Supporting your research with our capabilities

BD Accuri™ C6 Plus Personal Flow Cytometer

BD FACSCelesta™ Cell Analyzer

BD LSRFortessa™ X-20 Cell Analyzer

BD FACSMelody™ Cell Sorter

One of the largest portfolios of reagents

Learn more>

BD

IJC
International Journal of Cancer

Serum insulin-like growth factor (IGF)-I and IGF 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
[2] Section of Population Science, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ
[3] Division of Health Policy and Management, College of Health Science, Korea University, Seoul, Republic of Korea
[4] U.S. Food and Drug Administration, Silver Spring, MD
[5] McGill University, Montreal, Québec, Canada
[6] Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, IN
[7] Mayo Clinic, Jacksonville, FL
[8] Usher Institute of Population Health Sciences and Informatics, Institute of Genomics and Molecular Medicine, Edinburgh Cancer Research Centre, University of Edinburgh, UK

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 and 2012 (75% premenopausal), serum concentrations of IGF-I and binding protein (IGFBP)-3 were quantified using enzyme-linked immunosorbent assay. Hematoxylyn and 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 (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.

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

Key words: Insulin-Like Growth Factor I, insulin-like somatomedin peptide I, somatomedin C, IGF-I, IGFBP-3, TDLU, normal breast tissue, lobular involution

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: The authors declare that they have no potential conflicts of interest.

[†]G.L.G. and J.D.F. contributed equally to this work.

Grant sponsor: Intramural Research Program of the National Cancer Institute; **Grant number:** ZIA CP010126-16; **Grant sponsor:** Susan G. Komen; **Grant sponsor:** Breast Cancer Research Foundation; **Grant sponsor:** Oracle Giving; **Grant sponsor:** Catherine Peachey Fund

DOI: 10.1002/ijc.31333

History: Received 21 July 2017; Accepted 7 Feb 2018; Online 23 Feb 2018

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

What's new?

Insulin-like growth factor (IGF)-I signaling plays an important role in stimulating cell proliferation and inhibiting apoptosis. In this study, the authors examined normal mammary tissue in Caucasian and African American women, and found that increased levels of IGF-binding protein (IGFBP)-3 were associated with decreased involution of the mammary ducts in both groups. Because decreased involution is a known risk factor for breast cancer, and because these two groups express different levels of IGF-I and IGFBP-3, these results may help to explain the biological underpinnings of racial disparities in breast cancer.

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 *versus* 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, this 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 >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) 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 (one follicular phase, two luteal phase and three 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 described previously.[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 and 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 (ZIP) regression[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 percentage 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 between 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 $p \geq 0.07$) (data not shown). BMI ($\geq$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 and $\geq$35 kg/m^2) (data not shown). Compared with nulliparous women, parous women who had their first birth at age $\geq$25 years had higher levels of IGF-I (GM = 140 *vs.* 129, $p = 0.001$). Age at menarche ($\geq$14 *vs.* $\leq$12 years: GM = 4753 *vs.* 4474 ng/mL) and current alcohol consumption ($\geq$7 *vs.* 0 drinks/week: GM = 4970 *vs.* 4456 ng/mL) were positively associated with IGFBP-3 (all p-trend $\leq$ 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 (Supporting Information Table S1).

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) (Supporting Information Table S2). Acini count/TDLU alone was not associated with IGF measures among women with $\geq$1 TDLU (data not shown).

There were no statistically significant interactions by race or BMI (p-interaction $\geq$ 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.* $\geq$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

Table 1. Characteristics of study population in the Komen Tissue Bank, stratified by race and menopausal status, $N = 998$

	Premenopausal			Postmenopausal		
Characteristics	Caucasians ($N = 544$)	African Americans ($N = 203$) *N* (%)	*p* value	Caucasians ($N = 171$)	African Americans ($N = 80$) *N* (%)	*p* value
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
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)	

Table 1. Characteristics of study population in the Komen Tissue Bank, stratified by race and menopausal status, $N = 998$ (Continued)

	Premenopausal			Postmenopausal		
Characteristics	Caucasians ($N = 544$)	African Americans ($N = 203$)	p value	Caucasians ($N = 171$)	African Americans ($N = 80$)	p value
	N (%)			N (%)		
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/week	148 (27.2)	81 (39.9)	<0.0001	65 (38.0)	38 (47.5)	0.15[1]
<7 drinks/week	349 (64.2)	120 (59.1)		88 (51.5)	42 (52.5)	
≥7 drinks/week	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)	
Percentage 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						
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)	

Note: p values were estimated using χ^2 test.
[1]p values were estimated for 0 drink/week and >0 drink/week categories.
Abbreviations: H&E: hematoxylin and 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.

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, this 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 upregulating 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 *versus* 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] and body fat distribution[29,37,40]). The overall agreement of our results with previous findings supports the validity of our IGF data.

Table 2. Age-adjusted GMs and 95% CIs of IGF-I and IGFBP-3 concentrations (ng/mL) and the IGF-I:IGFBP-3 molar ratio by population characteristics: The Komen Tissue Bank

Characteristics	*N* (weighted percentage[1])	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I:IGFBP-3 molar ratio
Age[2]				
<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[3]		**<0.0001**[4]	**<0.0001**[4]	**<0.0001**[4]
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[5]		0.07[4]	**<0.0001**[4]	**<0.0001**[4]
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[5]		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[3]		**<0.0001**[4]	**0.03**	**<0.0001**[4]
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[3]		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[3]		0.16	**0.001**[4]	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)
Parous, ≥25 years	344 (34.1)	140 (135–144)	4665 (4539–4794)	0.108 (0.105–0.112)
p value[5]		**0.001**[4]	**0.02**	0.06[4]
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[5]		0.09	0.20	0.36

Table 2. Age-adjusted GMs and 95% CIs of IGF-I and IGFBP-3 concentrations (ng/mL) and the IGF-I:IGFBP-3 molar ratio by population characteristics: The Komen Tissue Bank (Continued)

Characteristics	*N* (weighted percentage[1])	IGF-I (ng/mL)	IGFBP-3 (ng/mL)	IGF-I:IGFBP-3 molar ratio
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[3]		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[5]		0.55	0.12	**0.01**[4]
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[5]		**0.03**	0.32	0.06
Current alcohol consumption				
0 drink/week	332 (32.2)	131 (126–135)	4456 (4336–4579)	0.106 (0.103–0.109)
<7 drinks/week	599 (60.8)	133 (129–138)	4561 (4477–4647)	0.106 (0.103–0.109)
≥7 drinks/week	67 (7.0)	134 (124–145)	4970 (4693–5262)	0.098 (0.092–0.103)
p-trend[3]		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[5]		0.89	0.96	0.91

Note: GMs and 95% CIs 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 4.
[1]Weighted percentage was estimated using inverse probability of sampling weights and refers to the overall Komen Tissue Bank study base.
[2]Not adjusted for age.
[3]*p*-trend was estimated using the Wald test for ordinal trend variables.
[4]$p < 0.05$ 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.
[5]*p* value was estimated using the F test for categories.
Abbreviations: CI: confidence interval; GM: geometric mean; IGF-I: insulin-like growth factor-I; IGFBP-3: insulin-like growth factor binding protein-3.

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] Using the same standardized, quantitative TDLU measures used in this 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 present analysis 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 this analysis, the inverse association between

Table 3. RRs and 95% CIs for relationships of IGF-I, IGFBP-3 and IGF-I:IGFBP-3 molar ratio with TDLU count, stratified by race and menopausal status: The Komen Tissue Bank

	All women				Premenopausal				Postmenopausal			
	Range (ng/mL)	*N*	Model 1[1] RR (95% CI)	Model 2[2] RR (95% CI)	Range (ng/mL)	*N*	Model 1[3] RR (95% CI)	Model 2[4] RR (95% CI)	Range (ng/mL)	*N*	Model 1[3] RR (95% CI)	Model 2[4] RR (95% CI)
Caucasians												
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												
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)
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

Table 3. RRs and 95% CIs for relationships of IGF-I, IGFBP-3 and IGF-I:IGFBP-3 molar ratio with TDLU count, stratified by race and menopausal status: The Komen Tissue Bank (Continued)

	All women				Premenopausal				Postmenopausal			
	Range (ng/mL)	*N*	Model 1[1] RR (95% CI)	Model 2[2] RR (95% CI)	Range (ng/mL)	*N*	Model 1[3] RR (95% CI)	Model 2[4] RR (95% CI)	Range (ng/mL)	*N*	Model 1[3] RR (95% CI)	Model 2[4] RR (95% CI)
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: RRs and 95% CIs were estimated using ZIP regression models, with a sandwich robust variance estimator. *p* values significant at the alpha level 0.05 are denoted in bold font.
[1]Model 1: Adjusted for age (10-year categories), BMI (<25, 25–29.9, ≥30 kg/m^2), and menopausal status (premenopausal, postmenopausal).
[2]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 percentage fat on the H&E slide (≤75, >75%).
[3]Model 1: Adjusted for age (10-year categories) and BMI (<25, 25–29.9, ≥30 kg/m^2).
[4]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 percentage fat on the H&E slide (≤75, >75%).
Abbreviations: CI: confidence interval; H&E: hematoxylin and 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; ZIP: zero-inflated Poisson.

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] Furthermore, 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 Ref. 50), body composition,[51] breast density,[52] IGF levels (Table 2 and Refs. 20–23) and other endogenous factors (*e.g.*, estrogens,[53,54] adipokines[55,56] and 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 this study does not have mammographic density data, AA women had higher BMI, a strong inverse correlate of mammographic density.[58] This study also showed suggestively stronger IGF-I and TDLU associations in Caucasian and AA women with lower BMI. Furthermore, the discrepancy in results with previous studies may be partially explained by differences in normal breast tissue from women with *versus* without BBD, and differences in study participant characteristics (*e.g.*, age and 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 in 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.

Acknowledgements

The authors thank all KTB staff and researchers. Most importantly, the authors also thank the volunteers who donated invaluable samples that made this study possible. This study was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics of the U.S. National Cancer Institute. The Komen Tissue Bank is supported by Susan G. Komen, the Breast Cancer Research Foundation, Oracle Giving, and the Catherine Peachey Fund, Inc.

References

1. Russo J, Russo IH. Development of the human breast. *Maturitas* 2004;49:2–15.
2. Milanese TR, Hartmann LC, Sellers TA, et al. Age-related lobular involution and risk of breast cancer. *J Natl Cancer Inst* 2006;98:1600–7., 3rd,.
3. Figueroa JD, Pfeiffer RM, Brinton LA, et al. Standardized measures of lobular involution and subsequent breast cancer risk among women with benign breast disease: a nested case-control study. *Breast Cancer Res Treat* 2016;159:163–72.
4. Deeks S, Richards J, Nandi S. Maintenance of normal rat mammary epithelial cells by insulin and insulin-like growth factor 1. *Exp Cell Res* 1988;174:448–60.
5. Ruan W, Kleinberg DL. Insulin-like growth factor I is essential for terminal end bud formation and ductal morphogenesis during mammary development. *Endocrinology.* 1999;140:5075–81.
6. Burren CP, Wilson EM, Hwa V, et al. Binding properties and distribution of insulin-like growth factor binding protein-related protein 3 (IGFBP-rP3/NovH), an additional member of the IGFBP Superfamily. *J Clin Endocrinol Metab* 1999;84: 1096–103.
7. Lewitt MS, Saunders H, Phuyal JL, et al. Complex formation by human insulin-like growth factor-binding protein-3 and human acid-labile subunit in growth hormone-deficient rats. *Endocrinol* 1994;134:2404–9.
8. Valentinis B, Bhala A, DeAngelis T, et al. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. *Mol Endocrinol* 1995;9:361–7.
9. 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.
10. Borugian MJ, Spinelli JJ, Sun Z, et al. Prostate cancer risk in relation to insulin-like growth factor (IGF)-I and IGF-binding protein-3: a prospective multiethnic study. Cancer. *Epidemiol Biomark Prevent* 2008;17:252–4.
11. Endogenous Hormones Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, et al. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *Lancet Oncol* 2010;11:530–42.
12. Dearth RK, Delgado DA, Hiney JK, et al. Parity-induced decrease in systemic growth hormone alters mammary gland signaling: a potential role in pregnancy protection from breast cancer. *Cancer Prevent Res* 2010;3:312–21.
13. Rowzee AM, Lazzarino DA, Rota L, et al. IGF ligand and receptor regulation of mammary development. *J Mammary Gland Biol Neoplasia* 2008;13:361–70.
14. Radisky DC, Hartmann LC. Mammary involution and breast cancer risk: transgenic models and clinical studies. *J Mammary Gland Biol Neoplasia* 2009;14:181–91.
15. Richards RG, Klotz DM, Walker MP, et al. Mammary gland branching morphogenesis is diminished in mice with a deficiency of insulin-like growth factor-I (IGF-I), but not in mice with a liver-specific deletion of IGF-I. *Endocrinology.* 2004;145:3106–10.
16. Kleinberg DL, Feldman M, Ruan W. IGF-I: an essential factor in terminal end bud formation and ductal morphogenesis. *J Mammary Gland Biol Neoplasia* 2000;5:7–17.
17. Neuenschwander S, Schwartz A, Wood TL, et al. Involution of the lactating mammary gland is inhibited by the IGF system in a transgenic mouse model. *J Clin Invest* 1996;97:2225–32.
18. Rice MS, Tamimi RM, Connolly JL, et al. Insulin-like growth factor-1, insulin-like growth factor binding protein-3 and lobule type in the Nurses' Health Study II. *Breast Cancer Res* 2012;14:R44
19. Horne HN, Sherman ME, Pfeiffer RM, et al. Circulating insulin-like growth factor-I, insulin-like growth factor binding protein-3 and terminal duct lobular unit involution of the breast: a cross-sectional study of women with benign breast disease. *Breast Cancer Res* 2016;18:24
20. Fowke JH, Matthews CE, Yu H, et al. Racial differences in the association between body mass index and serum IGF1, IGF2, and IGFBP3. *Endocr Relat Cancer* 2010;17:51–60.
21. DeLellis K, Rinaldi S, Kaaks RJ, et al. Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the multiethnic cohort. *Cancer Epidemiol Biomarkers Prev* 2004;13:1444–51.
22. Berrigan D, Potischman N, Dodd KW, et al. Race/ethnic variation in serum levels of IGF-I and IGFBP-3 in US adults. *Growth Horm IGF Res* 2009;19:146–55.
23. Higgins PB, Fernandez JR, Goran MI, et al. Early ethnic difference in insulin-like growth factor-1 is associated with African genetic admixture. *Pediatr Res* 2005;58:850–4.
24. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst* 2014;106:
25. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2013. Table 1. 12. Bethesda, MD: National Cancer Institute:based on November 2015 SEER data submission.
26. Sherman ME, Figueroa JD, Henry JE, et al. The Susan G. Komen for the cure tissue bank at the IU simon cancer center: a unique resource for defining the "molecular histology" of the breast. *Cancer Prevent Res* 2012;5:528–35.
27. Figueroa JD, Pfeiffer RM, Patel DA, et al. duct lobular unit involution of the normal breast: implications for breast cancer etiology. *J Natl Cancer Inst* 2014;106:
28. Diorio C, Pollak M, Byrne C, et al. Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. *Cancer Epidemiol Biomarkers Prev* 2005;14:1065–73.
29. Faupel-Badger JM, Berrigan D, Ballard-Barbash R, et al. Anthropometric correlates of insulin-like growth factor 1 (IGF-1) and

IGF binding protein-3 (IGFBP-3) levels by race/ethnicity and gender. *Ann Epidemiol* 2009;19:841–9.
30. Rohrmann S, Grote VA, Becker S, et al. Concentrations of IGF-I and IGFBP-3 and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Br J Cancer* 2012;106:1004–10.
31. Rosebrock A, Caban JJ, Figueroa J, Gierach G, Linville L, Hewitt S, Sherman M. Quantitative analysis of TDLUs using adaptive morphological shape techniques. *Proc Spie* 2013;8676
32. Khodr ZG, Sherman ME, Pfeiffer RM, et al. Circulating sex hormones and terminal duct lobular unit involution of the normal breast. *Cancer Epidemiol Biomarkers Prev* 2014;23: 2765–73.
33. Long JS. Count Outcomes: regression Models for Counts, 1st edn. Thousand Oaks: SAGE Publications, 1997.
34. Zeileis A. Econometric computing with HC and HAC covariance matrix estimators. *J Stat Soft* 2004;11:1–17.
35. Zeileis A. Object-oriented computation of sandwich estimators. *J Stat Soft* 2006;16:1–16.
36. Bradbury KE, Balkwill A, Tipper SJ, Million Women Study C, et al. The association of plasma IGF-I with dietary, lifestyle, anthropometric, and early life factors in postmenopausal women. *Growth Horm IGF Res* 2015;25:90–5.
37. Gram IT, Norat T, Rinaldi S, et al. Body mass index, waist circumference and waist-hip ratio and serum levels of IGF-I and IGFBP-3 in European women. *Int J Obes* 2006;30:1623–31.
38. Lukanova A, Toniolo P, Akhmedkhanov A, et al. A cross-sectional study of IGF-I determinants in women. *Eur J Cancer Prevent* 2001;10: 443–52.
39. Holmes MD, Pollak MN, Hankinson SE. Lifestyle correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev* 2002;11:862–7.
40. Parekh N, Roberts CB, Vadiveloo M, et al. Lifestyle, anthropometric, and obesity-related physiologic determinants of insulin-like growth factor-1 in the Third National Health and Nutrition Examination Survey (1988–1994). *Ann Epidemiol* 2010;20:182–93.
41. Landin-Wilhelmsen K, Lundberg PA, Lappas G, et al. Insulin-like growth factor I levels in healthy adults. *Horm Res Paediatr* 2004;62:8–16.
42. Landin-Wllhelmsen K, Wllhelmsen L, Lappast G, et al. Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. *Clin Endocrinol* 1994;41:351–7.
43. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* 1995;16:3–34.
44. Baxter RC, Turtle JR. Regulation of hepatic growth hormone receptors by insulin. *Biochem Biophys Res Commun* 1978;84:350–7.
45. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev* 1994;15:80–101.
46. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. *Proc Nutr Soc* 2001;60:91–106.
47. Tannenbaum GS, Guyda HJ, Posner BI. Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science* 1983;220:77–9.
48. Oh H, Khodr ZG, Sherman ME, et al. Relation of serum estrogen metabolites with terminal duct lobular unit involution among women undergoing diagnostic image-guided breast biopsy. *Horm Cancer* 2016;7:305–15.
49. Henderson KD, Goran MI, Kolonel LN, et al. Ethnic disparity in the relationship between obesity and plasma insulin-like growth factors: the multiethnic cohort. *Cancer Epidemiol Biomarkers Prev* 2006;15:2298–302.
50. Hales CM, Carroll MD, Fryar CD, et al. Prevalence of obesity among adults and youth: United States, 2015–2016. *NCHS Data Brief* 2017;1–8.
51. Rahman M, Temple JR, Breitkopf CR, et al. Racial differences in body fat distribution among reproductive-aged women. *Metabolism* 2009;58: 1329–37.
52. del Carmen MG, Hughes KS, Halpern E, et al. Racial differences in mammographic breast density. *Cancer* 2003;98:590–6.
53. Pinheiro SP, Holmes MD, Pollak MN, et al. Racial differences in premenopausal endogenous hormones. *Cancer Epidemiol BiomarkPrevent* 2005;14:2147–53.
54. Setiawan VW, Haiman CA, Stanczyk FZ, et al. Racial/ethnic differences in postmenopausal endogenous hormones: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2006;15: 1849–55.
55. Khan UI, Wang D, Sowers MR, et al. Race-ethnic differences in adipokine levels: the Study of Women's Health Across the Nation (SWAN). *Metabolism* 2012;61:1261–9.
56. Morimoto Y, Conroy SM, Ollberding NJ, et al. Ethnic differences in serum adipokine and C-reactive protein levels: the multiethnic cohort. *Int J Obes (Lond)* 2014;38:1416–22.
57. Park NJ, Kang DH. Inflammatory cytokine levels and breast cancer risk factors: racial differences of healthy Caucasian and African American women. *Oncol Nurs Forum* 2013;40:490–500.
58. Boyd NF, Martin LJ, Sun L, et al. Body size, mammographic density, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2006;15:2086–92.
59. Ghosh K, Hartmann LC, Reynolds C, et al. Association between mammographic density and age-related lobular involution of the breast. *J Clin Oncol* 2010;28:2207–12.
60. Gierach GL, Patel DA, Pfeiffer RM, et al. Relationship of terminal duct lobular unit involution of the breast with area and volume mammographic densities. *Cancer Prevent Res* 2016;9:149–58.
61. Byrne C, Colditz GA, Willett WC, et al. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. *Cancer Res* 2000;60:3744–8.
62. 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.
63. Hutson SW, Cowen PN, Bird CC. Morphometric studies of age related changes in normal human breast and their significance for evolution of mammary cancer. *J Clin Pathol* 1985;38:281–7.
64. Missmer SA, Spiegelman D, Bertone-Johnson ER, et al. Reproducibility of plasma steroid hormones, prolactin, and insulin-like growth factor levels among premenopausal women over a 2- to 3-year period. *Cancer Epidemiol Biomarkers Prev* 2006;15:972–8.