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Plasma IGF-1 and IGFBP-3 may be imprecise surrogates for breast concentrations: an analysis of healthy women

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Abstract

We investigated insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-3 concentrations in histologically normal breast tissues and assessed their association with plasma concentrations, and breast cancer risk factors. IGF-1 and IGFBP-3 were assessed in plasma and breast tissues of 90 women with no history of any cancer and undergoing reduction mammoplasty. Pearson correlations and ANOVAs were used to describe plasma-breast associations and biomarker differences by breast cancer risk factors, respectively. Multivariable regression models were used to determine associations between risk factors, and breast IGF-1 and IGFBP-3. The mean age of the study sample was 37.3 years, 58 % were white, and generally these women were obese (mean BMI = 30.8 kg/m²). We observed no plasma-breast correlation for IGF-1, IGFBP-3, or IGF-1/IGFBP-3 ($r = -0.08$, $r = 0.14$, and $r = 0.03$, respectively; p -values >0.05). Through age- and BMI-adjusted analysis, BMI and years of oral contraceptive (OC) use were inversely associated with breast IGF-1 (p -values = 0.02 and 0.003, respectively) and age was associated with breast IGFBP-3 ($p = 0.01$), while breast IGF-1/IGFBP-3 was higher in blacks than whites (1.08 vs. 0.68, $p = 0.04$) and associated with age and BMI (p -values = 0.03 and 0.002, respectively). In multivariable-adjusted models, some breast cancer risk factors studied herein explained 24, 10, and 15 % of the variation in breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3,

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This study is novel in that it is the first to investigate factors associated with breast IGF-1 and IGFBP-3 variation and assess plasma-breast correlations for these hormones in women without a history of any cancer. These data could have important implications for understanding the observed associations with breast cancer risk and could further our understanding of breast cancer etiology, potentially leading to new insights about the role of the IGF-axis in breast carcinogenesis.

respectively. While reasons for the lack of plasma-breast hormone correlations in these cancer-free women are unknown, several factors were shown to be associated with breast concentrations. The lack of correlation between blood and tissue IGF-1 and IGFBP-3 suggests that studies of breast cancer risk assessing blood IGF-1 and IGFBP-3 may have important limitations in understanding their role in breast carcinogenesis.

Keywords

IGF-1; IGFBP-3; IGF-1/IGFBP-3; Breast tissues; Plasma; Race; Oral contraceptive use

Introduction

Insulin-like growth factors (IGFs) are peptide hormones involved in mammalian growth and development. IGF-1 is essential for many of the growth-promoting effects of growth hormone and is involved in the development of normal breast tissue [1, 2]. IGF-1 has been implicated in breast carcinogenesis [3, 4] and shown to be anti-apoptotic and mitogenic [2, 5]. It binds to the IGF-1 receptor (IGF-1R) and this binding is regulated by the IGF binding proteins (IGFBPs). There is evidence that IGFBPs can induce apoptosis by sequestering IGF-1 from the IGF-1R [6]. IGFBP-3, the most abundant IGFBP in postnatal serum, binds ~75–90 % of circulating IGF-1 and is important in IGF-1 action and bioavailability [7]. IGFBP-3 has been shown to be growth inhibitory, apoptosis-inducing [8], and to have an effect on breast carcinogenesis independent of IGF-1 [9].

Several prospective studies have investigated associations among IGF-1, IGFBP-3, and breast cancer [10]. Positive associations were reported between plasma IGF-1 and breast cancer risk among pre- [10–14] and post-menopausal [10, 13–17] women in some studies. Similarly, positive associations between IGFBP-3 and breast cancer risk have also been reported in pre- [10–12] and post-menopausal women [10, 12, 15, 16]. Several studies [10, 17–24] have provided clues as to the determinants of plasma IGF-1 and IGFBP-3 concentrations, which may in turn provide insight into how these hormones influence breast cancer risk. Consistent associations between plasma IGF-1 and age [10, 17, 18, 21–24], race [18, 19, 25, 26], and BMI [10, 17–19, 23–26], have been reported. Similarly, associations between plasma IGFBP-3 and age [17, 18, 23, 24], race [18, 19], and BMI [17, 18, 24, 26] have also been observed. Conversely, little is known about what factors contribute to variation in IGF-1 and IGFBP-3 concentrations within the breast tissue itself. Consequently, published investigations of blood hormone concentrations in relation to breast cancer have assumed direct relevance to target tissue levels. In this study, we present results from our cross-sectional investigation of plasma-breast correlations for IGF-1 and IGFBP-3 as well as the associations of these hormones within the breast with other exposures and participant characteristics.

Materials and methods

Recruitment and eligibility

Women undergoing reduction mammoplasty at Georgetown University Medical Center, the University of Maryland, the Washington Hospital Center and the Center for Plastic Surgery, Buffalo, NY between 1997 and 2009 were eligible for participation as previously described [27, 28]. Any reduction mammoplasty patient age >18 (or 16–18 years where a parent/guardian was available to provide consent) with no history of any cancer were included, unless there was an observed benign breast lesion (e.g., fibroadenomas, epithelial hyperplasia, perilobular or ductal inflammation, focal microcalcification, etc.), a psychiatric disorder, or other reason that precluded understanding the consent. Written informed consent

was obtained from all study participants and approval for this study was obtained from the Institutional Review Boards of all participating institutions.

Data collection

Within 24 h of surgery, a baseline interview was administered which queried on demographics, lifestyle, medications, and recent exposures. A more extensive interview was administered either at that time, or shortly after surgery to assess personal and family medical history, lifestyle, and reproductive history. If a participant was eligible and willing to participate, but unable to schedule an interview, data were obtained from their medical record ($n = 23$; 26 %). For participants whose medical records were used to obtain data, only age, race, height, weight, smoking status, and menopausal status were obtained.

Biospecimen collection

Surgically removed breast tissues were collected from 90 study participants and determined to be free from gross pathological abnormalities by board certified pathologists. Pathology reports were used to identify benign breast disease, as above. Epithelial and stromal tissues were dissected (to remove adipose tissues) and snap frozen in liquid nitrogen within 1 h of surgery. Aliquots of tissues were stored at -80°C until analysis. Heparinized plasma samples were collected, separated from other blood components, aliquoted, and stored at -80°C until analysis.

IGF-1 and IGFBP-3 assays

Plasma and breast IGF-1 and IGFBP-3 concentrations were quantified using the IGF-1 and IGFBP-3 immunoassays on the Immulite 1000 system (Siemens Healthcare Diagnostics, Inc., Washington, DC). Each assay batch included commercial controls, participant samples, and blinded plasma controls. Samples were assayed randomly and in duplicate. Mean concentrations of duplicate samples were reported for IGF-1 and IGFBP-3. The coefficients of variation (CVs) for the plasma assays were 1.70 and 1.48 % for IGF-1 and IGFBP-3, respectively.

For breast hormone analyses, frozen tissues (0.80 g) were homogenized according to our tissue protein homogenization protocol described previously [27]. Prior to hormone analysis, samples were thawed on ice, mixed gently, and assayed for total protein using the Coomassie Plus Assay Kit (Pierce, Rockford, IL). Breast hormone concentrations were normalized by total protein concentration and are reported in nanograms of hormone per milligram of protein (ng/mg of protein). CVs for the breast hormone assays were 9.33 and 3.37 % for IGF-1 and IGFBP-3, respectively.

Breast hormone and breast tissue homogeneity

To assess homogeneity of IGF-1 and IGFBP-3 concentrations across the breast, fifteen subjects with available breast tissues from three different sections of the same breast (upper, center, and lower sections) were studied ($n = 45$ samples). Each frozen breast tissue specimen was homogenized as previously described [27]. IGF-1 and IGFBP-3 concentrations were measured in each breast specimen and differences by breast location were assessed to determine if hormone concentrations varied by location. In addition, breast tissue heterogeneity was assessed to determine if differing amounts of epithelial and stromal tissues versus adipose tissues (on H&E slides) affected IGF-1 and IGFBP-3 concentrations. The heterogeneity index was scored on a 5-point scale, representing the ratio of epithelial/stromal versus adipose tissues, as previously described [27]. Correlations between IGF-1 and IGFBP-3 concentrations and mean heterogeneity index were assessed to determine if amounts of epithelia/stroma versus adipose correlated with breast hormone concentrations.

Statistical analyses

Breast IGF-1 and IGFBP-3 concentrations were log transformed for normality. Transformed data were used in comparison analyses. Median values were used to categorize years of OC and alcohol use. Means and frequencies were used to assess distributions of sample characteristics. *T*-tests and analysis of variance (ANOVA) were used to evaluate differences in IGF-1 and IGFBP-3 concentrations. Pearson's correlation coefficients were used to describe plasma-breast associations for IGF-1 and IGFBP-3.

Multivariable linear regression models were used to investigate associations between selected factors and IGF-1 and IGFBP-3 concentrations. We used backward elimination, with significance of 0.20 to determine which variables would remain in the final models. All variables of interest (Table 1) were included in the initial model given their known or suspected association with breast cancer. In addition, we hypothesized a priori that there would be interaction between race and BMI, and race and menopausal status on the associations with breast IGF-1 and IGFBP-3. These interactions were tested by including cross-product terms as covariates in the model selection procedures.

Log-transformed hormone concentrations were modeled to satisfy regression assumptions and compared to untransformed models, yielding similar data; therefore, untransformed models are reported for ease of interpretation. SAS version 9.2 (SAS Institute, Cary, NC) and STATA version 12 (StataCorp LP, College Station, TX) were used for analyses. All *p*-values were two-sided and *p* < 0.05 was considered statistically significant.

Results

Characteristics of study sample

One hundred fifty-five women were enrolled in this study. For the results reported herein, a subset of 90 women who underwent reduction mammoplasty, completed interview-administered questionnaires, contributed plasma and had breast tissue available, is included. Comparisons between women who did and did not have available tissue (based on the covariates listed in Table 1) showed no differences (data not shown). The mean age of the sample of 90 women was 37.3 ± 12.3 , 58 % were white, 77 % were pre-menopausal, and mean BMI was 30.8 ± 7.2 kg/m² (Table 1). Among these women, mean plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 concentrations were 155.5 ± 66.3 ng/mL, 4.4 ± 1.3 µg/mL, and 36.9 ± 16.2 , respectively. Breast hormone concentrations exhibited wide interindividual variation (i.e., there were broad ranging values across each hormone). The mean concentrations of breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 were 24.8 ± 19.4 ng/mg (range 4.94–150.83 ng/mg), 32.2 ± 26.4 ng/mg (range 5.23–171.40 ng/mg), and 1.1 ± 0.8 (range 0.12–3.60 ng/mg), respectively. Separately, differences in characteristics and hormone concentrations by site of recruitment were also assessed and none were found (data not shown). As part of the questionnaire, women were queried for a history of diabetes and diabetes medications. None reported such.

Breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 concentrations

No differences in mean breast IGF-1, IGFBP-3, or IGF-1/IGFBP-3 concentrations were observed by breast location (Friedman test *p*-values = 0.51, 0.47, and 0.43, respectively). The mean concentrations of IGF-1 in center, lower, and upper breast locations were: 12.96 ± 12.42 , 8.29 ± 6.10 , and 7.61 ± 6.00 ng/mg, respectively, while those for IGFBP-3 were: 13.96 ± 7.00 , 10.96 ± 6.20 , and 12.68 ± 5.62 ng/mg, respectively. Pearson correlation coefficients comparing the three breast locations ranged from 0.2 to 0.6 for IGF-1 and 0.3–0.6 for IGFBP-3. Tissue heterogeneity index, assessing the ratio of epithelial and stromal to adipose tissues was not correlated with breast IGF-1 (*r* = 0.02, *p* = 0.87), IGFBP-3 (*r* = 0.21,

$p = 0.07$), or IGF-1/IGFBP-3 ($r = -0.17$, $p = 0.14$), indicating no effect of tissue composition on breast hormone concentrations.

Plasma and breast hormone concentrations were poorly correlated (Table 2). In age-adjusted analysis, plasma IGF-1 was modestly negatively correlated with breast concentrations ($r = -0.22$, $p = 0.04$). In the multivariable-adjusted model, this correlation was attenuated ($r = -0.08$, $p = 0.51$). No plasma-breast correlations were observed for IGFBP-3 or the IGF-1/IGFBP-3 ratio.

Associations between breast cancer risk factors and breast IGF-1, IGFBP-3 and IGF-1/IGFBP-3

Associations between potential breast cancer risk factors and breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 concentrations are shown in Table 3. Breast IGF-1 was higher among never users of OCs ($p = 0.003$) and inversely associated with age at menarche ($p = 0.06$). Breast IGFBP-3 was positively associated with age ($p = 0.01$). IGF-1/IGFBP-3 was positively associated with age ($p = 0.03$) and BMI ($p = 0.001$), and almost twofold higher among blacks than whites (1.08 vs. 0.68; $p = 0.04$).

There was no evidence of an interaction effect between BMI and race on breast IGF-1 ($F = 0.32$, $p = 0.58$) or IGFBP-3 ($F = 1.41$, $p = 0.23$). There was also no interaction between race and menopausal status on breast IGF-1 ($F = 1.90$, $p = 0.18$). There was a trend toward an association for IGFBP-3 ($F = 3.00$, $p = 0.09$), although in multiple regression analysis race*menopausal status was not found to be associated with either breast IGF-1 or IGFBP-3.

Race, age at menarche, family history of breast cancer, and duration of OC use explained 24 % of the interindividual variation in breast IGF-1 (Table 4). Blacks had breast IGF-1 concentrations that were 7.77 ng/mg higher than whites ($\beta = 7.77$, $p = 0.07$). A 1-year increase in age at menarche was associated with 2.85 ng/mg decrease in breast IGF-1 ($\beta = -2.85$, $p = 0.03$). Mean breast IGF-1 among women who used OCs ≤ 5 years, mean breast IGF-1 concentration was 14.68 ng/mg lower than never users ($\beta = -14.68$, $p = 0.01$), while among those who had used OCs > 5 years was 20.23 ng/mg lower than never users ($\beta = -20.23$, $p = 0.002$). Age and race explained 10 % of breast IGFBP-3 variation. There was a 0.66 ng/mg decrease in breast IGFBP-3 concentration for a 1-year increase in age ($\beta = -0.66$, $p = 0.006$) and mean breast IGFBP-3 concentration was 8.97 ng/mg lower among black women than white women ($\beta = -8.97$, $p = 0.12$). Race, age at menarche, and duration of alcohol use explained 15 % of the variation in the breast IGF-1/IGFBP-3 ratio. Mean breast IGF-1/IGFBP-3 was higher among blacks than whites ($\beta = 0.58$, $p = 0.005$). There was a 0.11 unit decrease in IGF-1/IGFBP-3 per 1-year increase in age at menarche ($\beta = -0.11$, $p = 0.06$). There was also indication of an inverse association between breast IGF-1/IGFBP-3 and duration of alcohol use, particularly at lower durations. Breast IGF-1/IGFBP-3 was 0.44 units lower among those using alcohol ≤ 17 years than never users ($\beta = -0.44$, $p = 0.06$).

Discussion

To our knowledge, this is the first study to investigate associations between plasma and breast IGF-1 and IGFBP-3, and report on factors associated with breast concentrations. The study revealed large interindividual variation of breast IGF-1 and IGFBP-3 concentrations. In this study of women who never had cancer, breast hormone concentrations were not correlated with plasma hormones suggesting that systemic levels of IGF-1 and IGFBP-3 are imprecise surrogates for concentrations in the breast microenvironment. Furthermore, it is possible that misclassification resulting from the use of plasma hormone concentrations as a proxy for tissue hormones could contribute to conflicting reports regarding the association

between plasma IGF hormones and breast cancer [10–16, 18, 29]. Only 10–24 % of variation in breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 was explained by factors investigated in this study. We found that race, age at menarche, family history of breast cancer, and duration of OC use were associated with variation in breast IGF-1 concentrations, age and race were associated with variation in IGFBP-3 concentrations, and race, age at menarche, and duration of alcohol use were associated with variation in IGF-1/IGFBP-3 in the breast.

Findings from some studies indicate higher plasma IGF-1 concentrations are associated with increased breast cancer risk [11–16, 30, 31], others are null [29, 32–36], and others provide evidence specifically for pre- [11–14] or post-menopausal women only [15, 16, 30, 31]. In the studies demonstrating a positive and significant association between plasma IGF-1 and breast cancer risk, the risk estimates comparing the highest category of plasma IGF-1 to the lowest have ranged from 2.3 to 5.3 for pre-menopausal women [11–14], and 1.2–3.1 among post-menopausal women [15, 16, 30, 31]. In a pooled analysis of 17 prospective studies [10], there was a positive association between IGF-1 and breast cancer, irrespective of menopausal status (OR = 1.28, 95 % CI: 1.1–1.4). Although studies have investigated largely white populations, there is evidence of a positive association for IGF-1 and breast cancer among black women as well [30]. Studies of the plasma IGFBP-3-breast cancer association have also been inconsistent. Some have shown no association between IGFBP-3 and breast cancer in pre- [13, 29, 33, 35, 36] or post-menopausal women [13, 29, 32, 33, 36], whereas others point toward a positive association [11, 12, 15, 16]. In a pooled analysis [10], plasma IGFBP-3 was associated with 23 % increased breast cancer risk for post-menopausal women (OR = 1.23, 95 % CI: 1.01–1.45).

The above studies rely upon the assumption that plasma IGF-1 and IGFBP-3 concentrations are surrogates for hormone exposures within the breast. Based on findings from the present study, this assumption appears to be invalid. No plasma-breast correlation for IGF-1, IGFBP-3, or IGF-1/IGFBP-3 was observed overall, although the plasma concentrations reported herein are consistent with those of other studies of cancer-free women [13, 24, 31, 33, 35].

Low correlations between blood and tissue measures for other hormones have also been reported. While Falk et al. [37] observed high serum-breast adipose correlations for androstenedione, testosterone, and estrone, they saw no correlation for estradiol. We [27] recently reported modest plasma-breast correlations for leptin and adiponectin. While we cannot be certain why there would be stronger plasma-breast correlations for some hormones than for others, it is possible that circulating concentrations result from cumulative exposures (i.e., hormones produced and secreted in various tissues) and are thus not representative of local tissue concentrations. Hormone metabolism within tissues may alter tissue-specific concentrations of some hormones, which are not measurable in the blood. Supporting this hypothesis, although the liver is the major source of circulating concentrations for IGF-1, it can be synthesized and exported by almost any tissue in the body [38]. The advantage of assessing plasma concentrations is ease of access compared with tissue biopsies; however, there is potential for misclassification of tissue exposures and thus breast cancer associations for some hormones might be more consistent if *breast* concentrations were assessed.

It has been shown that in the breast epithelium, IGFBPs are regulated by an intricate pathway involving estrogen and progesterone [39]. Consequently, endogenous exposure to estrogens within the breast, as a result of a woman's reproductive history and other factors such as OC use, may alter the action and bioavailability of IGFBPs in the breast. Our findings of an association of duration of OC use and both breast IGF-1 and IGFBP-3 may

support these findings. An inverse association between OC use and concentrations of both IGF-1 and IGFBP-3 has also been found for plasma concentrations [20, 40]. In this study, the lowest concentrations of IGF-1 were found in breast tissues of women with longer durations of OC use (>5 years). Therefore, while the estrogens in OCs may have an effect on breast epithelial and/or stromal tissues and use of certain types of OCs may increase breast cancer risk [41–43], their influence on breast carcinogenesis may involve a complex mechanism that is yet to be determined. It is well-established that, independent of BMI, blacks have higher plasma IGF-1 and lower IGFBP-3 concentrations [19, 25, 26, 44, 45]. We found variation in breast concentrations to be explained in part by race as well. It is unclear what drives racial differences in IGF hormones, but BMI could play a role as it is associated with plasma IGF-1 and IGFBP-3 [17, 19, 21, 23, 25, 26]; however, in our small study, when we adjusted for age- and BMI we found no association between race and breast concentrations of IGF-1 or IGFBP-3. However, there was evidence that the IGF-1/IGFBP-3 ratio in the breast was higher among blacks than whites (1.08 vs. 0.68, $p = 0.04$). In this study, blacks were heavier than whites (mean BMI = 34.7 vs. 28.2 kg/m², $p < 0.0001$) and had higher breast IGF-1 and IGF-1/IGFBP-3 (30.3 vs. 20.4 ng/mg, $p = 0.02$ and 1.5 vs. 0.9, $p = 0.002$, respectively), although, based on multivariable-adjusted regression analysis, there was no evidence to support an association between BMI and variation in these breast hormones.

Understanding the determinants of variation in IGF-1/IGFBP-3 is particularly interesting as this ratio represents free IGF-1 that has not bound IGFBPs, and thus thought to represent the biologically active pool of IGF-1 [7, 9]. First, we found that breast IGF-1/IGFBP-3 was higher among blacks than whites, which may indicate that there is a larger portion of biologically active IGF-1 within the breasts of these women. Given the prior findings supporting a role for increased IGF-1 and breast cancer risk, as well as those demonstrating racial disparities in breast cancer incidence and prognosis [46–48], we speculate that racial differences in IGF-1/IGFBP-3, particularly within the breast microenvironment could potentially contribute to the biologic mechanisms involved in racial differences in breast cancer incidence and prognosis. In addition, we found that age at menarche was associated with breast IGF-1/IGFBP-3, which may suggest that there are possible joint effects of longer duration of estrogen exposure and endogenous IGF hormones within the breast, which may modify breast cancer risk. These observations warrant further investigation.

There were several strengths of this study. The cross-sectional analysis of breast hormone concentrations in women without breast cancer as well as without benign breast disease permitted examination of variation in IGF-1 and IGFBP-3 in histologically normal breast tissues. Using tissues sampled from different parts of the breast allowed us to determine, while concentrations within the breast for each woman were modestly correlated, mean hormone concentrations across the breast were similar for both IGF-1 and IGFBP-3. This study has demonstrated that the ratio of epithelial/stromal to adipose tissues was not correlated with breast IGF-1 or IGFBP-3 concentrations. We were able to measure IGF-1 and IGFBP with low CVs. The study of the associations of concentrations in blood and breast for the IGF biomarkers was an additional strength. Accruing women without breast cancer and undergoing reduction mammoplasty enabled retrieval of large amounts of blood and histologically normal breast tissues, together with extensive data for demographic, medical, family history, and lifestyle risk exposures. The opportunity for studying relationships of breast hormone concentrations and breast cancer risk factors, with sufficient quantities of breast tissues is not feasible in any other setting for women without cancer.

It is important to also note the limitations of this study. We recognize that the small sample size was a limitation; nonetheless we observed significant findings that can be further investigated in larger studies. Another limitation is choice of study participants. Women

who undergo reduction mammoplasty may not be representative of all women due to larger breast size, higher BMI, and potentially increased breast cancer risk due to large breast size [49, 50]. While generalizability might be a potential limitation, this group may be considered a susceptible population, so study participant choice may be a strength rather than a limitation, because it allows for investigation of risk factors that may be inherent to this sub-group of women. The wide inter-individual variability in IGF-1 and IGFBP-3 levels in the breast demonstrated in this study is what would be expected in the general population, though possibly to a greater extent. We also had some limitations in the questionnaire data that were collected. Lack of information on additional confounders, as well as information pertaining to recency of exposures (e.g., amount of daily tobacco and alcohol use and current use of OCs and types used) could have enhanced our findings.

In summary, we demonstrated that plasma IGF-1 and IGFBP-3 concentrations were not well-correlated with breast concentrations and that duration of OC use is an important determinant of breast IGF-1 and IGFBP-3 variation. Separately, race was strongly associated with breast IGF-1/IGFBP-3. Racial differences in concentrations of breast IGF-1/IGFBP-3 and possibly other hormones, is an important, understudied area in breast cancer research. Further studies are essential to determine additional factors that contribute to variation in breast hormone concentrations and their relation to breast cancer risk.

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Table 1Characteristics of reduction mammoplasty study participants, $N = 90$

Characteristic	<i>n</i> (%)
Age (years), mean $\pm$ SD	37.3 $\pm$ 12.3
Menopausal Status	
Pre-menopausal	66 (76.7)
Post-menopausal	20 (23.3)
Race	
White	51 (58.0)
Black	37 (42.0)
BMI (kg/m ²), mean $\pm$ SD	30.8 $\pm$ 7.2
BMI (kg/m ²)	
18.5–24.99	19 (21.1)
25–29.99	25 (27.8)
30	46 (51.1)
Education	
<12 years	16 (18.2)
12–16 years	39 (44.3)
16 years	24 (27.3)
Family history of breast cancer	
No	54 (63.5)
Yes	31 (36.5)
Age at menarche (years), mean $\pm$ SD	12.4 $\pm$ 1.6
Parity	
Nulliparous	38 (54.3)
1–2	14 (20.0)
>2	18 (25.7)
Years of oral contraceptive use ^a	
Never user	27 (32.9)
5 years	34 (41.5)
>5 years	21 (25.6)
Years of alcohol use ^b	
Never drinker	22 (28.5)
17 years	30 (39.0)
>17 years	25 (32.5)
Smoking status	
Never smoker	52 (62.7)
Former smoker	20 (24.1)
Current smoker	11 (13.2)

Characteristic	<i>n</i> (%)
Biomarker concentrations	Mean $\pm$ SD
Plasma IGF-1 (ng/mL)	155.5 $\pm$ 66.3
Plasma IGFBP-3 (μ g/mL)	4.4 $\pm$ 1.3
Plasma IGF-1/IGFBP-3	36.9 $\pm$ 16.2
Breast IGF-1 (ng/mg)	24.8 $\pm$ 19.4
Breast IGFBP-3 (ng/mg)	32.2 $\pm$ 26.4
Breast IGF-1/IGFBP-3	1.1 $\pm$ 0.8

BMI/body mass index

^aMedian years of oral contraceptive use among ever users was 5 years

^bMedian years of alcohol use among ever drinkers was 17 years

Table 2

Plasma-breast correlations of IGF-1, IGFBP-3, and IGF-1/IGFBP-3

	Model 1		Model 2		Model 3	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Breast IGF-1						
Plasma IGF-1	−0.16	0.12	−0.11	0.31	−0.08	0.51
Breast IGFBP-3						
Plasma IGFBP-3	0.19	0.07	0.16	0.14	0.14	0.21
Breast IGF-1/IGFBP-3						
Plasma IGF-1/IGFBP-3	−0.09	0.38	0.06	0.56	0.03	0.76

Note: Model 1 unadjusted, *Model 2* age- and BMI-adjusted, *Model 3* multivariable-adjusted [age (years), BMI (kg/m²), race (white/black), and menopausal status]

Table 3Age- and BMI-adjusted associations between breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 and selected variables, $n = 90$

Variable	Breast IGF-1 (ng/mg)				Breast IGFBP-3 (ng/mg)				Breast IGF-1/IGFBP-3			
	n	LS Means	95 % CI	P ^a	LS Means	95 % CI	P ^a	LS Means ^a	95 % CI	P ^a		
Age (years)	8 9	20. 50	19.49–21.51	0.84	25.02	24.01–26.03	0.01	1.02	1.01–1.03	0.03		
Menopausal status				0.70			0.53			0.37		
Pre-menopausal	66	20.49	17.46–24.05		23.81	19.69–29.08		0.86	0.70–1.06			
Post-menopausal	20	19.11	13.74–26.31		27.66	18.73–40.85		0.69	0.47–1.04			
Race				0.12			0.38			0.04		
White	51	17.81	14.73–21.76		26.31	20.70–33.12		0.68	0.53–0.88			
White	51	17.81	14.73–21.76		26.31	20.70–33.12		0.68	0.53–0.88			
Black	37	23.57	18.54–29.67		21.76	16.28–29.08		1.08	0.80–1.46			
BMI (kg/m ²)	89	20.50	19.41–21.59	0.02	25.02	24.00–26.04	0.11	1.02	1.00–1.04	0.001		
Education				0.85			0.31			0.21		
<12 years	16	21.12	14.88–29.67		18.54	12.18–27.94		1.15	0.74–1.77			
12–16 years	39	19.30	15.64–23.57		26.58	20.91–34.12		0.73	0.56–0.93			
16 years	24	20.70	15.64–27.66		26.31	19.11–36.60		0.78	0.55–1.12			
Family history of breast cancer				0.12			0.44			0.62		
No	54	21.76	18.36–25.79		25.79	21.12–31.50		0.85	0.68–1.05			
Yes	31	17.46	14.01–21.76		22.65	17.29–29.37		0.77	0.58–1.03			
Age at menarche (years)	80	20.36	20.28–20.44	0.06	25.37	24.26–26.48	0.89	1.03	0.91–1.15	0.19		
Parity				0.14			0.58			0.45		
Nulliparous	38	21.54	17.46–26.58		26.84	20.91–34.12		0.81	0.62–1.05			

Variable	Breast IGF-1 (ng/mg)				Breast IGFBP-3 (ng/mg)				Breast IGF-1/IGFBP-3			
	n	LS Means	95 % CI	P ^a	LS Means	95 % CI	P ^a	LS Means ^a	95 % CI	P ^a		
1–2	14	17.12	13.74–21.33		21.98	16.78–28.79		0.78	0.59–1.03			
>2	18	25.03	17.12–36.23		22.87	14.59–35.87		1.09	0.68–1.77			
Years of oral contraceptive use				0.003			0.13			0.76		
Never user	27	28.79	8.17–37.34		32.14	23.10–44.26		0.90	0.64–1.28			
5 years	34	20.09	16.61–24.05		24.05	19.11–30.27		0.84	0.65–1.07			
>5 years	21	15.49	12.18–19.49		20.49	15.49–27.39		0.76	0.55–1.04			
Years of alcohol use				0.13			0.94			0.18		
Never drinker	22	25.28	19.49–33.12		23.81	17.29–32.79		1.06	0.76–1.49			
17 years	30	18.54	15.18–22.65		25.28	19.89–32.46		0.73	0.57–0.94			
>17 years	25	18.17	13.74–24.29		24.29	17.12–34.12		0.76	0.53–1.08			
Smoking status				0.10			0.44			0.56		
Never smoker	52	21.54	18.36–25.53		24.29	19.69–48.91		0.90	0.72–1.12			
Former smoker	20	15.64	12.06–20.49		21.76	15.64–30.27		0.73	0.51–1.03			
Current smoker	11	23.57	16.61–33.78		31.19	20.09–48.42		0.76	0.47–1.22			

Note: Breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 were log transformed for normality, but back-transformed data reported in table. For the associations of age, BMI, and age at menarche, the adjusted means of breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3 are given at the average value of each continuous risk factor

^a P-values reflect linear associations between biomarkers and risk factors, adjusted for age and BMI

Table 4

Multivariable regression models of breast IGF-1, IGFBP-3, and IGF-1/IGFBP-3

	Parameter estimate	Standard error	P	R ²
Breast IGF-1 (ng/mg protein) ^a				0.24
Race	7.77	4.29	0.07	
Age at menarche (years)	-2.85	1.33	0.03	
Family history of breast cancer	-6.28	4.32	0.15	
Years of oral contraceptive use			0.007	
5 years	-14.68	5.72		
> 5 years	-20.23	6.24		
Breast IGFBP-3 (ng/mg protein) ^b				0.10
Race	-8.97	5.85	0.12	
Age (years)	-0.66	0.24	0.006	
Breast IGF-1/IGFBP-3 ^c				0.15
Race	0.58	0.20	0.005	
Age at menarche (years)	-0.11	0.06	0.06	
Years of alcohol use			0.13	
17 years	-0.44	0.25		
>17 years	-0.13	0.26		

Multivariable-adjusted R^2 reported. Backward elimination was used to determine the variables included in final models. Significance of 0.20 was required for a variable to remain in the final model. Variables were modeled as follows: race [white (referent)/black], age at menarche (years), family history of breast cancer [no (referent)/yes], age (years), years of OC use [never user (referent), 5 years, >5 years], and years of alcohol use [never-drinker (referent), 17 years, >17 years]

^aDue to the missing data on some covariates, the breast IGF-1 model included 75/90 (83.3 %) participants

^bDue to the missing data on some covariates, the breast IGFBP-3 model included 84/90 (93.3 %) participants

^cDue to the missing data on some covariates, the breast IGF-1/IGFBP-3 model included 77/90 (85.6 %) participants