Insulin-like growth factor-1, insulin-like growth factor-binding protein-3, growth hormone, and mammographic density in the Nurses' Health Studies

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Abstract Higher circulating insulin-like growth factor I (IGF-1) levels have been associated with higher mammographic density among women in some, but not all studies. Also, few studies have examined the association between mammographic density and circulating growth hormone (GH) in premenopausal women. We conducted a cross-sectional study among 783 premenopausal women and 436 postmenopausal women who were controls in breast cancer case–control studies nested in the Nurses' Health Study (NHS) and NHSII. Participants provided blood samples in 1989–1990 (NHS) or in 1996–1999 (NHSII), and mammograms were obtained near the time of blood draw. Generalized linear models were used to assess the associations of IGF-1, IGF-binding protein-3 (IGFBP-3), IGF-1:IGFBP-3 ratio, and GH with percent mammographic density, total dense area, and total non-dense area. Models were adjusted for potential confounders including age and body mass index (BMI), among others. We also assessed whether the associations varied by age or BMI. In both pre- and postmenopausal women, percent mammographic density was not associated with plasma levels of IGF-1, IGFBP-3, or the IGF-1:IGFBP-3 ratio. In addition, GH was not associated with percent density among premenopausal women in the NHSII. Similarly, total dense area and non-dense area were not significantly associated with any of these analytes. In postmenopausal women, IGF-1 was associated with higher percent mammographic density among women with BMI <25 kg/m^2, but not among overweight/obese women. Overall, plasma IGF-1, IGFBP-3, and GH levels were not associated with mammographic density in a sample of premenopausal and postmenopausal women.

Keywords Insulin-like growth factor 1 · Insulin-like growth factor-binding protein-3 · Mammographic density · Breast cancer

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Abbreviations

NHS   Nurses' Health Study
IGF-1   Insulin-like growth factor-1
IGFBP-3 Insulin-like growth factor binding protein-3
GH   Growth hormone
BMI   Body mass index
PMH   Postmenopausal hormone
BBD   Benign breast disease

Introduction

Mammographic density, or the radiographic appearance of the breast on a mammogram, is a strong predictor of breast cancer risk [1]. Epithelial and stromal tissue in the female breast is radiodense and appears light on a mammogram. In contrast, fat is radiolucent and appears dark. Women with over 75 % dense tissue on a mammogram have 4–6 times the risk of developing breast cancer compared with women with little to no dense tissue [1, 2]. Mammographic density is associated with a number of reproductive factors and has been hypothesized to represent cumulative exposure to hormones and growth factors. Studies have observed that women using postmenopausal hormones have increased density, whereas premenopausal women on tamoxifen have reduced density [3]. These drugs may act via effects on estrogen or by altering the IGF-1 axis [4].

IGF-1 is anti-apoptotic and mitogenic, and has been identified as a factor in tumor development and progression [5, 6]. IGF-1 is regulated by GH and modulated by IGF-binding proteins, primarily IGFBP-3 [6]. While epidemiologic studies suggest that IGF-1 is positively associated with both pre- and postmenopausal breast cancer risk [7], studies of mammographic density have been inconsistent. Higher circulating IGF-1 levels have been associated with an increase in mammographic density among premenopausal women in some studies [8–10]; however, others have not observed an association [11, 12]. In general, IGF-1 levels have not been associated clearly with mammographic density among postmenopausal women [9–14]. Few studies have examined GH in relation to mammographic density in premenopausal women. In one study, GH was significantly positively associated with percent water, a correlate of mammographic density, among women under 30 years of age [15]. However, in another study among older premenopausal women, GH was not associated with percent density after adjustment for body mass index (BMI) [10]. Therefore, the purpose of this cross-sectional study was to examine the association between circulating IGF-1, IGFBP-3, IGF-1: IGFBP-3 ratio, and GH levels with percent mammographic density in a large sample of pre- and postmenopausal women in the nurses' health study (NHS) and the NHSII.

Materials and methods

Study population

In 1976, 121,700 female registered nurses, 30–55 years of age and residing in 11 U.S. states completed an initial questionnaire, forming the NHS cohort. The NHSII began in 1989, when 116,430 female registered nurses, aged 25–42, from 14 U.S. states completed an initial questionnaire. Both cohorts have been followed via biennially mailed questionnaires to update exposure and covariate information and to ascertain incident diseases.

In 1989–1990, we obtained blood samples from 32,826 NHS participants, ages 43–70. Between 1996 and 1999, 29,611 NHSII members, aged 32–45 years, provided a blood sample. Characteristics of both cohorts and blood collection details have been described previously [16–19]. In brief, in the NHSII, premenopausal women who had not taken any type of hormones, been pregnant, or breastfed in the previous 6 months (n = 18,521), provided a 30-ml blood sample drawn 7–9 days before the anticipated start of their next menstrual cycle (luteal blood draw). All NHS participants as well as NHSII women who were ineligible to provide timed samples (i.e., perimenopausal, postmenopausal, had a simple hysterectomy, currently used oral contraceptives or other hormones, or declined to give timed samples; n = 11,090) provided a single 30-ml blood sample (referred to as "untimed" samples). For both luteal and untimed samples, women shipped the blood to our laboratory, with an ice-pack, via overnight courier, where the samples were processed, separated into plasma, red blood cell, and white blood cell components, and aliquoted into labeled cryotubes. All samples have been stored in the vapor phase of continuously monitored liquid nitrogen freezers (<-130 °C) since collection.

We restricted our analysis to women who were controls in the NHS or NHSII nested breast cancer case–control studies [16, 20]. Breast cancer cases were matched to one or two controls on age, menopausal status at blood draw and diagnosis, and current postmenopausal hormone use (PMH) as well as month, time of day, and fasting status at time of blood collection. We collected mammograms conducted as close as possible to the date of blood collection for women in the nested breast cancer case–control studies. Among controls in the NHS and NHSII case–control studies, 1,816 had valid measures of plasma hormone levels and mammographic density. We excluded 12 women without a valid measure of BMI. We further restricted our study population to the 783 women in the NHS and NHSII who were premenopausal at both blood collection and mammogram as well as 436 NHS women who were postmenopausal at both blood collection and mammogram and who reported no current PMH use.

The median time between mammography and blood collection was 8 months for premenopausal women and 10 months for postmenopausal women. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital. Informed consent was implied by receipt of completed questionnaires and blood samples.

Laboratory assays

IGF-1 and IGFBP-3 levels were assayed in six batches for untimed samples in the NHS and in two batches for luteal and untimed samples in the NHSII. GH was assayed in two batches for luteal and untimed samples among premenopausal women the NHSII. IGF-1, IGFBP-3, and GH were assayed by ELISA after acid extraction at the Department of Medicine and Oncology at McGill University, using reagents from Diagnostic Systems Laboratory (Webster, TX, USA). Cases and matched controls were assayed in the same batch and laboratory personnel were masked to case status. In the NHS, all breast cancer cases (and their matched controls) diagnosed between the 1990 blood draw and 1996 were assayed for IGF-1 and IGFBP-3. In addition, breast cancer cases (and their matched controls) who were premenopausal at blood collection and were diagnosed between 1996 and 2004 were assayed. In NHSII, all breast cancer cases (and their matched controls) diagnosed between blood collection and 2003 were assayed for IGF-1, IGFBP-3, and GH. Coefficients of variation (CVs) for the assay batches ranged from 4 to 11 % for IGF-1, IGFBP-3, and GH.

Mammographic density

The craniocaudal views of both breasts were digitized with a Lumysis 85 laser film scanner. We measured absolute dense area, absolute non-dense area (the total area minus the dense area), and percent dense area (the dense area divided by the total area) using the Cumulus software for computer-assisted thresholding. The observer was blinded to case–control status. The within-person intraclass correlation coefficient was 0.90. We averaged the density parameters of both breasts for this analysis.

Covariate data

We used the covariate information from the biennial questionnaire preceding the mammogram date to obtain

Table 1 Characteristics of 783 premenopausal and 436 postmenopausal women by quartile of percent mammographic density, NHS and NHSII, mean (SD) or N (%)

	Premenopausal				Postmenopausal			
	Percent mammographic density				Percent mammographic density			
	Q1 (<24 %) $N = 195$	Q2 (24–40 %) $N = 196$	Q3 (40–55 %) $N = 196$	Q4 (55+ %) $N = 196$	Q1 (<8 %) $N = 109$	Q2 (8–18 %) $N = 109$	Q3 (18–33 %) $N = 109$	Q4 (33+ %) $N = 109$
Mean (SD)								
Age (year)	48.2 (4.0)	47.6 (4.0)	46.5 (4.2)	45.8 (4.2)	62.3 (5.6)	61.4 (5.8)	60.9 (6.4)	58.3 (7.8)
BMI (kg/m²)	29.1 (6.3)	25.4 (4.1)	23.8 (3.5)	22.4 (2.9)	29.9 (5.7)	27.0 (3.9)	25.1 (4.1)	23.4 (3.3)
Age at menarche	12.1 (1.3)	12.4 (1.3)	12.7 (1.4)	12.6 (1.4)	12.5 (1.3)	12.4 (1.4)	12.7 (1.5)	12.7 (1.4)
Parity (among parous)	2.7 (1.1)	2.7 (1.0)	2.6 (1.0)	2.3 (0.9)	3.8 (1.6)	3.8 (1.8)	3.5 (1.5)	3.3 (1.7)
Age at first birth (among parous)	24.8 (3.8)	25.9 (3.9)	26.2 (3.6)	25.8 (3.8)	25.1 (3.1)	25.2 (3.3)	25.6 (3.3)	25.8 (3.2)
Alcohol (gm/day)	4.3 (8.6)	4.8 (7.2)	4.8 (7.3)	4.6 (8.6)	5.5 (9.4)	6.3 (10.4)	5.8 (9.1)	6.9 (9.5)
N (%)								
Nulliparous	18 (9.2)	14 (7.1)	21 (10.7)	29 (14.8)	4 (3.7)	4 (3.7)	8 (7.3)	15 (13.8)
History of BBD	80 (41.0)	87 (44.4)	104 (53.1)	118 (60.2)	36 (33.0)	39 (35.8)	38 (34.9)	52 (47.7)
Family history of breast cancer	13 (6.7)	23 (11.7)	16 (8.2)	13 (6.6)	16 (14.7)	14 (12.8)	16 (14.7)	17 (15.6)
Smoking								
Never	107 (54.9)	103 (52.6)	117 (59.7)	127 (64.8)	49 (45.0)	39 (35.8)	59 (54.1)	60 (55.1)
Past	71 (36.4)	72 (36.7)	65 (33.2)	54 (27.6)	52 (47.7)	56 (51.4)	42 (38.5)	34 (31.2)
Current	17 (8.7)	21 (10.7)	14 (7.1)	15 (7.7)	8 (7.3)	14 (12.8)	8 (7.3)	15 (13.8)
Past PMH use	–	–	–	–	34 (31.2)	41 (37.6)	41 (37.6)	37 (33.9)

data on the following covariates: body mass index (BMI) (kg/m^2), history of benign breast disease (BBD), family history of breast cancer, age at menarche, parity, age at first birth, smoking, and alcohol intake.

Statistical analysis

Owing to batch-to-batch variation over time, we recalibrated IGF-1 and IGFBP-3 levels from all batches to have a comparable distribution to an average batch according to methods outlined by Rosner et al. [21]. In brief, we assumed that all batches combined represented an average batch. We then regressed levels of IGF-1 (or IGFBP-3) on age and BMI (the strongest predictors of these hormones) as well as indicator variables for each batch. Within each batch, IGF-1 and IGFBP-3 levels were recalibrated by adding the resulting value of the coefficients for that batch minus the average of the batch coefficients. Therefore, these recalibrated levels accounted for the variability between batches independent of varying age and BMI distributions between batches. We then classified the recalibrated values into quartiles based on the overall

distribution. For GH, 40 % of women had levels below the limit of detection (0.14 ng/ml for batch 1 and 0.18 ng/ml for batch 2). Therefore, all women with values below the limit of detection comprised the reference group. The remaining women were classified into batch-specific tertiles resulting in four total categories.

We used generalized linear models adjusting for matching factors to evaluate the association between plasma hormone levels and mammographic density measures. These models accounted for potential correlation between controls that were matched to the same case. Square-root transformed dense area and non-dense area on the mammogram were used as outcomes as both distributions were skewed. For each category of the hormones, we estimated the least-square mean percent density, square-root dense area, and square-root non-dense area. We tested for linear trend using Wald tests by ordinally modeling the median of the batch-specific categories. When conducting trend tests for GH, we used the median of the midpoint between zero and the limit of detection for women whose values were below the limit of detection. Our primary model adjusted for age (continuous) and matching factors

Table 2 Mean percent density by quartiles of plasma IGF-1, IGFBP-3, IGF-1:IGFBP-3 ratio, and growth hormone among premenopausal women in the NHS and NHSII

	Mean percent density				*p*-trend
	Q1	Q2	Q3	Q4	
IGF-1					
N	196	195	196	196	
Model 1[a]	38.1	42.1	39.5	38.7	0.94
Model 2[b]	39.2	41.1	39.1	39.2	0.81
IGFBP-3					
N	196	195	196	196	
Model 1[a]	40.9	40.3	39.2	37.8	0.11
Model 2[b]	40.5	40.5	38.3	39.2	0.33
IGF-1/IGFBP-3 ratio					
N	195	197	195	196	
Model 1[a]	37.8	40.3	40.8	39.6	0.36
Model 2[b]	39.8	40.2	39.6	38.8	0.53
Growth hormone (NHSII only)					
N	146	77	75	76	
Model 1[a]	42.5	39.5	43.7	44.6	0.31
Model 2[b]	43.9	38.9	43.2	42.8	0.90

Quartile/category cutpoints: IGF-1 ng/ml (156.6, 193.8, 236.5), IGFBP-3 ng/ml (3364.8, 3797.2, 4208.8), IGF-1:IGFBP-3 molar ratio (0.16, 0.19, 0.22), GH ng/ml (batch 1: <0.14, 0.14–0.57, 0.57–2.14, 2.14+ ; batch 2: <0.18, 0.18–0.45, 0.45–1.30, 1.30+)

[a] Model 1: age at mammography (continuous), month of blood draw (continuous), fasting status (<8 h or unknown, ≥8 h since last meal), time of day of blood collection (12 am–5:59 am, 6:00 am–7:59 am, 8:00 am–11:59 pm), and cohort (except for growth hormone)

[b] Includes covariates in Model 1 and history of bbd (yes/no), family history of breast cancer (yes/no), age at menarche (<12, 12, 13, 14+), parity and age at first birth (nulliparous, 1–2 children age at first birth <25 years, 1–2 children age at first birth 25–29 years, 1–2 children age at first birth ≥30 years, 3+ children age at first birth <25 years, 3+ children age at first birth 25+ years), alcohol consumption (0, <5, 5–<15, ≥15 g/day, missing), smoking status (never, past, current), and BMI (continuous), also adjusts for IGFBP-3 (quartiles) in the IGF-1 model and for IGF-1 (quartiles) in the IGFBP-3 model

Table 3 Mean percent density by quartiles of plasma IGF-1, IGFBP-3, and GH among premenopausal women in NHS and NHSII by categories of age and BMI

	Q1	Q2	Q3	Q4	*p*-trend	*p*-het
IGF-1						
Mean percent density: by age						
N	35	39	66	72		
Age: <45 years[a]	45.3	45.8	43.6	45.9	0.91	0.92
N	161	156	130	124		
Age: 45+ years[a]	36.8	39.3	37.9	36.4	0.79	
Mean percent density: by BMI						
N	99	125	127	125		
BMI: <25 kg/m^{2a}	48.0	48.7	46.7	45.5	0.24	0.16
N	97	70	69	71		
BMI: 25+ kg/m^{2a}	26	28.7	28.2	28.4	0.44	
IGFBP-3						
Mean percent density: by age						
N	45	56	54	57		
Age: <45 years[a]	46.6	46.2	43.8	45.9	0.67	0.98
N	151	139	142	139		
Age: 45+ years[a]	38.0	39.0	35.4	37.9	0.65	
Mean percent density: by BMI						
N	124	118	121	113		
BMI: <25 kg/m^{2a}	48.6	47.3	45.8	46.8	0.39	0.25
N	72	77	75	83		
BMI: 25+ kg/m^{2a}	26.6	30.5	26.2	27.3	0.90	
GH						
Mean percent density: by age						
N	78	32	28	37		
Age: <45 years[a]	44.9	41.1	47.9	44.4	0.89	0.82
N	68	45	47	39		
Age: 45+ years[a]	43.5	36.7	39.4	42.2	0.76	
Mean percent density: by BMI						
N	88	52	46	47		
BMI: <25 kg/m^{2a}	50.0	48.8	52.9	51.8	0.51	0.21
N	58	25	29	29		
BMI: 25+ kg/m^{2a}	33.8	19.7	28.5	29.4	0.85	

Quartile/category cutpoints: IGF-1 ng/ml (156.6, 193.8, 236.5), IGFBP-3 ng/ml (3364.8, 3797.2, 4208.8), IGF-1:IGFBP-3 molar ratio (0.16, 0.19, 0.22), GH ng/ml (batch 1: <0.14, 0.14–0.57, 0.50–2.14, 2.14+ ; batch 2: <0.18, 0.18–0.45, 0.45–1.30, 1.30+))

[a] Adjusted for age at mammography (continuous), month of blood draw (continuous), fasting status (<8 h or unknown, ≥8 h since last meal), time of day of blood collection (12 am–5:59 am, 6:00 am–7:59 am, 8:00 am–11:59 pm), cohort, history of bbd (yes, no), family history of breast cancer (yes, no), age at menarche (continuous), parity and age at first birth (nulliparous, 1–2 children age at first birth <25 years, 1–2 children age at first birth 25–29 years, 1–2 children age at first birth ≥30 years, 3+ children age at first birth <25 years, 3+ children age at first birth 25+ years), alcohol consumption (0, <5, 5– <15, ≥15 g/day, missing), smoking status (never, past, current), BMI (continuous), also adjusts for IGFBP-3 (quartiles) in the IGF-1 model and for IGF-1 (quartiles) in the IGFBP-3 model

from the original nested case–control study, and then we subsequently adjusted for breast cancer risk factors, including history of BBD, family history of breast cancer, age at menarche, parity, age at first birth, smoking status, alcohol use, BMI, and IGFBP-3 (for the IGF-1 analysis), and IGF-1 (for the IGFBP-3 analysis). Tests for trend for GH also were adjusted for assay batch. To determine if the associations between IGF-1, IGFBP-3, and GH levels and percent density varied by age (<45, 45+ years for premenopausal; <60, 60+ for postmenopausal) and BMI (<25, 25+ kg/m^2), we conducted Wald tests for obtaining the interaction term between the ordinal median variable for the hormone and the binary age and BMI variables. We used SAS 9.2 software (SAS Institute, Cary, NC, USA) for

all analyses and considered a *p* value less than 0.05 to be statistically significant.

Results

For both pre- (*n* = 783) and postmenopausal (*n* = 436) women, those in the highest category of percent mammographic density were younger, had a lower BMI, were more likely to have a history of BBD, and were more likely to be nulliparous compared to women in the lowest quartile of mammographic density (Table 1).

Among premenopausal women, we did not observe a statistically significant association between percent mammographic density and IGF-1, IGFBP-3, the IGF-1: IGFBP-3 ratio, or GH in multivariate models (*p*-trend ≥0.33) (Table 2). For example, women in the highest quartile of IGF-1 levels had the same least squares mean percent mammographic density of 39.2 % as women in the lowest quartile. These associations did not vary by age or BMI (*p*-heterogeneity ≥ 0.16, Table 3). In addition, there were no associations between the selected hormones and dense area or non-dense area (Supplementary Tables 1 and 2).

Among postmenopausal women in the NHS, IGF-1 was not associated with percent mammographic density (*p*-trend = 0.79; Table 4). However, IGFBP-3 was inversely associated with percent mammographic density (*p*-trend = 0.03) and the IGF-1:IGFBP-3 ratio was positively associated with percent density (*p*-trend = 0.01) in models adjusting for age and matching factors only. These associations were attenuated after adjustment for other potential confounders, particularly BMI (*p*-trend = 0.23 and 0.92, respectively). Similarly, we did not observe any statistically significant associations between these hormones and dense area or non-dense area on the mammogram (Supplementary Tables 3 and 4). Among postmenopausal women, the association between IGF-1 and percent density varied by BMI (*p* for heterogeneity = 0.01) (Table 5). Increasing IGF-1 levels were associated with higher percent mammographic density among women with a BMI below 25 kg/m² (*p*-trend = 0.01), but not among women with a BMI above 25 kg/m² (*p*-trend = 0.47). For example, the least squared mean percent density was 35.5 in the highest quartile and 25.0 in the lowest quartile in leaner women. In contrast, we did not observe effect modification by BMI for IGFBP-3. There was no difference in the associations by age.

Discussion

In this analysis of pre- and postmenopausal women, we did not observe evidence of any overall association between

plasma levels of IGF-1, IGFBP-3, or GH and mammographic density. However, in stratified analyses, IGF-1 levels were associated with higher percent mammography density among leaner postmenopausal women. Our findings did not vary by age or BMI in premenopausal women, or by age in postmenopausal women.

While we previously observed a positive association between IGF-1 and mammographic density in postmenopausal women, our earlier NHS analysis was comprised of only 65 women [8]. In our larger updated analysis (N = 783 premenopausal women), we did not observe a clear association. The results of the current study are consistent with a pooled analysis of 525 premenopausal women studied by Maskarinec et al. [11] in which no association was observed between IGF-1 and IGFBP-3 and percent mammographic density. While there was a suggestion of an association between IGF-1 and dense area in a cross-sectional study of 215 premenopausal UK women, no statistically significant associations were noted for percent density [12]. However, two other studies reported

Table 4 Mean percent density by quartiles of plasma IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 ratio among postmenopausal women in the NHS

	Q1	Q2	Q3	Q4	*p* value
Mean percent density					
IGF-1					
N	109	110	108	109	
Model 1[a]	19.2	23.5	24.9	21.9	0.34
Model 2[b]	21.6	22.9	22.4	22.5	0.79
IGFBP-3					
N	109	109	109	109	
Model 1[a]	24.6	23.3	21.8	19.5	0.03
Model 2[b]	24.4	21.9	21.8	21.3	0.23
IGF-1/IGFBP-3 ratio					
N	109	109	109	109	
Model 1[a]	18.2	22.7	24.5	24.3	0.01
Model 2[b]	21.9	22.5	22.8	22.2	0.92

Quartile/category cutpoints: IGF-1 (140.2, 173.6, 226.0), IGFBP-3 (3406.8, 4050.0, 4783.7), IGF-1:IGFBP-3 ratio (0.14, 0.16, 0.20)

[a] Model 1: age at mammography (continuous), month of blood draw (continuous), fasting status (<8 h or unknown, ≥8 h since last meal), time of day of blood collection(12 am–5:59 am, 6:00 am–7:59 am, 8:00 am–11:59 pm), and cohort

[b] Includes covariates in Model 1 and history of bbd (yes, no), family history of breast cancer (yes, no), age at menarche (<12, 12, 13, 14+), parity and age at first birth (nulliparous, 1–2 children age at first birth <25 years, 1–2 children age at first birth 25–29 years, 1–2 children age at first birth ≥30 years, 3+ children age at first birth <25 years, 3+ children age at first birth 25+ years), alcohol consumption (0, <5, 5– <15, ≥15 g/day, missing), smoking status (never, past, current), BMI (continuous),and past PMH use (yes, no), also adjusts for IGFBP-3 (quartiles) in the IGF-1 model and for IGF-1 (quartiles) in the IGFBP-3 model

Table 5 Mean percent density by quartiles of plasma IGF-1 and IGFBP-3 among postmenopausal women in NHS by categories of age and BMI

	Q1	Q2	Q3	Q4	*p*-trend	*p*-het
IGF-1						
Mean percent density: by age						
N	34	40	52	45		
Age: <60 years[a]	24.1	26.9	27.4	27.2	0.55	0.96
N	75	70	56	64		
Age: 60+ years[a]	19.0	19.3	19.0	20.9	0.49	
Mean percent density: by BMI						
N	46	44	69	48		
BMI: <25 kg/m^{2a}	25.0	29.5	31.7	35.5	0.01	0.01
N	63	66	39	61		
BMI: 25+ kg/m^{2a}	15.7	16.1	14.0	14.0	0.47	
IGFBP-3						
Mean percent density: by age						
N	42	39	53	37		
Age: <60 years[a]	26.8	29.4	27.5	23.0	0.40	0.59
N	67	70	56	72		
Age: 60+ years[a]	21.0	18.1	19.1	20.0	0.92	
Mean percent density: by BMI						
N	56	54	51	46		
BMI: <25 kg/m^{2a}	33.9	30.4	30.3	27.6	0.11	0.56
N	53	55	58	63		
BMI: 25+ kg/m^{2a}	16.4	15.4	13.7	14.9	0.56	

Quartile/category cutpoints: IGF-1 (140.2, 173.6, 226.0), IGFBP-3 (3406.8, 4050.0, 4783.7), IGF-1:IGFBP-3 ratio (0.14, 0.16, 0.20)

[a] Adjusted for age at mammography (continuous), month of blood draw (continuous), fasting status (<8 h or unknown, ≥8 h since last meal), time of day of blood collection(12 am–5:59 am, 6:00 am–7:59 am, 8:00 am–11:59 pm), cohort, history of bbd (yes, no), past pmh use (yes, no), family history of breast cancer (yes, no), age at menarche (<12, 12, 13 14+), parity and age at first birth (nulliparous, 1–2 children age at first birth <25 years, 1–2 children age at first birth 25–29 years, 1–2 children age at first birth ≥30 years, 3+ children age at first birth <25 years, 3+ children age at first birth 25+ years), alcohol consumption (0, <5, 5– <15, ≥15 g/day, missing), smoking status (never, past, current), BMI (continuous), also adjusts for IGFBP-3 (quartiles) in the IGF-1 model and for IGF-1 (quartiles) in the IGFBP-3 model

significant positive associations between IGF-1 levels and percent density in premenopausal women [9, 10]. Given these inconsistent results, the role of mammographic density in the relationship between IGF-1 and premenopausal breast cancer risk remains unclear.

Among postmenopausal women, several studies have observed no association between IGF-1 levels and mammographic density, particularly after adjusting for BMI [9–13]. For example, in a study of about 800 postmenopausal women, neither IGF-1 (*p*-trend = 0.57) nor IGFBP-3 (*p*-trend = 0.67) was associated with mammographic density [11]. These results, in conjunction with our data, suggest that IGF-1 may influence postmenopausal breast cancer risk through mechanisms other than by altering breast density.

Few studies have examined the association between GH and percent mammographic density in premenopausal women. Boyd et al. [10] observed a positive association between GH levels and percent density (*n* = 193); however

this was attenuated after adjustment for age and waist circumference. These results are consistent with our data; however, given the relatively small sample sizes of both studies, additional research is warranted.

Our study has several limitations. While the CVs for the plasma hormones were low, there is some modest measurement error, which would be non-differential as the laboratory was blinded to density measurements (and case–control status). Similarly, we only have a single measurement of hormones levels, which may not be representative of long-term levels. However the three-year intra-class correlation coefficient among 113 premenopausal women in NHSII was 0.86 for IGF-1 and 0.82 for IGFBP-3, irrespective of menstrual cycle phase [22]; results were similar for postmenopausal women [23]. These data suggest that a single measurement of IGF-1 and IGFBP-3 is representative of levels over at least a three-year period. The ICC for GH in the NHS2 was somewhat lower (ICC = 0.44) as GH has a pulsatile secretion, which may have precluded observing an

association. The strengths of our study include the centralized collection and reading of mammograms, which is highly reproducible, high quality hormone assays, and detailed adjustment for correlates of mammographic density.

Conclusion

Plasma IGF-1, IGFBP-3, and GH levels were not associated with mammographic density in a sample of pre- and postmenopausal women from the Nurses' Health Studies.

Conflicts of interest The authors declare that they have no conflict of interest.

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