

International Journal of Cancer [What is RSS?](#)**Early View** (Articles online in advance of print)

Published Online: 22 May 2007

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Funded by:

▪ NCI; Grant Number: R03 CA105948

▪ NIH

▪ R01 CA80843; Grant Number: R01 CA85265, R03 CA81620

▪ U.S. Army Medical Research Materiel Command; Grant Number: DAMD17-00-1-0281

▪ Ministry of Education, Science, Sports, and Culture, Japan

▪ Susan G. Koman Breast Cancer Foundation; Grant Number: BCTR 2000 538

▪ Department of Clinical Research, University Hospital of North Norway, the Norwegian Cancer Society, the Aakre Foundation

▪ Norwegian Women's Public Health Association; Grant Number: H3-02

▪ Research Council of Norway; Grant Number: 148365/300

▪ University of Tromsø, Norway

KEYWORDS

breast density • IGFs • prolactin • ethnicity • breast cancer

ABSTRACT

Insulin-like growth factor (IGF-I) and prolactin have been found to be associated with breast cancer risk and with mammographic density. In a pooled analysis from 4 geographic locations, we investigated the association of percent mammographic density with serum levels of IGF-I, IGFBP-3 and prolactin. The pooled data set included 1,327 pre- and

postmenopausal women: Caucasians from Norway, Arizona and Hawaii, Japanese from Hawaii and Japan, Latina from Arizona, and Native Hawaiians from Hawaii. Serum samples were assayed for IGF-I, IGFBP-3 and prolactin levels using ELISA assays. Mammographic density was quantified using a computer-assisted density method. After stratification by menopausal status, multiple regression models estimated the relation between serum analytes and breast density. All serum analytes except prolactin among postmenopausal women differed significantly by location/ethnicity group. Among premenopausal subjects, IGF-I levels and the molar ratio were highest in Hawaii, intermediate in Japan and lowest in Arizona. For IGFBP-3, the order was reversed. Among postmenopausal subjects, Norwegian women had the highest IGF-I levels and women in Arizona had the lowest while women in Japan and Hawaii had intermediate levels. We observed no significant relation between percent density and IGF-I or prolactin levels among pre- and postmenopausal women. The significant differences in IGF-I levels by location but not ethnicity suggest that environmental factors influence IGF-I levels, whereas percent breast density varies more according to ethnic background than by location. Based on this analysis, the influence of circulating levels of IGF-I, IGFBP-3, and prolactin on percent density appears to be very small. © 2007 Wiley-Liss, Inc.

Received: 1 February 2007; Accepted: 4 April 2007

DIGITAL OBJECT IDENTIFIER (DOI)

10.1002/ijc.22834 [About DOI](#)

ARTICLE TEXT

Breast cancer incidence differs more than 4-fold between women in Japan and in the United States, but several generations after migration, Japanese-Americans experience a similar risk as Caucasians.[1][2] Despite the inconsistent evidence for dietary factors,[3] nutritional changes are thought to contribute to the gradual increase in breast cancer risk among migrants. Insulin-like growth factor I (IGF-I) has been proposed as a link between diet and breast cancer risk.[4] Despite some inconsistencies, there is considerable evidence for a positive association between premenopausal breast cancer risk and circulating IGF-I levels, measured either as absolute concentrations or relative to IGFBP-3.[5][6][7] In addition, a modestly elevated breast cancer risk was observed for pre- and postmenopausal women with higher levels of prolactin.[8][9] Because of its independent association with breast cancer,[10][11] breast density has been examined in relation to IGF-I and prolactin. In 4 reports, direct associations with IGF-I and inverse associations with IGFBP-3 were observed for premenopausal women,[12][13][14][15] whereas only 1[16] out of 6 studies[12][13][14][15][17] showed a positive association of percent density with IGF-I in postmenopausal women. Serum prolactin levels were positively related to breast density among postmenopausal women in 2 reports,[12][18] but not in another one[19] or in premenopausal women.[12] The present study examined the hypothesis that breast density is associated with serum levels of IGF-I, IGFBP-3 and prolactin among women of Caucasian, Japanese, Latina and Native Hawaiian ancestry residing in Hawaii, Arizona, Japan and Norway. In a previous analysis of this pooled data set, we described that mammographic density differed significantly by ethnicity ($p < 0.001$), whereas location appeared to have only a weak relation to breast density ($p = 0.19$) after adjustment for covariates.[20]

Material and methods



Study design and population

The participants of this study were recruited as part of previous epidemiologic studies in 4 locations: Hawaii and Arizona in the United States, Gifu in Japan and Tromsø in Norway. The women in all locations were recruited through mammography clinics, had a screening mammogram that was found to be without suspicious lesions by a radiologist,[21] and had no history of cancer and breast surgery. Women taking HRT were included in all locations except Arizona.

The Caucasian, Japanese and Native Hawaiian women in Hawaii were part of 4 previous investigations with different eligibility criteria. Two studies included 169 premenopausal women not taking any hormone medication and reporting low self-reported soy intake.[22][23] The other 2 Hawaii projects comprised 128 pre- and postmenopausal women with and without hormone use.[24][25][26][27] In Japan, the 376 pre- and postmenopausal women included a small proportion of women on HRT.[28][29] In Arizona, the 221 pre- and postmenopausal subjects came from the Women's Breast and Bone Density study[30] that excluded subjects reporting any kind of hormone medication, while some of the 433 postmenopausal women from Norway reported HRT use.[16][31]

In all geographic locations, questionnaire data were collected for demographic variables, medical history, anthropometric characteristics, reproductive behavior, physical activity and a dietary history using validated food frequency questionnaires.[32][33][34][35] Only in Arizona and Norway, height and weight were measured.

The Committee on Human Studies at the University of Hawaii approved the overall project and the respective Institutional Review Boards at all 4 institutions had approved the original projects. All study participants gave written informed consent.

Mammographic density assessment

Standard mammography techniques were used at all participating mammography clinics. All mammograms were ordered

as part of routine health care. Whereas in Hawaii, Arizona and Norway each screening mammography consisted of both craniocaudal (CC) and mediolateral (ML) views, the usual practice in Japan is to take ML views only. Thus, for women in the second Gifu study who were recruited as controls in a case-control study,[29] only ML views were available, but CC views were available for half of the Japanese women. Based on a comparison of CC and ML images,[36] we computed an estimated value for the CC images using the formula $ML = 2.6 + 0.86 CC$ to correct for the slightly lower percent densities in ML than CC images. Because all previous projects analyzed CC views and most of the breast density literature is based on CC views, using ML for the entire project was not considered an option.

To standardize the measurable outcomes, mammographic density assessment was performed with an identical method in all of the substudies. For comparability, the Japanese images and a proportion of the Arizona mammograms that had been obtained with digital mammography (34% of the 1,327 mammograms) were printed on films. Mammograms for all study subjects were mailed to Hawaii and scanned into a computer using a Kodak LS 85 digitizer from the Eastman Kodak Company (formerly Lumisys Company). The mammograms were scanned at a resolution of 260 $\mu\text{m}/\text{pixel}$, approximately equivalent to 97.7 dots per inch. Only the left mammograms were used because it has been shown that the measurements for the right and the left breast are highly correlated.[36] For density assessment, we used the computer-assisted method developed in Toronto.[37][38] One of the authors (GM), who was blinded as to the study participant's identity and place of residence, performed the density assessments. After the mammograms were quantified, mammographic percent density was computed as the ratio of the dense area to the total area of the breast. The size of the nondense areas was computed as the difference between total and dense area of the breast. Duplicate readings by the same rater of a random sample of mammograms ($n = 145$) indicated a high level of reproducibility of total area, dense area, and percent density readings with respective correlation coefficients of 0.996, 0.97 and 0.98 ($p < 0.0001$ for all).

Blood collection and analysis

In Hawaii and Arizona, fasting blood samples were collected in the morning. For the majority of premenopausal women in Hawaii, the collection occurred 5 days after ovulation using an ovulation detection kit.[22][39] In Gifu, nonfasting serum samples not timed to the menstrual cycle were collected in the afternoon, while in Norway, blood collections were performed at the time of screening during any part of the day.[16][29] In all locations, the samples were processed as soon as possible, centrifuged, aliquoted and stored at -70°C or below. Levels of IGF-I and IGFBP-3 were measured by ELISA assays and prolactin by immunoradiometric assay (both from Diagnostic Systems Laboratories, Webster, TX) at the International Agency for Research on Cancer (Lyon, France) over a 1-year period following standardized procedures. The Hawaii and half of the Japan samples were done first, the Arizona and the other half of the Japan samples next and the Norway samples last. The intra-assay coefficient of variation (CV) was 3.1% for IGF-I at a concentration of 110 ng/ml and 4.1% for IGFBP-3 at a concentration of 4,900 ng/ml. The respective inter-assay CVs based on blinded samples that were added to all batches were 15.2% for IGF-I and 12.6% for IGFBP-3. The intra-assay CVs for prolactin were 8.5% at 7 ng/ml, 4.0% at 18 ng/ml and 5.0% at 40 ng/ml; the inter-assay CVs based on a pooled sample from women not taking HRT were 6.5% (at a concentration of 9.3 ng/ml), and 5.9% from a pooled sample from women taking HRT (at a concentration of 18 ng/ml). The molar ratio of IGF-I to IGFBP-3 was calculated after converting the IGF-I and IGFBP-3 values from ng to nmol using the respective factors of 0.13 and 0.035.

Statistical analysis

All data analyses were performed using the SAS statistical software package, version 9 (SAS Institute, Cary, NC, 2001). [40] Depending on the availability of comparable information, we combined data from the 4 study populations in a common format. Because the distribution of percent density was not normal, we used square-root transformed values and density categories (<10 , 10-24.9, 25-49.9 and 50%+). Biologically implausible values of serum analytes ($n = 5$), *i.e.*, values more than 5 standard deviations from the mean, were replaced with missing values. Categorical variables for location and ethnicity had 4 levels each. A binary variable was created to distinguish between digital and regular mammograms from Japan and Arizona. Age at menarche was categorized as <13 and 13+ years. Two binary variables were used to describe parity: nulliparous vs. parous and <3 vs. 3+ children. Age at first live birth was classified as <25 vs. 25+ years, or none. Body mass index (BMI) was calculated as the ratio of weight in kg divided by the square of height in meters. Women who stated that their menstruation had permanently stopped or were without a menstrual cycle in the past 12 months, women who reported an oophorectomy and women 50 years and older with a hysterectomy or missing information were considered postmenopausal. HRT use was classified as never, past and current. Because the type of HRT was only known for Norway and Hawaii, this information was not included into the database. We imputed a small number of missing values ($<1\%$ for BMI and parity, 1-2% for HRT use, 5% for age at menarche and age at first live birth) using the mean or the most common category for the respective location-ethnicity group.

Based on the following reasoning, we applied generalized linear models (PROC GLM) for all analyses.[40] We tested for a random effect of study location using a multi-level model[41] and found that the association between breast density and IGF-I did not differ significantly among the 4 locations despite differences in serum analytes and breast density across populations. Because the pooled data set represents an incomplete design for ethnicity and location, *i.e.*, there were no complete replicates of ethnic-comparisons by location or replicates of location-comparisons by ethnicity, it was not possible to include a random effect for location and keep ethnicity in a model at the same time. Therefore, including an indicator variable that distinguished the 7 groups by ethnicity and location (Hawaii-Caucasian, Hawaii-Japanese, Hawaii-Hawaiian, Arizona-Caucasian, Arizona-Latina, Japan-Japanese and Norway-Caucasian) in all models was considered the optimal method to control for the variability by study group.

Covariate-adjusted means for mammographic measures and serum analytes were estimated for each subgroup using the LSMEANS option in PROC GLM.[40] The models were adjusted for covariates that were found to be significantly related to breast density in our data set including age, BMI and reproductive variables. A nonlinear association between BMI and IGF levels has been reported[42][43][44] and a quadratic model component was statistically significant ($p < 0.001$) for IGF-I and IGFBP-3 among pre- and postmenopausal women in our data. Therefore, we also included a quadratic term for BMI into all regression models. We did not adjust for smoking status, physical activity, alcohol intake and education because these variables did not make a significant contribution to the models. All analyses were stratified by menopausal status. We repeated the analysis stratified by normal weight vs. overweight, using quartiles of dense areas instead of percent density categories, and for postmenopausal women who had never taken HRT because exogenous estrogens reduce serum IGF-I levels.[45]

Results



The study population included 1,327 subjects, with women from Norway ($n = 433$), Arizona ($n = 221$), Hawaii ($n = 297$) and Japan ($n = 376$) (Table I). Because all Norwegian women were postmenopausal, their mean age was higher than for the other locations. Mean BMI was highest for Native Hawaiians, followed by women from Arizona and Norway, and lowest for Japanese. The use of HRT was uncommon in Japan, whereas ~35% of women in the Norwegian study were current HRT users. For Hawaii, the proportion was 10% and, due to exclusion criteria, no woman in Arizona was taking HRT. The total breast area was largest among Caucasians and Native Hawaiians. The latter group also had the largest dense areas, whereas age-adjusted percent density was highest among Japanese in Hawaii (41%), followed by Native Hawaiians (36%) and Japanese in Japan (35%). Caucasians in Hawaii and Arizona had intermediate densities (32%) followed by Latinas in Arizona (29%). Because of their postmenopausal status, women in Norway had the lowest percent densities (26%). Premenopausal women had higher age adjusted IGF-I and IGFBP-3 levels across all BMI deciles than postmenopausal women (Fig. 1). The association between BMI and IGF-I was slightly curvilinear, with highest serum IGF-I levels among women within a BMI range of 23-27 kg/m², and lower IGF-I levels for women with BMI levels either above or below this range. The Spearman correlation coefficient between BMI and percent density was -0.51 ($p < 0.0001$); IGFBP-3 was significantly related to the nondense areas ($r_s = 0.06$, $p = 0.04$).



Figure 1. IGF-I and IGFBP-3 levels by BMI and menopausal status. IGF-I and IGFBP-3 levels were adjusted for age and BMI decile. The median BMI for each decile is shown on the x-axis.
[Normal View 20K | Magnified View 53K]

Table I. Characteristics of the Study Population¹

Characteristic	Hawaii			Arizona		Japan	Norway	All
	Caucasian	Japanese	Hawaiian	Caucasian	Latina	Japanese	Caucasian	
Sample size	130	105	62	140	81	376	433	1327
Age at mammogram	49.0	52.6	51.8	53.1	50.0	49.0	61.2	53.9
Body mass index	25.9	24.1	29.3	27.2	27.9	22.7	27.7	25.8
Education (years)	15.7	14.9	14.0	14.9	13.0	11.9	9.4	12.2
Current smoking (%)	3.1	8.6	9.7	9.3	9.9	6.9	24.0	12.8
Physical activity (PAL)	2.15	1.93	1.97	2.16	2.12	1.37	1.37	1.65
Family history (%)	15.4	11.4	22.6	na	na	3.2	6.9	8.0
Age at menarche (years)	13.1	12.8	12.8	13.1	13.1	13.7	13.7	13.4
Age at first live	27.9	28.5	25.1	25.9	24.7	26.0	23.9	25.5

birth (years)								
Number of children	1.7	2.0	2.9	1.8	2.4	2.2	2.6	2.3
Premenopausal (%)	73.8	62.9	56.4	48.6	69.1	54.3	0.0	39.6
Oral contraceptives (%)	86.5	78.8	88.6	79.4	41.1	7.8	na	36.5
Postmenopausal (%)	26.2	37.1	43.6	51.4	30.9	45.7	100.0	60.4
Age at menopause	48.8	49.2	46.2	49.5	48.5	49.8	48.8	49.0
Current HRT use (%)	11.5	9.5	9.7	0	0	0	34.6	13.6
Past HRT use (%)	6.2	10.5	8.1	31.4	9.9	4.8	15.5	12.1
Percent density ²	32.3	40.6	36.2	31.9	28.6	34.9	25.8	31.5
Dense area (cm ²) ²	36.4	32.0	42.8	31.5	31.0	28.3	30.1	31.2
Breast area (cm ²) ²	134.4	85.1	139.8	119.6	125.7	95.5	154.5	124.2

¹ Means are shown for continuous variables and percentages for categorical variables.

² Adjusted for age; na = not available.

After full adjustment, significant differences across the 7 location/ethnic groups were observed for all serum analytes before and after menopause except for prolactin among postmenopausal women (Table II). When we included a separate variable for location and ethnicity into the model, location was statistically significant ($p < 0.001$ for pre- and postmenopausal women) but ethnicity was not ($p = 0.57$ for pre- and $p = 0.95$ for postmenopausal women). Among premenopausal subjects, women in Hawaii had the highest IGF-I levels, whereas these values were lowest in Arizona and intermediate in Japan. For IGFBP-3, the order was reversed. Levels were highest in Arizona, intermediate in Japan and lowest in Hawaii. Among postmenopausal subjects, women in Norway had the highest IGF-I levels, followed by women in Japan and Hawaii, while IGF-I levels were lowest in Arizona.

Table II. Mean Serum Analytes by Location and Ethnicity¹

Location	Ethnicity	IGF-I (ng/ml)		IGFBP-3 (ng/ml)		IGF molar ratio		Prolactin (ng/ml)	
		Age + BMI	Full	Age + BMI	Full	Age + BMI	Full	Age + BMI	Full
Premenopausal									
Arizona	Caucasian	244	220	4,810	4,888	0.188	0.185	14.0	13.4
	Latina	223	208	4,660	4,812	0.177	0.177	16.4	16.4
Hawaii	Caucasian	308	309	4,280	4,084	0.265	0.264	11.5	11.2
	Japanese	320	316	4,337	4,097	0.278	0.274	13.4	13.2
	Hawaiian	310	321	4,106	3,920	0.279	0.279	11.6	12.2
Japan	Japanese	242	253	4,196	4,330	0.218	0.221	12.8	13.1
<i>p</i> for group difference		<0.0001	<0.0001	0.97	<0.0001	<0.0001	<0.0001	0.06	0.002
Postmenopausal									
Arizona	Caucasian	186	155	4,869	5,145	0.141	0.133	9.32	9.85
	Latina	174	156	4,580	4,897	0.140	0.133	8.95	9.44
Hawaii	Caucasian	172	201	3,629	3,853	0.175	0.182	9.16	8.67

	Japanese	167	193	3,617	3,926	0.173	0.174	9.39	9.45
	Hawaiian	159	194	3,310	3,667	0.190	0.192	9.25	9.02
Japan	Japanese	206	201	4,152	4,329	0.189	0.177	8.61	9.96
Norway	Caucasian	234	232	4,372	4,217	0.202	0.203	10.3	10.3
<i>p</i> for group difference									

¹ Least-square means were obtained through general linear models while adjusted for age, BMI, quadratic BMI term, parity, age at menarche, age at first live birth, IGF-I or IGFBP-3 and HRT use, where appropriate.

We observed no significant association between percent density and any of the serum analytes in the fully adjusted models (Table III). The differences in IGF-I between the highest and lowest category of percent density were 16 and -3 ng/ml for pre- and postmenopausal women, respectively. For prolactin, the respective differences were 1.7 and 0.2 ng/ml. Although the respective *r*-squares for the overall pre- and postmenopausal models were 29.7 and 33.9%, IGF-I explained less than 1% of the variation in percent density. Stratification by group indicated that the association between IGF-I and percent density category was not statistically significant in any subgroup although mean IGF-I levels differed by location (Fig. 2). Among Hawaii Caucasians and Native Hawaiians, the *p*-values for trend approached significance (*p* = 0.07 and 0.06), but the association was inverse for the latter group.

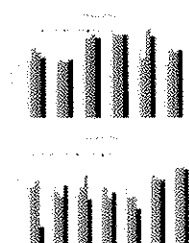


Figure 2. Adjusted mean IGF-I levels by percent density category and menopausal status. Adjusted for age, BMI, quadratic BMI term, digital mammogram, IGFBP-3, parity, age at menarche, age at first live birth and HRT use, where appropriate.
[Normal View 53K | Magnified View 189K]

Table III. Mean Serum Analytes by Percent Density Category¹

Density Category	N	Premenopausal				N	Postmenopausal			
		IGF-I (ng/ml)	IGFBP-3 (ng/ml)	IGF molar ratio	Prolactin (ng/ml)		IGF-I (ng/ml)	IGFBP-3 (ng/ml)	IGF molar ratio	Prolactin (ng/ml)
<10	44	250	4335	0.217	11.5	301	189	4255	0.169	9.8
10-<25	75	267	4383	0.233	12.4	225	192	4274	0.173	9.3
25-<50	165	270	4406	0.230	14.2	201	192	4285	0.172	9.4
≥50	241	266	4335	0.231	13.2	75	186	4411	0.165	10.0
p for trend ²		0.83	0.91	0.67	0.48		0.57	0.67	0.67	0.92

¹ Mean serum analytes were adjusted for ethnic/location group, age, BMI, quadratic BMI term, digital mammogram, IGF-I or IGFBP-3, parity, age at menarche, age at first live birth and HRT use, where appropriate.

² For trend test, square root of percent density was included in the model.

Restricting the analysis to postmenopausal women who had never used HRT (*n* = 460) resulted in mean IGF-I levels of 190, 197, 201 and 197 ng/ml (*p* for trend = 0.13) by percent density category. The respective *p*-values for trend for IGFBP-3, the molar ratio and prolactin were 0.40, 0.13 and 0.42. Stratification by body weight status, *i.e.*, BMI of less than 25 vs. 25+ kg/m² did not reveal a differential relation between percent density and any of the serum analytes. No significant associations emerged among pre- or postmenopausal women when the analyses were repeated with the size of the dense areas instead of percent density.

Discussion



This pooled analysis of women from 4 locations did not show a significant relation of percent mammographic density with IGF-I, IGFBP-3, the molar IGF-I/IGFBP-3 ratio or prolactin before or after menopause. IGF-I levels and the molar IGF-I/IGFBP-3 ratio differed significantly by location but not by ethnicity after adjustment for anthropometric and reproductive covariates (Fig. 2). These findings are contrary to our hypothesis and to reports of positive associations with IGF-I in premenopausal subjects,[12][13][14][15] 1 study with a significant relation between breast density and IGF-I among postmenopausal women,[16] 2 reports of an association prolactin and breast density in postmenopausal women,[12][18] and a recent investigation showing an association of breast density with common genetic variation for IGF-I.[46] On the other hand, this analysis agrees with findings among postmenopausal women that observed no relation of breast density with IGF-I[12][13][14][15][17] or prolactin.[19]

There are many possible reasons for the discrepancies. Our previous report from Hawaii with a weakly positive relation included 169 premenopausal women from the current study, but also subjects with other ethnic backgrounds.[13] Many of the positive findings in previous studies were based on small sample sizes and reported relatively weak associations. Variations in timing between mammograms and blood collection and incomplete control for confounding variables may have also affected the results. Since BMI does not completely reflect body fat, residual confounding by adiposity may have been present in previous analyses.[47] A weak relation between percent density and IGF-I could result from the significant correlation of IGFBP-3 with the nondense areas and the strong inverse relation of BMI with percent density. The inclusion of a quadratic BMI term in our models may be partially responsible for the negative results.

One or more of the following limitations may have contributed to the lack of an association. As is often the case in a pooled analysis, the original studies used different eligibility criteria in order to meet their primary objectives. These criteria may have led to selection bias and strong variability across studies. Mammograms were taken on different machines, some of them were digital, and for a subset from Japan, only ML views were available. Although we tried to adjust for these differences by adding an indicator variable for the type of film and by applying a conversion factor to ML density, some bias may have remained because the formula used for the conversion may not be accurate for the Japanese population.[36] However, little heterogeneity across population and different types of mammograms was apparent; no significant positive associations were seen for individual populations (Fig. 2). Adjustment for study group by ethnicity and location should have provided sufficient control for differences in study design.

Differences in timing of blood collection, *i.e.*, time of the day and day during the menstrual cycle, may also have introduced error. However, serum IGF-I levels showed no diurnal variation in children and several adult studies,[48] no difference between morning and afternoon collections in a British study,[49] and less than 10% change during day time in another report.[50] Compared with levels in the follicular phase, IGF-I concentrations were only 8.6 and 7.6% higher in the luteal phase among premenopausal women,[51][52] whereas daily blood collections in 9 women showed no significant day to day differences across the menstrual cycle.[53] These small differences do not appear large enough to affect our study results given the sizeable differences across populations. Also, luteal phase blood draws were obtained for at least one third of premenopausal women.[23] For prolactin, median levels in the follicular and luteal phase were very similar in the Nurses' Health Study, but the strong circadian rhythm of prolactin may have introduced error.[54] Because slightly higher prolactin levels were observed in postmenopausal women with recent HRT use,[18] misclassification of HRT would have led to biased findings.

Strengths of our analysis are the inclusion of women with different ethnic groups and from different geographic locations resulting in a study population that covers a wide range of breast cancer risk factors, in particular body weight. The relatively large sample size of this pooled data set should have made chance findings less likely. As the CVs based on blinded samples indicate, the laboratory produced stable assays results over time. Blood samples were combined in batches and analyzed at the same laboratory. To avoid inter-assay variation, the serum analytes of the 169 women in the previous report were measured again together with the samples from the other locations.[13] Similarly, the chance of misclassification was minimized for the breast density measures by assessing all films in mixed batches by 1 reader.

The fact that IGF-I levels differed significantly by location suggests that IGF-I levels are more influenced by dietary or environmental factors than breast density which has a large genetic component.[55] In this pooled analysis, mammographic densities differed considerably by ethnicity independent of location.[20] The fact that IGF-I levels were lowest among Latina in Arizona and Japanese in Japan, whereas the Japanese-Americans in Hawaii had similar levels as Caucasians suggests the possibility of shared environmental and nutritional influences. As migrant populations experience an increasing risk for breast cancer, the differences in IGF-I levels with the host population may decrease over time. The women in Japan and the Latinas in Arizona also have the lowest breast cancer incidence rates within this study population: 32.5 and 50.3 per 100,000 according to the Osaka and the Arizona cancer registry.[20]

In conclusion, IGF-I and prolactin levels showed little association with breast density in this study population from 4 locations and in the individual studies. Given the methodologic issues involved in a pooled analysis, the negative findings could be due to bias. However, given the discrepancies among published studies,[12][13][14][15][16][17][18][19] it remains unclear whether the possible effects of IGF-I and prolactin on breast cancer risk are mediated through breast density.

Significant differences in IGF-I levels by geographic location suggest that IGF-I levels may represent environmental influences such as nutrition on breast cancer risk,^[4] while percent density reflects more of the ethnic and genetic background.^[55]

Acknowledgements



We are grateful to Dr. Laurence N. Kolonel and Dr. Brian E. Henderson for access to the Multiethnic Cohort study, to Dr. Eiliv Lund for the collaboration with the NOWAC study and the use of the diet questionnaire, to Ms. Josiane Bouzac for performing the lab assays, to Ms. Ilene Brill for her programming efforts, and to Dr. Lynne Wilkens for her statistical advice. Most importantly, we want to thank the women participating in the studies.

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