.87-1.29)	0.099-0.124	182	1.23 (0.96-1.57)	1.10 (0.87-1.38)	0.079-0.095	57	0.87 (0.56-1.35)	0.83 (0.55-1.26)
T3	≥0.119	238	**1.34 (1.06-1.71)**	1.16 (0.93-1.46)	≥0.125	181	**1.33 (1.02-1.75)**	1.15 (0.90-1.48)	≥0.096	57	0.99 (0.66-1.48)	0.97 (0.65-1.44)
p-trend			**0.01**	0.18			**0.04**	0.27			0.99	0.92
	African Americans											
	All women				Premenopausal				Postmenopausal			
	Range (ng/mL)	N	Model 1[a] RR (95% CI)	Model 2[b] RR (95% CI)	Range	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)	Range	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)
IGF-I												
T1	<117	94	1.0 (ref)	1.0 (ref)	<131	67	1.0 (ref)	1.0 (ref)	<97	26	1.0 (ref)	1.0 (ref)
T2	117-158	95	0.79 (0.57-1.10)	0.76 (0.57-1.01)	131-167	68	1.13 (0.73-1.75)	1.03 (0.70-1.52)	97-124	27	0.89 (0.42-1.91)	0.58 (0.30-1.12)
T3	≥159	94	0.76 (0.49-1.17)	0.76 (0.51-1.13)	≥168	68	0.75 (0.44-1.12)	0.75 (0.46-1.23)	≥125	27	0.71 (0.35-1.44)	0.62 (0.32-1.20)

	Caucasians											
	All women				Premenopausal				Postmenopausal			
	Range (ng/mL)	N	Model 1[a] RR (95% CI)	Model 2[b] RR (95% CI)	Range (ng/mL)	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)	Range (ng/mL)	N	Model 1[c] RR (95% CI)	Model 2[d] RR (95% CI)
p-trend			0.22	0.20			0.29	0.24			0.37	0.20
IGFBP-3												
T1	<3915	94	1.0 (ref)	1.0 (ref)	<3941	67	1.0 (ref)	1.0 (ref)	<3820	26	1.0 (ref)	1.0 (ref)
T2	3915-4519	95	0.84 (0.59-1.19)	0.75 (0.55-1.03)	3941-4574	68	0.79 (0.51-1.21)	0.69 (0.47-1.03)	3820-4400	27	0.97 (0.54-1.72)	0.98 (0.61-1.57)
T3	≥4520	94	0.98 (0.67-1.43)	0.86 (0.61-1.22)	≥4575	68	1.08 (0.71-1.64)	0.93 (0.63-1.37)	≥4401	27	**0.49 (0.28-0.84)**	0.55 (0.33-0.91)
p-trend			0.93	0.45			0.65	0.85			**0.04**	0.05
IGF-I:IGFBP-3												
T1	<0.101	94	1.0 (ref)	1.0 (ref)	<0.111	67	1.0 (ref)	1.0 (ref)	<0.091	26	1.0 (ref)	1.0 (ref)
T2	0.101-0.135	95	0.99 (0.70-1.40)	0.97 (0.72-1.31)	0.111-0.144	68	0.99 (0.67-1.46)	1.05 (0.75-1.46)	0.091-0.112	27	0.77 (0.39-1.54)	**0.49 (0.28-0.86)**
T3	≥0.136	94	0.75 (0.51-1.12)	0.87 (0.61-1.24)	≥0.145	68	0.65 (0.42-1.00)	0.75 (0.50-1.11)	≥0.113	27	0.79 (0.42-1.49)	0.69 (0.40-1.17)
p-trend			0.14	0.43			0.05	0.16			0.47	0.27

Note: Relative risks and 95% confidence intervals were estimated using zero-inflated Poisson regression models, with a sandwich robust variance estimator. P-values significant at the alpha level 0.05 are denoted in bold font.

[a]Model 1:Adjusted for age (10-year categories), BMI (<25, 25-29.9, ≥30 kg/m^2), and menopausal status (premenopausal, postmenopausal)

[b]Model 2:Adjusted for age (10-year categories), BMI (<25, 25-29.9, ≥30 kg/m^2), menopausal status (premenopausal, postmenopausal), parity/age at first birth (nulliparous, parous/<25 years, parous/≥25 years), and percent fat on the H&E slide (≤75, >75%)

[c]Model 1: Adjusted for age (10-year categories) and BMI (<25, 25-29.9, ≥30 kg/m^2)

[d]Model 2: Adjusted for age (10-year categories), BMI (<25, 25-29.9, ≥30 kg/m^2), parity/age at first birth (nulliparous, parous/<25 years, parous/≥25 years), and percent fat on the H & E slide (≤75, >75%)

Abbreviations: CI=confidence interval, H & E=hematoxylin & eosin, IGF-I=insulin-like growth factor-I, IGFBP-3=insulin-like growth factor binding protein-3, RR=relative risk, TDLU=terminal duct lobular unit