Fasting insulin and endogenous hormones in relation to premenopausal breast density (Canada)

Marilyn J. Borugian · John J. Spinelli · Paula B. Gordon · Zenaida Abanto · Angela Brooks-Wilson · Michael N. Pollak · Linda J. Warren · T. Gregory Hislop · Richard P. Gallagher

Abstract

Purpose Mammographic breast density (BD) is associated with increased risk of breast cancer. This study asks which circulating metabolic and reproductive biomarkers are associated with BD, particularly dense breast area, in premenopausal women not taking exogenous hormones.

Methods In a cross-sectional study, 299 premenopausal women aged 40–49 completed questionnaires, provided a fasting blood sample, had height, weight, percentage body fat, waist and hip measurements taken, and attended a screening mammogram. Multivariate linear regression was used to calculate adjusted means for percentage BD, absolute dense and non-dense area, across categories of covariates, adjusted for day of menstrual cycle, age, parity, body mass index, percentage body fat, and ethnicity.

Results Fasting insulin levels were inversely associated, and insulin-like growth factor-binding protein 1 levels directly associated with percentage BD, but lost statistical significance after multivariate adjustment. Sex hormone-binding globulin levels were directly associated with percentage BD, still significant after multivariate adjustment ($p = 0.03$). A significant inverse dose–response association was observed between progesterone levels and dense area ($p < 0.01$).

Conclusions Breast density in premenopausal women seems unrelated or inversely related to insulin resistance, levels of insulin-like growth factor 1 and its binding proteins, and levels of sex steroids; therefore, the mechanism by which radiodensity on a mammogram is related to breast cancer risk remains unclear.

Keywords Mammographic breast density · Insulin resistance · Premenopausal · Insulin-like growth factors · Sex steroid hormones · Breast cancer

M. J. Borugian (✉) · J. J. Spinelli · Z. Abanto · A. Brooks-Wilson · T. G. Hislop · R. P. Gallagher
Cancer Control Research, British Columbia Cancer Agency, 675 West 10th Avenue, Vancouver, BC V5Z 1L3, Canada
e-mail: mborugian@bccrc.ca

M. J. Borugian · J. J. Spinelli · T. G. Hislop · R. P. Gallagher
School of Population and Public Health, University of British Columbia, Vancouver, BC, Canada

P. B. Gordon · L. J. Warren
Screening Mammography Program of British Columbia, Vancouver, BC, Canada

A. Brooks-Wilson
Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada

A. Brooks-Wilson
Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada

M. N. Pollak
Departments of Oncology and Medicine, McGill University, Montreal, QC, Canada

Introduction

Although advances in treatment and screening have resulted in improved outcomes, breast cancer remains the most common cancer in women worldwide, accounting for about 23 % of all cancers in women [1]. Mammographic breast density (BD) is a significant breast cancer risk factor [2] and can vary with other breast cancer risk factors including age, parity, menopausal status, and body mass index (BMI) [3–7]. BD is also an independent risk factor, as shown by its robust association with breast cancer risk even after adjustment for these other risk factors [8]. One problem is

that BD varies inversely with some breast cancer risk factors such as age and BMI, so the biological pathway from high BD to increased breast cancer is complex, and there is a clear need for an improved understanding of the factors and processes involved [9].

Insulin resistance (IR) and hyperinsulinemia have been associated with breast cancer risk [10, 11] though a recent meta-analysis has raised the issue of possible inadequate control for adiposity [12]. Excess insulin may be associated with BD and an association between IR (which is modifiable) and BD may suggest a critically needed new avenue for primary prevention of breast cancer. In a 2005 study, Diorio et al. [13] reported no association between BD and C-peptide after adjustment for BMI and waist-to-hip ratio, while more recently Conroy et al. [14] reported a modest inverse association between BMI-adjusted BD and metabolic syndrome, implying that these two risk factors may not act through the same mechanism, however, only percentage density was evaluated in both studies, not absolute dense area. In addition, the role of the endogenous sex steroid hormones and insulin-like growth factors in any potential association of IR and BD has not been examined in premenopausal women as it has in postmenopausal women [15].

We looked at the association of BD and IR in light of several potential confounders, including body size, estradiol, progesterone, total testosterone (TT), sex hormone-binding globulin (SHBG), insulin-like growth factor 1 (IGF-1), insulin-like growth factor-binding proteins 1 and 3 (IGF-BP1, IGF-BP3). We examined both absolute area of dense breast tissue and absolute area of non-dense tissue, as well as percentage density, to allow comparison of factors affecting these different measures of BD. All analyses were adjusted for day of menstrual cycle. We examined two measures of adiposity, measured waist-hip ratio and measured percentage body fat, in addition to measured BMI, to reduce the residual confounding of both body size and body composition on BD.

Methods

Study population

Participants were ascertained from the Screening Mammography Program of British Columbia (SMPBC) database, from consecutive women due for a screening mammogram. Each participant provided written informed consent. Ethical review was conducted by the British Columbia Cancer Agency/University of British Columbia Research Ethics Board, with peer review by the SMPBC Academic Committee and the British Columbia Cancer Agency Breast Tumour Group.

We invited 1,154 healthy women, 40–49 years, English speaking, with at least one previous screening mammogram, who had agreed to be contacted for research. Premenopausal status was defined as having had at least one menstrual cycle within the last 12 months. Exclusions were age (3), language (15), postmenopausal (55), hysterectomy or bilateral oophorectomy (36), history of breast cancer (2), breast implants (1), breast reconstruction surgery (27), pregnant or lactating (10), oral contraceptives in last 6 months (108), exogenous estrogen or hormone replacement therapy in last 6 months (30), diabetic medications including insulin and insulin sensitizers (9), not doing a mammogram or completed mammogram before study letter arrived (72), or moved (57). We were unable to contact 214 women, leaving 515 women available for recruitment. Of these, 133 were not interested, nine refused for personal reasons, six for health reasons, 48 did not have time to join, seven did not want to give a blood sample, and 13 either canceled or did not show up for their appointment, leaving 299 women who were recruited and who completed the protocol between January 2007 and October 2008 (58 % of the 515 eligible women contacted).

Data collection

Women were sent a study invitation package, right after the SMPBC mammogram reminder letter. The study project coordinator followed up to confirm eligibility, or determine reasons for non-participation. On the day of the woman's regular mammogram, fasting participants went first to the screening center for the mammogram, and then to the BC Cancer Research Centre, where they signed the study consent forms. Then, the fasting blood draw was done by a phlebotomist and the body measurements were done by a research assistant, followed by a light snack and time to complete the questionnaires with study staff available to resolve any questions or omissions.

Sample processing and storage

The fasting blood samples were collected, processed, and stored at one location, allowing very fast time-to-freezer for the samples. The maximum time from blood draw to freezer was 2 h. Serum was collected using a glass collection tube with no additives and allowed to clot for 1 h before centrifuging for 10 min at 2,000*g*. Plasma was collected using the fluoride/oxalate tube for glucose determination. Samples were aliquoted to 1.0-mL Sarstedt Micro tubes, frozen at −80°, a maximum of 10 min after centrifuging.

Measurements

Mammograms

The outcomes of interest were absolute dense area, absolute non-dense area, and percentage BD. Assessment of BD was done using Cumulus, a computer-assisted quantitative method [16, 17]. The screening mammograms were conducted using the normal procedure of two-view screen-film mammography at SMPBC screening centers. Images were read by a SMPBC radiologist blinded to group status. Each cranio-caudal view of a randomly selected breast was digitized using the Hologic R2 DigitalNow system and software. One of us (PG), a practicing breast radiologist with over 20 years of experience, took training on the Cumulus software and conducted the assessments in three batches over a period of about 3 weeks. We did not measure variability across batches, however, intra-class correlations of quantitative assessments over time by an experienced evaluator usually exceed 0.9 (0.915 adjusted for case status, age, weight, and film type [18]; 0.897 for an unadjusted estimate [19]).

Serum assays

Aliquots were shipped to the laboratory of one of us (MP) for measurement of fasting glucose, insulin, IGF-1, IGF-BP1, IGF-BP3, estradiol, progesterone, TT, and SHBG, methods similar to those previously described [20], using enzyme immunoassay techniques, except for the glucose which was done with a quantitative colorimetric assay. Inter-assay precision values, measured by CV %, are as follows: glucose (<3 %), insulin (4.5 %), IGF-1 (6.7 %), IGF-BP1 (6.9 %), IGF-BP3 (10.0 %), estradiol (12.5 %), progesterone (8.2 %), TT (6.2 %), SHBG (8.9 %). Free androgen index was calculated as the ratio of TT to SHBG, after conversion to like units. Given the variability of some analytes with the menstrual cycle, we recorded the self-reported day of menstrual cycle at the time of the blood draw.

Insulin resistance

We used the homeostasis model assessment measurement of insulin resistance (HOMA-IR), calculated by a modification of the method of Matthews et al. [21] developed by Levy [22] using fasting plasma insulin (FPI in mU/L) and fasting plasma glucose (FPG in mmol/L). HOMA insulin resistance (HOMA-IR) = FPI × FPG/22.5. High HOMA-IR indicates low insulin sensitivity.

Demographics

We collected data on reproductive history, family history of cancer, breast health history, smoking, and lifestyle, using a modified version of the health and lifestyle questionnaire of the Alberta Tomorrow Project (with permission). The variables for analysis were age, years in school, marital status, household income, ethnicity, BMI, WHR, body fat percent (BF percentage), day of menstrual cycle, age at first live birth, parity, age at menarche, smoking (Y/N, number/day, years smoked), family history of breast or ovarian cancer in first-degree relatives.

Body size

Height and weight were measured by project staff using a balance scale/stadiometer, and waist and hip circumference were measured with a standard measuring tape. Body fat percentage was measured using an Omron hand-held bio-electrical impedance analysis body fat analyzer (Model HBF-306INT, Omron Healthcare Inc, Japan). BMI was calculated as weight in kilograms divided by height in meters squared. Waist-to-hip ratio was calculated as waist circumference divided by hip circumference.

Data analysis

Using IBM SPSS Statistics version 21 (IBM Inc., Armonk, NY, USA), we built multivariate linear regression models to determine the association of independent variables, including insulin resistance and endogenous hormone levels with the continuous measures of percentage BD, dense area, non-dense area, and total area of the breast. The density measures were approximately normally distributed, and no transformations were used to improve normality. Multivariate linear regression was used to calculate adjusted means for percentage BD, absolute dense and non-dense area, across categories of covariates. Potential breast cancer risk factors, including age, BMI, WHR, BF percentage, age at menarche, number of births, smoking, education, income, ethnicity, and family history of breast cancer among first-degree relatives were included in the model and analyzed using general linear model (GLM) multivariate procedure. The final group of variables was selected based on significant ($p \leq 0.05$) F test. The day of menstrual cycle variable was calculated by taking the date of enrollment and subtracting the self-reported date of the first day of the woman's last menstrual cycle. The menstrual cycle phase categories were 1–14 days as follicular phase, 15–36 days as luteal phase, and 37+ days as oligomenorrhea (irregular cycles). One subject was omitted due to lost mammogram film, and two were omitted due to lost samples, leaving 296 for analysis. In analyses involving control variables, four had missing values for ethnicity ($n = 292$), and three had missing values for years in school ($n = 289$). Two subjects with extremely high TT values were omitted from TT analyses. All p values are two-sided.

Results

Study population

In Table 1, the study population is described in relation to mutually adjusted demographic, body size, and reproductive factors that have been associated with risk of breast cancer. The data compare adjusted means of three measures of BD: percentage BD, absolute dense area, and non-dense area.

We did not observe statistically significant differences in any BD measure by age at enrollment, age at menarche, age at first full-term pregnancy, household income, years in school, family history, or smoking in this age-restricted premenopausal Canadian population.

Regardless of whether BMI, BF percentage, or WHR was examined, there was a significant direct association of body size with non-dense area, but not with dense area. Percent BD, influenced by the usually larger non-dense component in its denominator, was strongly and inversely associated with BMI ($p = 0.02$) and BF percentage ($p < 0.001$), but did not reach statistical significance with WHR.

Number of pregnancies was inversely associated with percentage BD ($p = 0.02$), but the other reproductive variables (age at menarche, age at first pregnancy) were not associated with any measures of BD. Phase of menstrual cycle was not significantly associated with BD; however, the women in the oligomenorrhea group had less dense area and more non-dense area, resulting in a lower percentage density than women in either luteal or follicular phase.

Although the Asian sub-group was relatively small ($n = 41$), Asian ethnicity was associated with much smaller non-dense area, but interestingly almost the same mean dense area as the Caucasian group, resulting in significantly higher percentage BD for the Asian women ($p = 0.002$).

Multivariate models

Adjusted for age, parity, BMI, BF percentage, day of menstrual cycle, and ethnicity, an inverse association of fasting insulin and percentage BD exists, but is not statistically significant (Table 2). There is a similar inverse relationship of insulin with mean dense area. Fasting glucose levels and insulin resistance in the adjusted model were not associated with any of the density measures.

A direct association of unadjusted IGF-BP1 with BD (data not shown) did not persist after adjustment for the control variables, notably BMI and BF percentage. No association with BD was observed in the adjusted model for IGF-1 or IGF-BP3.

After adjustment, progesterone levels were inversely associated with mean dense area ($p < 0.01$), but were not associated with non-dense area or percentage BD. Estradiol levels showed a similar but not statistically significant inverse association with dense area ($p = 0.20$), particularly in the highest quartile. Free androgen index and TT also displayed this pattern, and although the P trend was not significant, pairwise comparisons (not shown in table) show significant differences between quartiles for TT ($p = 0.03$) and FAI ($p = 0.02$). With respect to SHBG, a direct association with percentage BD is significant in the adjusted model ($p = 0.03$).

The women with irregular menstrual periods (oligomenorrhea) may have been perimenopausal, however, the group was small ($n = 24$), and we were not able to effectively analyze them separately. Analyses excluding these women did not change our results.

Discussion

This study examined circulating metabolic and reproductive biomarkers potentially affecting BD in premenopausal women, including insulin, IGF-1, IGF-BP1, IGF-BP3, estrogen, testosterone, progesterone, and SHBG. Most known influences on BD are not themselves directly modifiable (age for example), or are influenced by many other factors (parity for example), and do not provide practical opportunities for intervention.

We found no association of BD and insulin resistance, while the fasting insulin component was associated with percentage BD in univariate analyses, but lost statistical significance after adjustment for control variables including BMI and BF percentage. There was an inverse but not statistically significant association of insulin levels with dense area, supporting others who have hypothesized different mechanisms for BD and factors like body size or insulin resistance in the etiology of breast cancer [23]. Once adjusted for body size and composition, we observed no significant association of IGF-1, IGF-BP1, or IGF-BP3 with BD, similar to some previous reports, including a recent one using data from the Nurses' Health Study [24].

Inversely correlated with insulin resistance [25], SHBG is considered a marker for the metabolic syndrome in patients with polycystic ovary syndrome (PCOS) [26]. An androgenic profile of high testosterone and low SHBG is common in women with PCOS, who also may have a lower risk of developing breast cancer [27]. Our observation of low SHBG being associated with lower BD, unchanged by adjustment for body size, is consistent with lower risk of breast cancer among women with this androgenic profile and supports earlier work on endogenous sex hormone levels and BD among premenopausal women [28]. Our observation is

Table 1 Multivariate adjusted mean percentage breast density, dense and non-dense breast area, premenopausal Canadians, 2007–2008

Variables	*n*	Percentage density Mean	Dense area (cm^2) Mean	Non-dense area (cm^2) Mean
Age				
40–44	104	33.5	33.0	89.9
45–50	185	36.6	33.1	87.7
p value[a]		0.23	0.97	0.67
Waist-to-hip ratio				
<0.73	70	39.2	32.7	80.0
0.73 to <0.77	73	35.0	32.7	84.6
0.77 to <81	72	32.4	32.7	98.0
≥0.81	74	33.7	34.2	92.6
p value		0.23	0.98	0.05
Body fat percent				
<27	67	47.8	38.0	73.2
27 to <31	75	30.6	29.0	98.3
31 to <35	75	32.1	32.0	91.1
35+	72	29.8	33.4	92.6
p value		<0.001	0.14	0.005
Body mass index				
≤Normal (≤24.9)	190	42.5	36.1	51.1
Overweight (25–29.9)	62	36.7	35.7	77.7
Obese (30+)	37	26.0	27.5	137.6
p value		0.02	0.31	<0.001
Age at menarche				
<12	43	33.8	34.4	92.5
12	71	33.3	31.6	92.6
13	102	39.1	35.0	81.9
14+	73	34.0	31.2	88.2
p value		0.21	0.65	0.33
Number of pregnancies				
Nulliparous	100	42.6	38.4	77.2
1	51	38.6	33.7	86.0
2	49	34.4	31.0	87.6
3	57	31.8	32.0	90.2
4	18	32.5	35.0	96.6
5–9	14	30.5	28.4	95.2
p value		0.02	0.31	0.24
Age at first full-term pregnancy[b]				
≤24	21	34.1	32.3	100.1
25–29	40	29.3	24.6	92.0
≥30	86	30.7	28.8	94.2
p value		0.70	0.46	0.78
Menstrual phase				
Follicular	137	37.8	35.1	81.1
Luteal	128	35.8	34.2	87.5
Oligomenorrhea	24	31.6	29.9	97.9
p value		0.35	0.59	0.14

Table 1 continued

Variables	*n*	Percentage density Mean	Dense area (cm^2) Mean	Non-dense area (cm^2) Mean
Household income				
<$50,000	57	35.2	35.1	89.6
$50,000–$99,000	92	34.9	32.5	91.3
≥$100,000	140	35.1	31.6	85.5
p value		0.99	0.64	0.55
Years in school				
≤13	17	33.2	33.9	87.2
14–15	197	35.9	33.0	89.6
16+	75	36.1	32.4	89.7
p value		0.87	0.97	0.97
Family history of breast or ovarian cancer				
No	231	33.5	31.8	90.9
Yes	58	36.7	34.4	86.7
p value		0.29	0.43	0.48
Ethnicity				
White/other	248	29.3	33.4	102.7
Asian	41	40.8	32.8	74.9
p value		0.002	0.89	<0.001
Ever smoked ≥100 cigarettes				
Yes	119	34.4	33.0	90.9
No	170	35.7	33.2	86.7
p value		0.62	0.93	0.41

[a] *p* values based on pairwise comparisons among the estimated marginal means

[b] Excluding 102 nulliparous women

not consistent, however, with studies that have linked higher androgen levels with higher breast cancer risk in premenopausal women [29–31], illustrating the difficulty of using intermediate markers of risk such as BD when so little is known of the biologic mechanism of action.

The inverse association of endogenous progesterone levels with BD provides some support to studies which have shown mitigation of estrogen-induced breast epithelial proliferation, but there is also support for progesterone increasing risk (reviewed in Hankinson 2010) [31], so the association of endogenous progesterone with breast cancer risk remains unclear. A review by Boyd et al. [32] reported that most studies of percentage BD and circulating ovarian hormones in premenopausal and postmenopausal women found either no association or an inverse association. It is important to note that endogenous progesterone data are distinct from data relating to exogenous or synthetic hormones called progestins. Developed for contraception or to mitigate the effects of estrogen on endometrial proliferation, progestins may be quite different both structurally and functionally from endogenous progesterone.

In this study, the reproductive, metabolic, and body size variables examined had very different associations with dense versus non-dense breast area, highlighting the importance of examining dense area in analyses of BD. Dense area is increasingly being recognized as the better measure, compared with percentage BD [33, 34]. Indeed, non-dense area has also garnered some attention as a potentially protective factor and may be a more important factor than originally thought [35, 36]. Percent BD is confounded by body size [23], however, remains useful to report for comparability with older studies.

It is important to consider the individual-level factors that can affect mammographic density. These factors were identified in a reliability study to be age, weight, and disease status, and the study concluded that most of the variability arises from biological differences among women, rather than from variation in assessment [18]. We controlled for the individual factors of age and weight; disease status is not relevant in this healthy population.

Technician-measured body size variables maximized the reliability compared with self-measurement [37]. The fasting status of the participants helped to reduce possible variation from recent meals. WHR, BF percentage, and BMI were all tested in the statistical models because WHR and BF percentage are important measures of adiposity that may better capture central adiposity, while BMI provides comparability with many previous studies.

Table 2 Multivariate adjusted mean percentage density, dense and non-dense breast area by quartiles of laboratory values, premenopausal Canadians, 2007–2008

Variables[a]	*n*	Percentage density Mean	Dense area (cm^2) Mean	Non-dense area (cm^2) Mean
Insulin mIU/mL				
Q1 (<4.1)	73	39.3	37.3	85.9
Q2 (4.1 to <5.3)	73	31.4	31.4	92.9
Q3 (5.3 to <6.9)	73	31.7	29.7	95.0
Q4 (≥6.9)	73	32.8	29.8	90.4
p for trend[b]		0.30	0.11	0.45
Glucose mg/dL				
Q1 (<72.1)	73	31.4	31.3	90.9
Q2 (72.1 to <77.4)	72	29.8	25.5	90.2
Q3 (77.4 to <83.3)	74	39.9	38.6	91.4
Q4 (≥83.3)	73	32.3	30.8	91.5
p for trend		0.56	0.62	0.27
Insulin resistance				
Q1 (<0.74)	73	35.3	33.0	88.3
Q2 (0.74 to <1.02)	73	31.9	32.2	95.2
Q3 (1.02 to <1.3)	73	28.8	26.5	96.8
Q4 (≥1.3)	73	37.2	34.4	86.3
p for trend		0.92	0.91	0.89
IGF-1 quartiles				
Q1 (<184.6)	73	31.2	33.3	98.6
Q2 (184.6 to <228.3)	73	33.2	30.1	93.4
Q3 (228.3 to <276.9)	73	37.6	32.1	85.3
Q4 (≥276.9)	73	31.3	31.3	90.7
p for trend		0.72	0.71	0.22
IGF-BP1 quartiles				
Q1 (<15.7)	73	32.3	30.2	92.1
Q2 (15.7 to <27.6)	73	36.1	33.8	92.1
Q3 (27.6 to <41.7)	73	34.0	31.8	87.1
Q4 (≥41.7)	73	33.7	32.2	91.6
p for trend		0.92	0.74	0.58
IGF-BP3 quartiles				
Q1 (<3,998.4)	72	32.6	33.0	96.3
Q2 (3,998.4 to <4,431.8)	74	34.5	32.1	87.4
Q3 (4,431.8 to <4,917.3)	73	39.5	34.5	83.1
Q4 (≥4,917.3)	73	30.5	28.0	94.1
p for trend		0.98	0.48	0.76
Progesterone ng/mL				
Q1 (<1.9)	73	35.5	35.2	91.9
Q2 (1.9 to <3.1)	73	32.0	32.7	92.2
Q3 (3.1 to <8.0)	73	35.6	30.3	88.0
Q4 (≥8.0)	73	31.1	25.4	93.5
p for trend		0.50	<0.01	0.90
Estradiol pg/mL				
Q1 (<58.7)	73	35.0	36.4	93.7
Q2 (58.7 to <81.6)	73	36.1	32.2	84.4
Q3 (81.6 to <113.8)	73	34.3	34.0	92.2

Table 2 continued

Variables[a]	*n*	Percentage density Mean	Dense area (cm^2) Mean	Non-dense area (cm^2) Mean
Q4 (≥113.8)	73	29.5	25.6	95.4
p for trend		0.17	0.20	0.55
SHBG nmol/L				
Q1 (<47.3)	73	31.6	31.5	93.2
Q2 (47.3 to <69.3)	73	32.7	29.3	93.4
Q3 (69.3 to <91.4)	73	37.1	32.1	85.2
Q4 (≥91.4)	73	37.2	34.7	88.6
p for trend		0.03	0.25	0.20
Testosterone ng/mL				
Q1 (<0.40)	73	34.2	34.0	92.9
Q2 (0.40 to <0.51)	73	36.8	36.1	88.0
Q3 (0.51 to <0.68)	72	35.4	33.8	91.2
Q4 (≥0.68)	72	29.9	25.5	92.5
p for trend		0.35	0.20	0.86
Free androgen index				
Q1 (<0.5)	73	39.0	38.9	86.1
Q2 (0.5 to <0.8)	73	31.2	28.0	95.4
Q3 (0.8 to <1.3)	73	34.8	30.9	92.0
Q4 (≥1.3)	73	31.5	28.4	91.0
p for trend		0.39	0.42	0.70

IGF-1 insulin-like growth factor 1, *IGF-BP1* insulin-like growth factor-binding protein 1, *IGF-BP3* insulin-like growth factor-binding protein 3, *SHBG* sex hormone-binding globulin

[a] Adjusted for age, parity, BMI, body fat, and ethnicity, all modeled as categorical, and day of menstrual cycle, continuous

[b] *p* for trend across quartiles calculated using continuous variables with assigned values equal to median level within each category

The HOMA model has been compared with several well-validated methods to measure insulin resistance and there is good correlation, for example, between estimates of insulin resistance from HOMA and from the euglycemic clamp ($r = 0.88$, $p < 0.0001$) or from the minimal model ($r = 0.7$, $p < 0.001$). [22].

We were limited by doing only one measurement of BD and laboratory values. Lokate et al. [38], however, reported that the average change in BD over 10 years was not related to breast cancer risk. In addition, although insulin secretion is pulsatile and the use of the mean of three samples taken at 5-min intervals is theoretically better, in practice where population estimates are sought, it is acceptable to use a single sample and this has been found to yield a similar result in large population studies [22]. We recognize that women who attend screening, as well as those who volunteer to join research studies may differ from the general population. SMPBC, however, screens 49 % of the total female population of British Columbia, and about 33 % of those aged 40–49 [39], so these women are representative of a large segment of the female population. Although some of the women may have been perimenopausal, we did not omit those with irregular menstrual periods because without additional information, that one symptom is not enough by itself to rule in or rule out perimenopause [40, 41].

The study is strengthened by the focus on premenopausal women in their 40's, a group which has been less studied compared with postmenopausal women. In addition, this group was selected from women who were not taking any exogenous hormones (oral contraceptives or hormone therapies), insulin, or insulin sensitizers. Exogenous hormone use is particularly difficult to adjust for, given the many different variations possible, and the issue of recall with respect to dose and duration. Restriction, therefore, provides a clearer picture of the role of the endogenous hormones. We recorded and adjusted for day of menstrual cycle because the sex steroid levels vary, and also because differences in BD over the menstrual cycle have been reported [42]. Finally, fasting blood samples, mammograms, and measurements of height, weight, waist, hip, and body fat percentage were all done on the same day for each woman, eliminating the problem of time lag.

These data underline the necessity of examining absolute dense area when studying mammographic BD. The percentage BD metric is useful for comparability with older studies, but does not allow examination of associations with the two components of that percentage. Where it is not possible to measure dense and non-dense area, analyses should adjust for BMI and if possible a measure of adiposity such as BF percentage or WHR. We conclude that once adjusted for body size, mammographic BD is not

related to insulin resistance, or to IGF-1 and its binding proteins, and may be inversely related to levels of ovarian hormones in this premenopausal Canadian population. More research is indicated on the role of endogenous progesterone levels in female breast cancer risk.

Acknowledgments The research was sponsored by the Canadian Institutes of Health Research and the Canadian Breast Cancer Research Alliance. We thank Teresa Switzer, Treena McDonald, Rozmin Janoo-Gilani, Donna Kan, and June Song for help with operational aspects.

References

1. World Cancer Research Fund Panel/American Institute for Cancer Research (2007) Food, nutrition, physical activity and the prevention of cancer: a global perspective. WCRF/American Institute of Cancer Research, Washington
2. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, Jong RA, Hislop G, Chiarelli A, Minkin S, Yaffe MJ (2007) Mammographic density and the risk and detection of breast cancer. N Engl J Med 356(3):227–236
3. Boyd N, Martin L, Stone J, Little L, Minkin S, Yaffe M (2002) A longitudinal study of the effects of menopause on mammographic features. Cancer Epidemiol Biomarkers Prev 11(10 Pt 1):1048–1053
4. Kelemen LE, Pankratz VS, Sellers TA, Brandt KR, Wang A, Janney C, Fredericksen ZS, Cerhan JR, Vachon CM (2008) Age-specific trends in mammographic density: the Minnesota Breast Cancer Family Study. Am J Epidemiol 167(9):1027–1036.
5. Ursin G, Parisky YR, Pike MC, Spicer DV (2001) Mammographic density changes during the menstrual cycle. Cancer Epidemiol Biomarkers Prev 10(2):141–142
6. Vachon CM, Kuni CC, Anderson K, Anderson VE, Sellers TA (2000) Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States). Cancer Causes Control 11(7):653–662
7. Walker K, Fletcher O, Johnson N, Coupland B, McCormack VA, Folkerd E, Gibson L, Hillier SG, Holly JM, Moss S, Dowsett M, Peto J, dos Santos Silva I (2009) Premenopausal mammographic density in relation to cyclic variations in endogenous sex hormone levels, prolactin, and insulin-like growth factors. Cancer Res 69(16):6490–6499.
8. Vachon CM, van Gils CH, Sellers TA, Ghosh K, Pruthi S, Brandt KR, Pankratz VS (2007) Mammographic density, breast cancer risk and risk prediction. Breast Cancer Res 9(6):217
9. Martin LJ, Boyd NF (2008) Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Res 10(1):201.
10. Giovannucci E (2003) Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res 35(11–12):694–704
11. Kaaks R (1996) Nutrition, hormones, and breast cancer: is insulin the missing link? Cancer Causes Control 7(6):605–625
12. Autier P, Koechlin A, Boniol M, Mullie P, Bolli G, Rosenstock J, Boyle P (2013) Serum insulin and C-peptide concentration and breast cancer: a meta-analysis. Cancer Causes Control 24(5):873–883.
13. Diorio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Brisson J (2005) Levels of C-peptide and mammographic breast density. Cancer Epidemiol Biomarkers Prev 14(11 Pt 1):2661–2664
14. Conroy SM, Butler LM, Harvey D, Gold EB, Sternfeld B, Greendale GA, Habel LA (2011) Metabolic syndrome and mammographic density: the Study of Women's Health Across the Nation. Int J Cancer 129(7):1699–1707.
15. Woolcott CG, Courneya KS, Boyd NF, Yaffe MJ, McTiernan A, Brant R, Jones CA, Stanczyk FZ, Terry T, Cook LS, Wang Q, Friedenreich CM (2013) Association between sex hormones, glucose homeostasis, adipokines, and inflammatory markers and mammographic density among postmenopausal women. Breast Cancer Res Treat 139(1):255–265.
16. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ (1994) The quantitative analysis of mammographic densities. Phys Med Biol 39(10):1629–1638
17. Byng JW, Yaffe MJ, Jong RA, Shumak RS, Lockwood GA, Tritchler DL, Boyd NF (1998) Analysis of mammographic density and breast cancer risk from digitized mammograms. Radiographics 18(6):1587–1598
18. Benichou J, Byrne C, Capece LA, Carroll LE, Hurt-Mullen K, Pee DY, Salane M, Schairer C, Gail MH (2003) Secular stability and reliability of measurements of the percentage of dense tissue on mammograms. Cancer Detect Prev 27(4):266–274
19. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, Yaffe MJ (1995) Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 87(9):670–675
20. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351(9113):1393–1396
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412–419
22. Wallace TM, Levy JC, Matthews DR (2004) Use and abuse of HOMA modeling. Diabetes Care 27(6):1487–1495
23. Boyd NF, Martin LJ, Sun L, Guo H, Chiarelli A, Hislop G, Yaffe M, Minkin S (2006) Body size, mammographic density, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 15(11):2086–2092
24. Rice MS, Tworoger SS, Rosner BA, Pollak MN, Hankinson SE, Tamimi RM (2012) Insulin-like growth factor-1, insulin-like growth factor-binding protein-3, growth hormone, and mammographic density in the Nurses' Health Studies. Breast Cancer Res Treat 136(3):805–812.
25. Pugeat M, Crave JC, Elmidani M, Nicolas MH, Garoscio-Cholet M, Lejeune H, Dechaud H, Tourniaire J (1991) Pathophysiology of sex hormone binding globulin (SHBG): relation to insulin. J Steroid Biochem Mol Biol 40(4–6):841–849
26. Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL (2003) The biological variation of testosterone and sex hormone-binding globulin (SHBG) in polycystic ovarian syndrome: implications for SHBG as a surrogate marker of insulin resistance. J Clin Endocrinol Metab 88(4):1528–1533
27. Gammon MD, Thompson WD (1991) Polycystic ovaries and the risk of breast cancer. Am J Epidemiol 134(8):818–824
28. Yong M, Atkinson C, Newton KM, Aiello Bowles EJ, Stanczyk FZ, Westerlind KC, Holt VL, Schwartz SM, Leisenring WM, Lampe JW (2009) Associations between endogenous sex hormone levels and mammographic and bone densities in premenopausal women. Cancer Causes Control 20(7):1039–1053.

29. Dorgan JF, Stanczyk FZ, Kahle LL, Brinton LA (2010) Prospective case-control study of premenopausal serum estradiol and testosterone levels and breast cancer risk. Breast Cancer Res 12(6):R98
30. Zeleniuch-Jacquotte A, Afanasyeva Y, Kaaks R, Rinaldi S, Scarmo S, Liu M, Arslan AA, Toniolo P, Shore RE, Koenig KL (2012) Premenopausal serum androgens and breast cancer risk: a nested case–control study. Breast Cancer Res 14(1):R32.

31. Hankinson SE, Eliassen AH (2010) Circulating sex steroids and breast cancer risk in premenopausal women. Horm Cancer 1(1):2–10.
32. Boyd NF, Martin LJ, Bronskill M, Yaffe MJ, Duric N, Minkin S (2010) Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst 102(16):1224–1237
33. Haars G, van Noord PA, van Gils CH, Grobbee DE, Peeters PH (2005) Measurements of breast density: no ratio for a ratio. Cancer Epidemiol Biomarkers Prev 14(11 Pt 1):2634–2640
34. Stone J, Ding J, Warren RM, Duffy SW, Hopper JL (2010) Using mammographic density to predict breast cancer risk: dense area or percentage dense area. Breast Cancer Res 12(6):R97
35. Lokate M, Peeters PH, Peelen LM, Haars G, Veldhuis WB, van Gils CH (2011) Mammographic density and breast cancer risk: the role of the fat surrounding the fibroglandular tissue. Breast Cancer Res 13(5):R103
36. Pettersson A, Hankinson SE, Willett WC, Lagiou P, Trichopoulos D, Tamimi RM (2011) Nondense mammographic area and risk of breast cancer. Breast Cancer Res 13(5):R100
37. Kushi LH, Kaye SA, Folsom AR, Soler JT, Prineas RJ (1988) Accuracy and reliability of self-measurement of body girths. Am J Epidemiol 128(4):740–748
38. Lokate M, Stellato RK, Veldhuis WB, Peeters PH, van Gils CH (2013) Age-related changes in mammographic density and breast cancer risk. Am J Epidemiol 178(1):101–109.

39. SMPBC (2004) 2003/2004 Annual report. Screening Mammography Program of BC, Vancouver
40. Bastian LA, Smith CM, Nanda K (2003) Is this woman perimenopausal? JAMA 289(7):895–902
41. Prior JC (1998) Perimenopause: the complex endocrinology of the menopausal transition. Endocr Rev 19(4):397–428
42. Buist DS, Aiello E, Miglioretti DL, White E (2006) Mammographic breast density, dense area and breast area differences by phase in the menstrual cycle. Cancer Epidemiol Biomarkers Prev 15(11):2303–2306