Lifestyle, Behavioral, and Dietary Risk Factors in Relation to Mammographic Breast Density in Women at High Risk for Breast Cancer

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ABSTRACT

Background: Women at high risk for breast cancer due to genetics or risk factor profiles are counseled to adopt lifestyle, behavioral, and dietary changes to help reduce their risk. These recommendations are based on studies of women at average risk, so their effectiveness in high-risk women is unclear.

Methods: We evaluated the impact of physical activity, smoking, alcohol consumption, and intake of folate and carotenoids on mammographic breast density—a proxy for breast cancer risk—among 387 high-risk women. Exposures were self-reported on questionnaires. Breast dense area, nondense area, and percent dense area were measured from screening mammograms with Library for Breast Radiodensity Assessment software. Cross-sectional associations were estimated with multivariable quantile regression models.

Results: After adjusting for age, adiposity, reproductive history, and use of postmenopausal hormones, no breast density measure was associated with physical activity level, smoking status, alcohol consumption, or estimated intake of folate, alpha-carotene, beta-carotene, lutein/zeaxanthin, and beta-cryptoxanthin. Lycopene intake was associated with lower dense area when comparing the highest and lowest intake categories (adjusted difference in median = -14 cm^2, 95% confidence interval: -29 to 1.3 cm^2). This association may be explained by incomplete adjustment for adiposity.

Conclusions: Recommended lifestyle, behavioral, and dietary changes to mitigate personal risk of breast cancer do not substantially impact mammographic breast density measures.

Impact: Alternative strategies, such as increased uptake of chemoprevention, may better serve risk reduction efforts in women at high risk for breast cancer.

Introduction

Breast cancer risk is heterogeneous, with lifetime incidence ranging from about 12% for women with no underlying high-risk factors to about 85% for women who carry high-risk germline mutations (e.g., *BRCA1* and *BRCA2*; ref. 1). Women can also be at increased risk if they have a strong family history of breast cancer or a combination of risk factors such as highly dense breasts, higher adiposity (in postmenopausal women), longer menstrual history, lower parity and less breastfeeding, personal history of benign breast disease, and use of postmenopausal hormones (2). These factors are captured among others by a number of clinical models of individual breast cancer risk such as the Gail, Tyrer-Cuzick, and Breast Cancer Screening Consortium models, which are comprehensively reviewed by Cintolo-Gonzalez and colleagues (3) Women classified as high risk by such models are candidates for enhanced screening regimens, initiation of endocrine therapy for primary prevention, and—in the most extreme cases—prophylactic mastectomy (4–6). High-risk women are also routinely counseled to adopt lifestyles and behaviors that purportedly mitigate some of their risk (7, 8). These recommendations follow from decades of research into modifiable risk factors for breast cancer, which has identified (with varying degrees of evidence and consensus) associations with physical activity, smoking, alcohol consumption, dietary patterns, and specific micronutrients (e.g., folate and carotenoids; ref. 2). The vast majority of research informing these recommendations was carried out in general populations—that is, without consideration of individual breast cancer risk. Those studies that have considered high-risk strata have largely focused on women who carry *BRCA1* or *BRCA2* mutations (9). It is therefore unclear whether lifestyle and behavioral modifications are effective at reducing breast cancer risk among women at higher than average risk for developing breast cancer, especially those who are at intermediate levels of risk.

Studying breast cancer incidence in high-risk women is challenging due to the scarcity of well-characterized cohorts and to the limited sample sizes of those that exist. Despite their higher rate of breast cancer incidence, there are relatively few incident cases available for study of complex risk factors. We addressed this challenge by using mammographic breast density (MBD) as an intermediate endpoint for breast cancer (10, 11). MBD characterizes the relative contribution of dense and nondense tissue to total breast area, and is thought to reflect the cumulative influence of both genetic and environmental causes of breast tissues proliferation (10). Dense area, comprised of parenchymal and stromal tissue, is radiopaque and appears bright on mammograms; nondense area, comprised of fat tissue, is radiolucent and appears dark (12). The most prevalent MBD classification system in clinical practice is the Breast Imaging Reporting and Data System (BIRADS). Under this system, MBD is qualitatively assigned to one of four categories by the interpreting radiologist: (i) almost entirely fat, (ii) scattered fibroglandular densities, (iii) heterogeneously dense, and

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(iv) extremely dense (13). Women with "extremely dense" breasts have about 310% higher breast cancer risk than women whose breasts are "almost entirely fat" (14), making MBD one of the strongest known breast cancer risk factors. Few studies have evaluated the impact of breast cancer risk factors and treatments on MBD in high-risk women; existing evidence suggests that tamoxifen treatment, intake of folate, smoking behavior, and high levels of osteoprotegerin (a RANK signaling inhibitor) are all associated with lower breast density in high-risk women (15–18). Conversely, low body mass index (BMI) and nulliparity are associated with higher MBD in high-risk women (18).

Our goal was to estimate associations between lifestyle and behavioral breast cancer risk factors and mammographic breast density in a cohort of women at high risk for breast cancer, whose high-risk status is due to both genetic and nongenetic factors.

Materials and Methods

Ethical review

This project was approved by the Institutional Review Board at the University of Vermont (Burlington, VT; protocol number M04-004). All study participants provided written informed consent.

Study population

The High Risk Breast Program (HRBP) Cohort at the University of Vermont Medical Center (UVMMC, Burlington, VT) has enrolled more than 600 women since its inception in 2003. Women are eligible for enrollment if they have at least one of the following breast cancer risk factors: breast biopsy showing atypical ductal hyperplasia or lobular neoplasia, germline mutation in a breast cancer-associated gene (either personally or in a family member), personal history of chest irradiation, >20% calculated risk of breast cancer from either the Gail, Claus, Tyrer-Cuzick, or BRCAPRO model (19), or a strong family history of breast and/or ovarian cancer. Strong family history was defined as meeting at least one of the criteria listed in **Fig. 1**. Women are excluded from the cohort if they have a prior diagnosis of breast cancer, if they are symptomatic or recommended for short-term follow-up at the time of presentation, and if they have prior (or imminent plans for) bilateral total mastectomy. Participants provide blood samples and complete a set of questionnaires at baseline and again at 4, 8, and 12 years of follow-up. Our cross-sectional analysis included 387 HRBP participants who returned a baseline questionnaire and underwent at least one screening mammogram at a University of Vermont Health Network site.

Breast density measurement

For each member of our cohort, we collected Digital Imaging and Communications in Medicine (DICOM)-formatted images from the screening mammography procedure closest in time to the baseline questionnaire return date. Using images from craniocaudal views, we measured continuous breast density values (total breast area and absolute dense area, in square centimeters) with software from the Laboratory for Individualized Breast Radiodensity Assessment (LIBRA v1.0.4; refs. 20, 21). We used LIBRA output to calculate nondense area (total breast area—absolute dense area) and percent dense area (absolute dense area/total breast area) separately for each breast. For patients with bilateral images, we averaged values from the right and left breasts.

Demographic, lifestyle, and behavioral risk factors

HRBP participants reported demographic, medical, and behavioral characteristics on two questionnaires—a medical screening questionnaire (MSQ) and a general health and health practices questionnaire (GHHP). The MSQ gathered detailed information on breast cancer risk factors including reproductive history, comorbidities, medication usage, personal and family history of cancer, menopausal status, use of postmenopausal hormones, and duration and frequency of tobacco smoking and alcohol consumption. The GHHP included questions addressing insurance status, health care utilization, and participation in screening programs for breast and cervical cancer. We measured physical activity with the 7-day physical activity recall (PAR), on which participants reported the daily number of hours spent at various exertion levels ranging from sleep to "very hard activity" (22). We converted PAR responses into metabolic equivalent of task values, which we used to compute the average daily kilocalories expended per kilogram of body weight (22). Typical dietary habits were ascertained with the NCI Diet History Questionnaire (DHQ, version 1) from which we estimated daily nutrient intakes using the accompanying Diet*Calc software package (23, 24). We focused on average daily intake of six micronutrients that have previously been associated with breast cancer incidence (folate, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, and lycopene; refs. 25, 26).

Definitions of analytic variables

Age was calculated as the number of years between a participant's date of birth and the completion date for their baseline questionnaire battery. We used decade categories of age for descriptive purposes, but statistical models adjusted for continuous age using a linear spline with

- Two or more first-degree relatives diagnosed with breast or ovarian cancer
- One first-degree relative and at least two second- or third-degree relatives diagnosed with breast cancer
- One first-degree relative diagnosed with breast or ovarian cancer before age 50
- One first-degree relative with breast cancer and at least one relative with ovarian cancer
- Two second- or third-degree relatives with breast cancer and at least one relative with ovarian cancer
- One second- or third-degree relative with breast cancer and at least two relatives with ovarian cancer
- Three or more second- or third-degree relatives with breast cancer
- One first-degree relative with a second primary breast cancer (in either the ipsilateral or contralateral breast)
- One first- or second-degree relative with ovarian cancer

Figure 1.
Criteria used to define a strong family history of breast and/or ovarian cancer for high-risk cohort eligibility.

Table 1. Characteristics of the HRBP cohort.

Characteristic	*n* = 387
Age category, *n* (%)	
25–29	12 (3.1)
30–39	85 (22)
40–49	124 (32)
50–59	114 (29)
60–69	42 (11)
≥70	10 (2.6)
Menopausal status, *n* (%)	
Premenopausal	248 (64)
Postmenopausal	138 (36)
(Missing)	1
Postmenopausal hormones, *n* (%)	
Premenopausal/never users	332 (86)
Ever users	51 (13)
(Missing)	4
Qualifying risk factor, *n* (%)	
Strong family history	302 (78)
Radiation treatment	2 (0.5)
Atypical biopsy	66 (17)
High-risk gene mutation	33 (8.5)
BIRADS breast density, *n* (%)	
Almost entirely fatty	42 (11)
Scattered density	108 (30)
Heterogeneously dense	174 (48)
Extremely dense	42 (11)
(Missing)	21
BMI category, *n* (%)	
Underweight	4 (1.2)
Normal	177 (53)
Overweight	92 (27)
Obese I	40 (12)
Obese II+	23 (6.9)
(Missing)	51
Domestic status, *n* (%)	
Married or partnered	326 (84)
Divorced or widowed	32 (8.3)
Single	29 (7.5)
Education level, *n* (%)	
High school	66 (17)
Some college	69 (18)
College degree	134 (35)
Graduate degree	113 (30)
(Missing)	5
Age at menarche	
≤10	15 (4.2)
11	42 (12)
12	105 (29)
13	123 (34)
14	46 (13)
15 or older	29 (8.1)
(Missing)	27
Number of births, *n* (%)	
0	73 (19)
1	72 (19)
2	161 (42)
3	56 (15)
4 or more	18 (4.7)
(Missing)	7
Age at first birth, *n* (%)	
19 or younger	23 (9.0)
20 to 29	162 (63)
30 or older	71 (28)
(Missing)	131
Screening mammogram frequency, *n* (%)	
Never[a]	23 (6.1)
At least yearly	338 (89)
Every two years	14 (3.7)
Every five or more years	5 (1.3)
(Missing)	7

(Continued on the following column)

Table 1. Characteristics of the HRBP cohort. (Cont'd)

[a]Screening mammogram participation was based on self-report; the 23 women who reported never attending screening mammograms did have screening mammograms that contributed to this study. Their self-reported status was therefore incorrectly classified.

five equally spaced knots. We calculated BMI (kg/m^2) values using height and weight values recorded in electronic medical records during the office visit closest in time to return of the baseline questionnaire. For description, we defined the following categories of BMI: underweight (<18.5), normal weight (18.5–24.9), overweight (25.0–29.9), class I obese (30.0–34.9), and class II or higher obese (≥35.0). As with age, regression models adjusted for continuous BMI using a linear spline with five equally spaced knots. Self-reported age at first birth, age at menarche, and number of pregnancies were modeled as continuous variables. Use of postmenopausal hormones was defined as ever/never. Smoking status was defined as current, former, or never based on self-report, and modeled as a factor variable with never smokers as the reference group. Alcohol consumption was defined as the number of alcoholic beverages consumed in an average week, and was categorized as nondrinkers (0 beverages/week), 1 or 2 beverages per week, 3 to 5 beverages per week, 6 to 10 beverages per week, and >10 beverages per week; this was modeled as a factor variable with nondrinkers as the reference group. Physical activity (kcal/kg/day) was categorized into fifths (category medians and ranges are reported in **Table 2**), and modeled as a factor variable with the lowest category as the reference group. Micronutrients (estimated micrograms consumed per day) were treated as continuous variables for visualization, but were categorized into fifths for modeling as factor variables with the lowest consumption levels as the reference group.

Statistical analysis

We tabulated the frequency and proportion of cohort participants, including the frequency of missing observations, according to demographic characteristics and breast cancer risk factors. We used multiple imputation to account for missing BMI and age at first birth, generating 10 imputed, complete datasets. BMI values were imputed by regression on age, absolute nondense area on mammogram, smoking status, and whether the participant had developed breast cancer since enrolling in the cohort; age at first birth was imputed *via* predictive mean matching based on marital/partnered status, total number of pregnancies, and educational level. We visualized the distribution of continuous percent dense area measurements within BIRADS density categories with violin plots (27). We modeled associations between lifestyle/dietary factors and continuous breast density measures (absolute dense area, nondense area, and percent dense area) using quantile regression to estimate differences in median outcome values and associated 95% confidence intervals (CI) across exposure categories. We fit both crude and multivariable models; the latter of which were adjusted for age (linear spline), BMI (linear spline), age at first birth, age at menarche, number of pregnancies, and use of postmenopausal hormones. We conducted sensitivity analyses in which we either adjusted models for, or stratified models by, menopausal status. We

further evaluated dietary micronutrient associations by fitting kernel-smoothed local polynomials to scatter plots of percent dense area and estimated daily micronutrient intakes. No hypothesis testing was performed (28). Statistical analyses were performed with Stata, version 16.1 (StataCorp LLC).

Results

Characteristics of the cohort

Our analysis included 387 women with paired questionnaire data and screening mammograms. **Table 1** reports demographic and clinical characteristics of the cohort. The median age was 47 years (range: 25–76 years) and over 98% of the cohort identified as non-Hispanic White, which reflects the catchment population surrounding UVMMC (Burlington, VT). The median time elapsed from questionnaire return to paired screening mammogram was 7 days (10th percentile: −184 days; 90th percentile: 127 days), and 97% of participants had bilateral images available. Categorical mammographic density assessment according to the BIRADS clinical standard was missing for 21 women (5.4%); the most common BIRADS category was "heterogeneously dense" (48% of women), followed by "scattered density" (30%), and "entirely fatty" and "extremely dense" (each 11%). BMI was missing for 51 women (13% of the cohort); the majority of participants (53%) had BMI within the normal weight range, about one quarter were overweight (27%), and just under one-fifth were obese (18.9%). Most women were married or in life partnerships (84%), had a college or graduate degree (65%), and had given birth to at least one child (81%). Most parous women were in their 20s at the time of their first birth (63%).

Continuous mammographic breast density measurements

Figure 2 shows the distribution of LIBRA percent dense area measurements within BIRADS categories. There was a positive trend in the distribution of percent dense area values with increasing BIRADS density category (progressing from "almost entirely fatty" to "extremely dense"); nonetheless, there was considerable overlap and variability in these distributions, consistent with past observations of considerable variability in the assignment of BIRADS categories (29).

Lifestyle and behavioral factors and mammographic density

Table 2 reports crude and adjusted associations of physical activity, smoking status, and alcohol consumption with three continuous measurements of breast density: percent dense area, absolute dense area (cm^2), and absolute nondense area (cm^2). There was little difference between crude and adjusted estimates for the absolute dense area outcome, but estimates for the percent dense area and absolute nondense area outcomes were considerably attenuated in multivariable models. This attenuation was largely driven by adjustment for BMI, which was expected given the strong association of adiposity with nondense breast area (and by extension with percent dense area; ref. 30). Sensitivity analyses showed no impact of further adjustment for menopausal status, nor evidence of effect measure modification within strata of menopausal status.

No level of physical activity was associated with any of the breast density measures in multivariable models (**Table 2**). For example, when comparing women in the highest category of physical activity (46.5–114.6 kcal/kg/day) with women in the lowest category (30.9–35.2 kcal/kg/day), the adjusted difference in median percent dense area was 0.9 units (95% CI: −6.9 to 8.7 units); median differences were similarly null for both dense area (adjusted difference in median = 2.6 cm^2, 95% CI: −8.4 to 14 cm^2) and nondense area (adjusted difference in median = −4.6 cm^2, 95% CI: −27 to 18 cm^2).

Current and former smokers had about the same median percent dense area, absolute dense area, and absolute nondense area as never smokers. For example, comparing current smokers with never smokers, the adjusted difference in median percent dense area was −3.6 units (95% CI: −20 to 13 units), the adjusted difference in median dense area was 5.1 cm^2 (95% CI: −18 to 28 cm^2), and the adjusted difference in median nondense area was 14 cm^2 (95% CI: −36 to 35 cm^2).

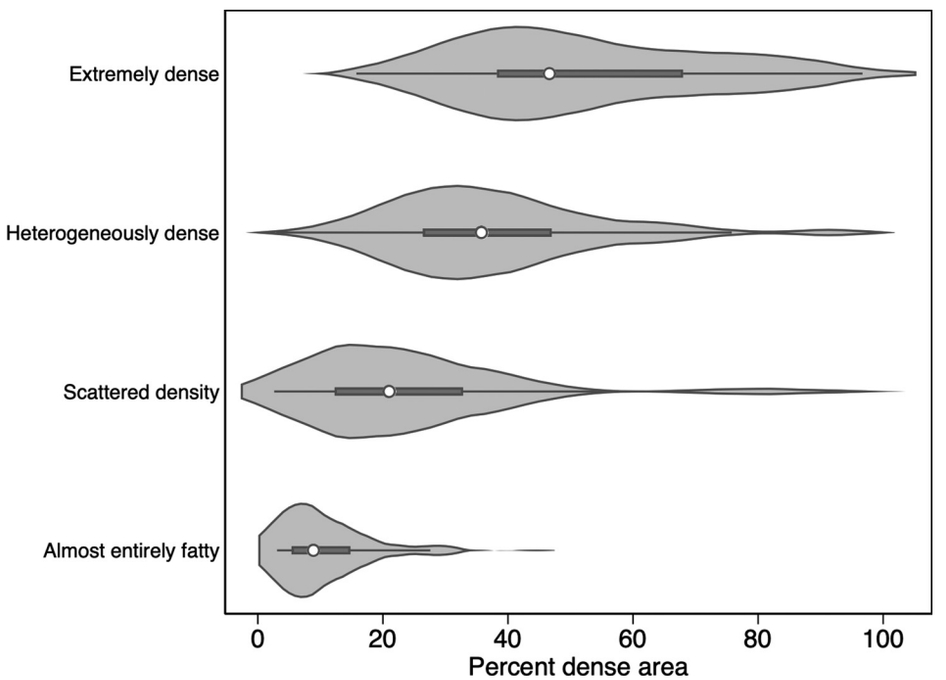

Figure 2.
Distribution of continuous percent dense area (average of measurements on left and right breasts, using the cranio-caudal mammographic view) according to BIRADS density category assigned by reading radiologists. White circles denote medians, dark gray boxes span interquartile ranges, and whiskers show the top and bottom adjacent values.

Table 2. Associations between lifestyle factors and continuous breast density measurements among women at high risk for breast cancer.

		Percent dense area Difference in median (95% CI)				Dense area, cm^2 Difference in median (95% CI)				Nondense area, cm^2 Difference in median (95% CI)			
Exposure	***N***	**Crude**		**Adjusted[a]**		**Crude**		**Adjusted[a]**		**Crude**		**Adjusted[a]**	
Physical activity (kcal/kg/day), category median (range)													
34.2 (30.9–35.2)	60	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference
36.6 (35.3–37.8)	59	5.5	(−3.3, 14)	2.1	(−5.7, 10)	4.7	(−5.2, 15)	3.8	(−7.2, 15)	−5.0	(−31, 21)	0.75	(−21, 22)
39.7 (37.9–41.7)	58	6.9	(−1.9, 16)	−1.2	(−9.3, 6.9)	3.6	(−6.4, 13)	−0.2	(−11, 11)	−24	(−50, 2.6)	−0.73	(−22, 21)
44.0 (41.8–46.3)	60	8.7	(−0.01, 17)	−0.7	(−8.3, 7.0)	3.8	(−6.1, 14)	1.4	(−9.8, 12)	−35	(−61, −8.6)	−2.3	(−25, 20)
52.4 (46.5–114.6)	57	9.2	(0.34, 18)	0.9	(−6.9, 8.7)	2.2	(−7.7, 12)	2.6	(−8.4, 14)	−33	(−59, −6.0)	−4.6	(−27, 18)
Smoking status													
Never smoker	176	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference
Former smoker	195	−3.8	(−9.1, 1.5)	−2.3	(−8.3, 3.7)	−3.0	(−8.7, 2.6)	−4.2	(−12, 3.4)	11	(−3.7, 26)	−2.0	(−17, 13)
Current smoker	16	−5.3	(−19, 8.1)	−3.6	(−20, 13)	4.2	(−9.9, 18)	5.1	(−18, 28)	42	(4.8, 79)	14	(−36, 65)
Alcohol consumption, (drinks/week)													
0 (nondrinker)	61	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference	0	Reference
1 or 2	57	13	(4.5, 22)	6.7	(−2.0, 15)	0.36	(−9.0, 9.7)	1.8	(−9.1, 13)	−37	(−65, −8.4)	−13	(−35, 9.6)
3 to 5	54	10	(1.2, 19)	6.3	(−2.5, 15)	10	(0.93, 20)	8.7	(−2.6, 20)	−36	(−65, −7.6)	−3.5	(−26, 19)
6 to 10	65	9.3	(0.91, 18)	5.3	(−3.2, 14)	−4.6	(−14, 4.4)	−2.5	(−13, 8.4)	−43	(−70, −15)	−11	(−32, 10)
>10	41	8.6	(−0.94, 18)	2.0	(−7.8, 12)	1.6	(−8.7, 12)	2.9	(−9.5, 15)	−22	(−54, 8.8)	−0.11	(−25, 25)

Note: Associations are reported as differences in median measurements between categories, with accompanying 95% confidence limits, estimated from crude and multivariable quantile regression models.

[a]All adjusted models were run on a multiply imputed dataset (10 imputations) to account for missing data. All adjusted models adjusted for age and BMI (continuous; linear splines), age at first birth (continuous), age at menarche (continuous), number of pregnancies (continuous), and use of postmenopausal hormones (ever/never); smoking status and alcohol consumption models were mutually adjusted.

Compared with nondrinkers, no level of alcohol consumption was associated with breast density outcomes in multivariable models. Comparing women in the highest category of alcohol consumption (>10 drinks per week) with non-drinkers, the adjusted difference in median was 2.0 units for percent dense area (95% CI: −7.8 to 12 units), 2.9 cm^2 for absolute dense area (95% CI: −9.5 to 15 cm^2), and −0.11 cm^2 for absolute nondense area (95% CI: −25 to 25 cm^2).

Micronutrient intake and mammographic density

Figure 3 depicts crude trends in percent dense area as a function of estimated daily intake of folate, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, and lycopene. Trends appeared flat, with the exception of increased percent dense area with higher estimated alpha-carotene consumption and decreased percent dense area with higher estimated lycopene consumption (**Fig. 3**). **Table 3** reports multivariable-adjusted associations between fifths of estimated micronutrient intake and percent dense area, absolute dense area, and absolute nondense area. The adjusted alpha-carotene association was not strongly consistent with the trend observed in the crude data; comparing the highest level of alpha-carotene intake with the lowest level, the difference in median percent dense area was 6.4 units (95% CI: −9.3 to 22 units). Alpha-carotene was not associated with absolute dense area, nor with absolute nondense area (**Table 3**). The adjusted lycopene association was somewhat more consistent with the crude trend observed in **Fig. 3**. Comparing the highest and lowest categories of lycopene intake, the difference in median percent dense area was –11 units (95% CI: −23 to 1.6 units). The association with percent dense area appears to be a consequence of lower absolute dense area in higher categories of lycopene intake (e.g., adjusted difference in median = –14 cm^2, 95% CI: −29 to 1.3 cm^2 when comparing the highest and lowest categories). There was no association between lycopene intake and absolute nondense area (**Table 3**).

Discussion

In our cohort of women at increased risk for breast cancer, we observed no differences in median breast density measures across levels of physical activity, tobacco smoking, and alcohol consumption. Our null tobacco smoking association is in contrast to findings from the International Breast Cancer Intervention Study, which showed lower median percent dense area among current smokers than in never smokers (38% vs. 70%, respectively; ref. 18). Other comparable studies of high-risk women evaluated associations with breast cancer incidence, for which our MBD measures only serve as a proxy. Our findings are concordant with a 2006 study of French-Canadian women at high breast cancer risk due to *BRCA1/2* mutations which found no associations between alcohol consumption, smoking, or physical activity and breast cancer incidence (31). Other studies focused on women at high risk due to *BRCA* mutations were heterogeneous with respect to the association between alcohol consumption and breast cancer incidence, with one finding no association (32), and two finding positive associations (33, 34). A 2015 Swedish study, which characterized participants' breast cancer risk using the Tyrer–Cuzick model (35), observed increased breast dense volume as a function of alcohol consumption among both high- and low-risk women, but not among women at moderate risk (36). Regarding smoking, an international, pooled cohort study of *BRCA1/2* mutation carriers reported a positive association between smoking for at least 5 years before a first full-term pregnancy and incident breast cancer (9), but a case-only gene–environment interaction analysis reported no modification of breast cancer incidence among *BRCA* mutation carriers by smoking status (33).

Of the dietary micronutrients we evaluated, only lycopene consumption was associated with breast density measures, showing a negative association with absolute dense area—and, by extension, with percent dense area—in a multivariable model adjusted for other

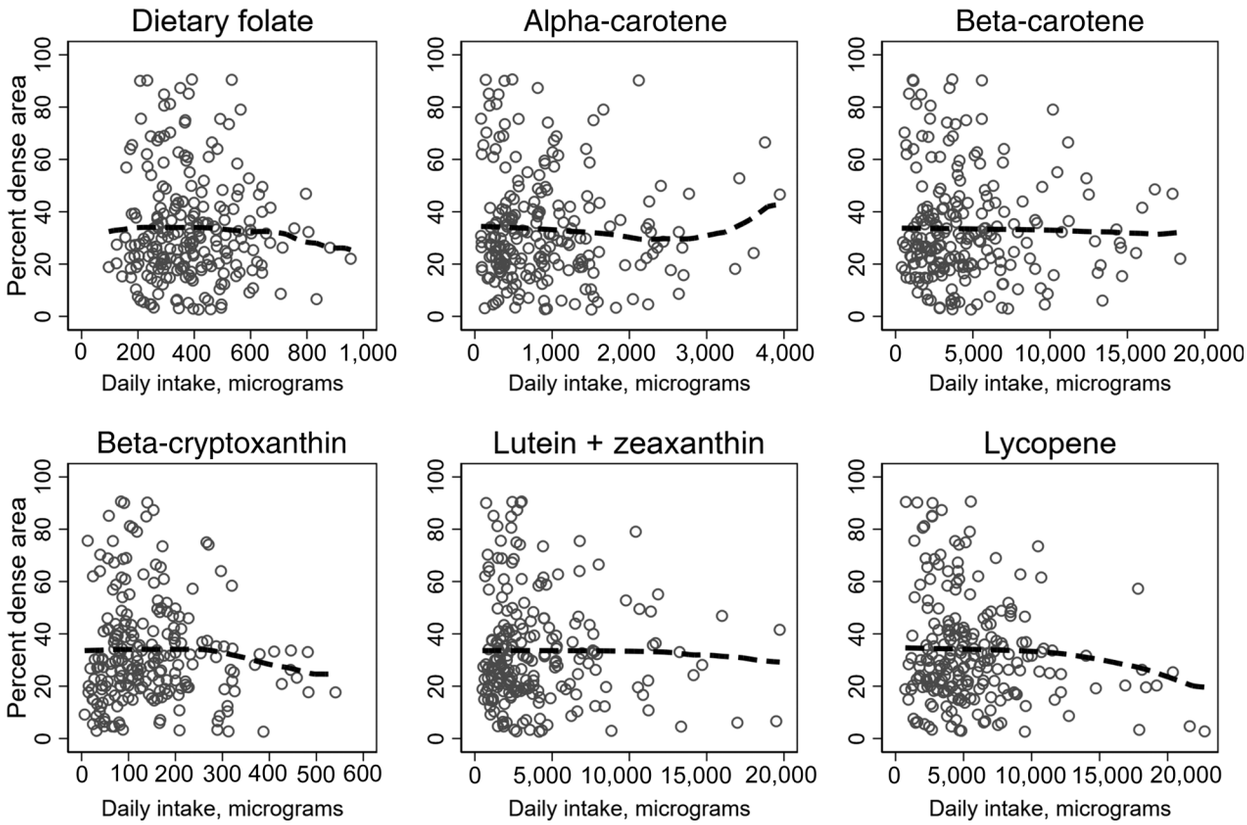

Figure 3.

Crude trends in percent dense breast area as a function of estimated daily intake of folate and carotenoids (alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein and zeaxanthin, and lycopene) among women at high risk for breast cancer. Trend lines reflect kernel-smoothed local polynomial functions.

micronutrients, age, BMI, reproductive characteristics, and use of postmenopausal hormones. This finding is consistent with some earlier studies, all of which were conducted in groups of women that were not selected on the basis of predicted breast cancer risk. Eliassen and colleagues carried out a pooled analysis of eight prospective studies in which carotenoids were assayed from plasma or serum; the highest fifth of lycopene level was negatively associated with breast cancer incidence (OR = 0.78, 95% CI: 0.62–0.99), which is consistent with the direction of our associations with percent dense area and absolute dense area. However, the Eliassen study also reported negative associations for alpha-carotene, beta-carotene, and lutein+zeaxanthin, whereas we did not (26). Other studies have found no association between lycopene and breast cancer risk, with lycopene either directly measured in blood or estimated *via* dietary recall (37–39). Lycopene is negatively correlated with age and BMI (40), so incomplete adjustment for these determinants of breast density could at least partially explain the negative associations we observed. Our null beta-carotene association in high-risk women contrasts with a negative association with semiquantitative mammographic density observed in an Italian cohort (41) and with a recent analysis in the Nurses' Health Study cohorts which demonstrated a protective association between total carotenoid intake and breast cancer incidence that was stronger for high-risk women, as defined by a polygenic risk score and higher MBD (42). Our null folate association is also in contrast with a suggestive negative association with high breast density in a cohort of women with familial breast cancer (16).

To our knowledge, ours is the first study of high-risk breast cancer that includes a large proportion of women whose elevated risk is largely a function of nongenetic factors, whereas much existing research in high-risk groups has focused on women who carry a *BRCA1* or *BRCA2* mutation. There are several important limitations to bear in mind when weighing the evidence from our study. Our cohort was relatively small, resulting in imprecise estimation of associations, particularly in multivariable models. Another consequence of our study size was that an insufficient number of breast cancer cases had occurred over available follow-up to permit statistical modeling of breast cancer incidence; we therefore examined associations with mammographic breast density, a strong but imperfect proxy for breast cancer. We improved on the common but highly variable BIRADS density characterization by using the LIBRA software package to estimate continuous measures of breast density from raw mammography images. While there is currently no standard against which to validate LIBRA's continuous breast density estimates, measurements from LIBRA compare favorably with measurements from other common MBD measurement algorithms (43). We were also limited to cross-sectional ascertainment of lifestyle/behavioral exposures and breast density measurements. While we expect diet and lifestyle reports from baseline questionnaires to be strongly correlated with past behaviors, these self-reported measures may not reflect actual exposure status during time periods that are etiologically relevant for effects on breast density. Cuzick and colleagues showed that tamoxifen therapy impacted breast density measures after 12–18 months of treatment (44),

Table 3. Multivariable-adjusted associations between estimated daily dietary micronutrient intake and continuous mammographic density measurements among women at high risk for breast cancer.

Micronutrient, μg/day, category Median (range)	Percent dense area Adjusted difference in median (95% CI)		Dense area (cm^2) Adjusted difference in median (95% CI)		Nondense area (cm^2) Adjusted difference in median (95% CI)	
Dietary folate						
206 (97–256)	0	Reference	0	Reference	0	Reference
289 (258–335)	0.24	(−11, 11)	0.08	(−13, 13)	−3.7	(−29, 22)
370 (336–396)	2.7	(−9.1, 14)	7.3	(−6.7, 21)	−4.2	(−34, 25)
449 (397–507)	−3.0	(−15, 9.3)	−1.3	(−17, 14)	8.4	(−23, 39)
598 (508–956)	5.2	(−8.3, 19)	2.5	(−15, 20)	−6.0	(−39, 26)
Alpha-carotene						
221 (81–320)	0	Reference	0	Reference	0	Reference
409 (324–523)	2.8	(−7.4, 13)	4.6	(−8.3, 17)	−4.6	(−29, 20)
770 (524–907)	1.9	(−10, 14)	0.10	(−15, 15)	−20	(−48, 8.9)
1,123 (909–1,481)	11	(−3.8, 26)	9.3	(−9.7, 28)	−26	(−60, 6.8)
2,254 (1,482–9,098)	6.4	(−9.3, 22)	5.2	(−15, 26)	−12	(−48, 25)
Beta-carotene						
1,340 (407–1,888)	0.	Reference	0.	Reference	0.	Reference
2,445 (1,903–2,999)	−3.6	(−17, 9.6)	−17	(−33, −0.22)	−7.4	(−41, 27)
3,723 (3,019–4,416)	−7.6	(−24, 8.6)	−17	(−37, 3.7)	4.7	(−35, 44)
5,588 (4,439–6,921)	−15	(−36, 5.5)	−22	(−49, 4.5)	16	(−37, 68)
10,350 (6,948–25,276)	−21	(−47, 6.3)	−26	(−60, 8.5)	19	(−44, 81)
Beta-cryptoxanthin						
41 (6.5–64)	0	Reference	0	Reference	0	Reference
82 (65–98)	7.9	(−4.0, 20)	22	(7.4, 37)	−1.9	(−30, 26)
120 (99–154)	11	(−0.03, 23)	21	(7.6, 35)	−11	(−37, 15)
180 (155–212)	11	(−1.6, 23)	19	(4.0, 34)	−8.0	(−35, 19)
304 (213—1,091)	8.1	(−5.5, 22)	13	(−4.6, 31)	−14	(−44, 16)
Lutein + zeaxanthin						
1,062 (499–1,475)	0	Reference	0	Reference	0	Reference
1,876 (1,489–2,224)	0.77	(−12, 14)	−0.33	(−15, 15)	5.3	(−26, 36)
2,690 (2,274–3,465)	6.6	(−7.7, 21)	8.2	(−9.8, 26)	−11	(−48, 25)
4,476 (3,496–6,244)	6.5	(−9.4, 22)	−1.1	(−21, 19)	−7.1	(−46, 32)
8,665 (6,324–25,692)	12	(−9.4, 33)	12	(−15, 39)	−11	(−64, 41)
Lycopene						
2,111 (720–2,695)	0	Reference	0	Reference	0	Reference
3,539 (2,696–4,114)	−5.1	(−15, 4.8)	−13	(−26, −0.35)	−11	(−33, 11)
4,790 (4,170–5,506)	−9.2	(−21, 2.1)	−13	(−26, 0.41)	2.7	(−21, 26)
6,734 (5,513–8,252)	−8.1	(−20, 4.1)	−9.1	(−25, 7.0)	4.9	(−22, 32)
10,717 (8,302–91,524)	−11	(−23, 1.6)	−14	(−29, 1.3)	14	(−14, 41)

Note: Associations are reported as differences in median measurements between categories, with accompanying 95% confidence limits, estimated from multivariable quantile regression models. Crude trends for these associations appear in **Fig. 3**.
Models were run on a multiply imputed dataset (10 imputations) to account for missing data. All models adjusted for all micronutrients, age and BMI (continuous; linear splines), age at first birth (continuous), age at menarche (continuous), number of pregnancies (continuous), and use of postmenopausal hormones (ever/never).

suggesting that even very recent exposures—which we expect to be well captured by baseline self-report—could impact our study outcomes. All of our exposures were measured by self-report on questionnaires, and are therefore susceptible to misclassification in any time frame. Estimation of micronutrient intake relied on the DHQ instrument developed by the U.S. NCI for dietary assessment in cancer epidemiology studies (24). Studies have validated the DHQ against other food frequency questionnaires (45) and against objective biomarkers of reported intake (46, 47). These studies found that DHQ performed better overall than the comparable Block and Willett food frequency questionnaires (45), but led to substantial underestimation of energy and protein intake compared with gold standard biomarkers (46, 47). The 7-day PAR that we used to estimate energy expenditure was validated against accelerometer data in a cohort of 159 female patients with breast cancer (48). The validation data showed that the PAR overestimated physical activity duration by only 22 minutes per week, on average, and that it could classify women as having met the American College of Sports Medicine's physical activity guideline with a sensitivity of 100% and a specificity of 84% (48). Misclassification of micronutrient intake by the DHQ and misclassification of energy expenditure by the 7-day recall did not likely impact the comparison of relative intake/expenditure levels under our exposure definitions, though the actual values (medians and ranges) reported within our exposure categories are more likely to have been impacted. Finally, our study population was composed almost entirely of White women and included a high proportion of participants with college or graduate degrees. Our results may therefore not be generalizable to non-White women or to women with lower education level.

We recommend that future research on lifestyle/behavioral and dietary modifiers of breast cancer incidence in high-risk women invest

resources in (i) focusing on individuals whose elevated risk is not due to carrying a qualifying genetic mutation, (ii) pooling data from similar cohorts to allow precise estimation of breast cancer incidence associations—especially among Black, indigenous, and other women of color, and (iii) incorporating longitudinal measurement of behavioral and dietary exposures using robust measurements such as electronic fitness monitors for physical activity, and using intake/exposure biomarkers to validate self-reported diet, smoking, and alcohol consumption.

Authors' Disclosures

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Authors' Contributions

T.P. Ahern: Conceptualization, data curation, formal analysis, funding acquisition, visualization, methodology, writing–original draft, writing–review and editing. **B.L. Sprague:** Conceptualization, data curation, investigation, methodology, writing–review and editing. **N.H. Farina:** Conceptualization, investigation, methodology, writing–review and editing. **E. Tsai:** Resources, data curation, investigation, methodology, writing–review and editing. **M. Cuke:** Resources, data curation, investigation, writing–review and editing. **D. Kontos:** Resources, software, investigation, methodology, writing–review and editing. **M.E. Wood:** Conceptualization, resources, data curation, supervision, investigation, writing–review and editing.

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References

1. Wood ME, Farina NH, Ahern TP, Cuke ME, Stein JL, Stein GS, et al. Towards a more precise and individualized assessment of breast cancer risk. Aging 2019;11:1305–16.
2. Hankinson S, Tamimi R, Hunter D. Breast cancer. In: Textbook of cancer epidemiology. 2nd ed. Oxford University Press; 2008. p. 403–45.
3. Cintolo-Gonzalez JA, Braun D, Blackford AL, Mazzola E, Acar A, Plichta JK, et al. Breast cancer risk models: a comprehensive overview of existing models, validation, and clinical applications. Breast Cancer Res Treat 2017;164:263–84.
4. Hartmann LC, Schaid DJ, Woods JE, Crotty TP, Myers JL, Arnold PG, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med 1999;340:77–84.
5. Shieh Y, Tice JA. Medications for primary prevention of breast cancer. JAMA 2020;324:291–2.
6. Daly MB, Pilarski R, Berry M, Buys SS, Farmer M, Friedman S, et al. NCCN guidelines insights: genetic/familial high-risk assessment: breast and ovarian, version 2.2017. J Natl Compr Canc Netw 2017;15:9–20.
7. LoConte NK, Gershenwald JE, Thomson CA, Crane TE, Harmon GE, Rechis R. Lifestyle modifications and policy implications for primary and secondary cancer prevention: diet, exercise, sun safety, and alcohol reduction. Am Soc Clin Oncol Educ Book 2018;38:88–100.
8. Cummings SR, Tice JA, Bauer S, Browner WS, Cuzick J, Ziv E, et al. Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. J Natl Cancer Inst 2009;101:384–98.
9. Li H, Terry MB, Antoniou AC, Phillips K-A, Kast K, Mooij TM, et al. Alcohol consumption, cigarette smoking, and risk of breast cancer for BRCA1 and BRCA2 mutation carriers: results from The BRCA1 and BRCA2 Cohort Consortium. Cancer Epidemiol Biomark Prev 2020;29:368–78.
10. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, et al. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6:798–808.
11. Ramón y Cajal T, Chirivella I, Miranda J, Teule A, Izquierdo Á, Balmaña J, et al. Mammographic density and breast cancer in women from high risk families. Breast Cancer Res 2015;17:93.
12. Boyd N, Martin L, Bronskill M, Yaffe M, Duric N, Minkin S. Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst 2010;102:1224–37.
13. Sprague BL, Gangnon RE, Burt V, Trentham-Dietz A, Hampton JM, Wellman RD, et al. Prevalence of mammographically dense breasts in the United States. J Natl Cancer Inst 2014;106:dju255.
14. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomark Prev 2006;15:1159–69.
15. Cuzick J, Warwick J, Pinney E, Warren RML, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. J Natl Cancer Inst 2004;96:621–8.
16. Tseng M, Byrne C, Evers KA, Daly MB. Dietary intake and breast density in high-risk women: a cross-sectional study. Breast Cancer Res 2007;9:R72.
17. Moran O, Zaman T, Eisen A, Demsky R, Blackmore K, Knight JA, et al. Serum osteoprotegerin levels and mammographic density among high-risk women. Cancer Causes Control 2018;29:507–17.
18. Warwick J, Pinney E, Warren RML, Duffy SW, Howell A, Wilson M, et al. Breast density and breast cancer risk factors in a high-risk population. Breast Edinb Scotl 2003;12:10–6.
19. McCarthy AM, Guan Z, Welch M, Griffin ME, Sippo DA, Deng Z, et al. Performance of breast cancer risk-assessment models in a large mammography cohort. J Natl Cancer Inst 2020;112:489–97.
20. Keller BM, Nathan DL, Wang Y, Zheng Y, Gee JC, Conant EF, et al. Estimation of breast percent density in raw and processed full field digital mammography images via adaptive fuzzy c-means clustering and support vector machine segmentation. Med Phys 2012;39:4903–17.
21. Keller BM, Chen J, Daye D, Conant EF, Kontos D. Preliminary evaluation of the publicly available Laboratory for Breast Radiodensity Assessment (LIBRA) software tool: comparison of fully automated area and volumetric density measures in a case-control study with digital mammography. Breast Cancer Res 2015;17:117.
22. Sallis JF, Haskell WL, Wood PD, Fortmann SP, Rogers T, Blair SN, et al. Physical activity assessment methodology in the Five-City Project. Am J Epidemiol 1985;121:91–106.
23. Diet*Calc Analysis Program; 2005. Available from: https://epi.grants.cancer.gov/dhq/dietcalc/.
24. NIH, Applied Research Program, NCI. Diet history questionnaire.; 2007. Available from: https://epi.grants.cancer.gov/dhq/.
25. Buja A, Pierbon M, Lago L, Grotto G, Baldo V. Breast cancer primary prevention and diet: an umbrella review. Int J Environ Res Public Health 2020;17:4731.
26. Eliassen AH, Hendrickson SJ, Brinton LA, Buring JE, Campos H, Dai Qi, et al. Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. J Natl Cancer Inst 2012;104:1905–16.
27. Hintze JL, Nelson RD. Violin plots: a box plot-density trace synergism. Am Stat 1998;52:181–4.
28. Rothman KJ. Six persistent research misconceptions. J Gen Intern Med 2014;29:1060–4.
29. Sprague BL, Conant EF, Onega T, Garcia MP, Beaber EF, Herschorn SD, et al. Variation in mammographic breast density assessments among radiologists in clinical practice: a multicenter observational study. Ann Intern Med 2016;165:457–64.
30. Pettersson A, Graff RE, Ursin G, dos Santos Silva I, McCormack V, Baglietto L, et al. Mammographic density phenotypes and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 2014;106:dju078.
31. Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P. Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 2006;98:285–94.

32. McGuire V, John EM, Felberg A, Haile RW, Boyd NF, Thomas DC, et al. No increased risk of breast cancer associated with alcohol consumption among carriers of BRCA1 and BRCA2 mutations ages <50 years. Cancer Epidemiol Biomark Prev 2006;15:1565–7.
33. Moorman PG, Iversen ES, Marcom PK, Marks JR, Wang F, Lee E, et al. Evaluation of established breast cancer risk factors as modifiers of BRCA1 or BRCA2: a multi-center case-only analysis. Breast Cancer Res Treat 2010;124: 441–51.
34. Friebel TM, Domchek SM, Rebbeck TR. Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju091.
35. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med 2004;23:1111–30.
36. Trinh T, Christensen SE, Brand JS, Cuzick J, Czene K, Sjölander A, et al. Background risk of breast cancer influences the association between alcohol consumption and mammographic density. Br J Cancer 2015;113:159–65.
37. Bakker MF, Peeters PHm, Klaasen VM, Bueno-de-Mesquita HB, Jansen EH, Ros MM, et al. Plasma carotenoids, vitamin C, tocopherols, and retinol and the risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition cohort. Am J Clin Nutr 2016;103:454–64.
38. Aune D, Chan DSm, Vieira AR, Navarro Rosenblatt DA, Vieira R, Greenwood DC, et al. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr 2012;96:356–73.
39. Sesso HD, Buring JE, Zhang SM, Norkus EP, Gaziano JM. Dietary and plasma lycopene and the risk of breast cancer. Cancer Epidemiol Biomark Prev 2005;14: 1074–81.
40. Casso D, White E, Patterson RE, Agurs-Collins T, Kooperberg C, Haines PS. Correlates of serum lycopene in older women. Nutr Cancer 2000;36:163–9.
41. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, Palli D. Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population. Int J Cancer 2006;118:1782–9.
42. Peng C, Gao C, Lu D, Rosner BA, Zeleznik O, Hankinson SE, et al. Circulating carotenoids and breast cancer among high-risk individuals. Am J Clin Nutr 2021; 113:525–33.
43. Gastounioti A, Kasi CD, Scott CG, Brandt KR, Jensen MR, Hruska CB, et al. Evaluation of LIBRA software for fully automated mammographic density assessment in breast cancer risk prediction. Radiology 2020;296:24–31.
44. Cuzick J, Warwick J, Pinney E, Duffy SW, Cawthorn S, Howell A, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. J Natl Cancer Inst 2011;103:744–52.
45. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol 2001;154:1089–99.
46. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. Am J Epidemiol 2003;158:1–13.
47. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, et al. Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol 2003;158:14–21.
48. Johnson-Kozlow M, Sallis JF, Gilpin EA, Rock CL, Pierce JP. Comparative validation of the IPAQ and the 7-Day PAR among women diagnosed with breast cancer. Int J Behav Nutr Phys Act 2006;3:7.