

# Associations of Mammographic Density with Dietary Factors in Japanese Women

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## Abstract

**Background:** A high percentage of mammographic dense area has been strongly associated with a risk of breast cancer. The present cross-sectional study evaluated the relations of percent density with dietary factors, such as fats, protein, dietary fiber, and soy isoflavones.

**Methods:** Study subjects were 601 (348 premenopausal and 253 postmenopausal) Japanese women who were recruited from a mammographic screening center. The size of the total breast area and the dense area were measured quantitatively using an automated mammographic mass detection method. Intakes of nutrients were estimated with a validated semi-quantitative food-frequency questionnaire.

**Results:** The crude means of the percent density were 39.2% and 18.9% in premenopausal and postmenopausal women, respectively. There were no significant associations of any dietary factors with the percent density in premen-

opausal women. In postmenopausal women, percent density was significantly positively associated with intakes of protein, total fat, and saturated fat after controlling for covariates; the increase in the means of percent density were 7.2%, 5.6%, and 9.2% in the highest versus lowest quartile of intakes for protein, total fat, and saturated fat, respectively ( $P$  for linear trend were 0.006, 0.04, and 0.01, respectively). Carbohydrate intake was inversely associated with percent density; the mean of percent density was 6.0% lower in the highest versus the lowest quartile of intake ( $P_{\text{trend}} = 0.03$ ). The associations of dietary factors with dense area were very similar to those with percent density.

**Conclusion:** These dietary factors may have implications for the risk of breast cancer in postmenopausal women. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2877–80)

## Introduction

Epidemiologic data consistently suggest that the proportion of mammographic dense area, which is called breast density, is a marker of breast cancer risk (1). Current computerized methods of quantitative assessment of breast density are reliable. A high percentage of dense area has been associated with an ~3- to 6-fold increased risk of breast cancer in most of the studies, which adopted quantitative assessment methods (1). We have developed an automated method for detecting mammographic density (2) and confirmed a positive association between breast density and breast cancer risk among Japanese women (3).

Factors that are associated with mammographic density may affect the risk of breast cancer. Dietary factors, such as the intake of fats, dietary fiber, and soy isoflavones, have been implicated in the development of breast cancer (4). We cross-sectionally examined the associations between these dietary factors and mammographic density in Japanese women. Most of the previous studies on diet and the percent density have been conducted in Western populations (5-9).

## Materials and Methods

Study subjects were women attending a mammographic breast cancer screening at a general hospital in Gifu, Japan. A total of

1,430 women participated in a study of mammographic breast density from 2000 to 2002 (the response rate was estimated to be 70.3%). A subset of this population ( $n = 659$ ) was selected as controls for a case-control study of breast density and breast cancer. Details of the study have been described elsewhere (3). From this group, we selected 601 women for the present cross-sectional study after excluding those who had incomplete or unreliable responses to a dietary questionnaire. The criteria for exclusion are shown in ref. (10); unreliable responses include intake frequency of <1.5 or  $\geq 5$  times/d for staple foods,  $\geq 7$  times/d for meat or fish, and >400 mL/d for ethanol. Informed consent was obtained from each woman. This study was approved by the institutional review board.

Women responded to a self-administered questionnaire seeking information about diet, basic demographic characteristics, physical activity, smoking and drinking habits, medical history, and reproductive history. They filled out the questionnaires while attending the screening. Diet was assessed with a validated 169-item semiquantitative food-frequency questionnaire. This questionnaire was developed for a Japanese population by modifying the one designed for a multiethnic cohort study in Hawaii and Los Angeles (11). The questionnaire asked participants how often on average they consumed each of the food items listed and what was the usual serving size of each item during the year before the study. The intakes of foods and nutrients were estimated from the frequency of ingestion and portion size using the Japanese Standard Tables of Food Composition, fourth and fifth editions, published by the Science and Technology Agency of Japan. Fatty acid intakes were evaluated using data published by Sasaki et al. (12). Detailed information on the questionnaire, including its validity and reproducibility, has been described elsewhere (13). For example, the Spearman correlation coefficients between the questionnaire and 12 daily diet records kept over a 1-year period for intakes of total energy, total and

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each type of fat, total protein, carbohydrate, dietary fiber, and soy isoflavones ranged from 0.45 to 0.63. Exercise was assessed by asking the average hours per week spent performing various kinds of activities during the past year. The details, including its validity, are described elsewhere (14).

Mammograms from the mediolateral oblique view were obtained from each woman. They were taken using a mammography machine (Senographe DMR) and read and recorded using a image reader Fuji Computed Radiography 3CS (model CR-IR331) and a recorder CR-LP415. Assessment of mammographic density consists of seven stages: (a) image digitalization (0.05 mm sampling pitch and 12-bit density resolution); (b) extraction of the breast border; (c) reduction of the image matrix; (d) extraction of the pectoralis muscle region; (e) determination of the breast area; (f) determination of the threshold; and (g) extraction of the dense area. The details of this procedure have been described elsewhere (2). The percentage of density was calculated as the number of pixels within the dense area divided by the number of pixels for the entire breast area. The mean percentage of the density of both breasts was calculated for each woman. The reliability of this measurement was evaluated among 38 women who revisited the screening ~1 year later. The intraclass correlation coefficients comparing the repeated mammograms were 0.96 for total breast area and 0.90 for percent density. Our mammogram measurements were compared with those assessed by researchers at the Cancer Research Center of Hawaii (15). They adopted the validated method described Byng et al. (16) and Ursin et al. (17). Based on mammograms from 131 women, the rank correlation coefficients between their method and our methods were 0.95 for total breast area and 0.80 for percent density.

Linear regression models determined associations of dietary variables with the absolute dense area and percent density. As the size of total breast area was significantly independently associated with risk of breast cancer in postmenopausal women in our previous study (3), the association of diet with total breast area was also studied. For statistical analyses, percentage of breast dense area (percent breast density) and sizes of total breast and dense area were square root transformed. Nutrient intakes were log-transformed and adjusted for total energy according the residual methods proposed by Willett (18). We used the analysis of covariance method to provide adjusted estimates of the means of square root-transformed mammographic measures according to the quartile of nutrient intakes among the entire group (premenopausal and postmenopausal combined). The values were squared and presented with their 95% confidence intervals. Potential breast cancer risk factors, such as age, body mass index (BMI), number of births, age at menarche, age at first birth, history of lactation, smoking, exercise, alcohol intake, age at menopause, use of hormone replacement therapy, and family history of breast cancer among first-degree relatives, were examined initially as potential confounders. Among them, only those showing a statistically significant or marginally significant association with each of mammographic measures in univariate analyses ( $P < 0.10$ ) were included into multivariate models as covariates. These variables were as follows: age, BMI, smoking status, the number of births, and history of breastfeeding for percent density and dense area of premenopausal women; BMI and the number of birth for total breast area of premenopausal women; age, BMI, the number of births, years of education, and age at menopause for percent density and dense area of postmenopausal women; and age, BMI, and age at menarche for total breast area of postmenopausal women.

Association between diet and breast density differed by menopausal status. Therefore, the analyses were done separately for premenopausal and postmenopausal women. BMI did not modify the associations between diet and breast

density. Women who had been without a menstrual cycle in the previous 12 months or who were ages  $\geq 55$  years and did not report their menstrual statuses were classified as postmenopausal. Actually, the latter group included six women who were all over 60 years of age. One woman who was age 65 years and was reported to be premenopausal without giving the date of the start of the last menses was assumed to be postmenopausal. The remaining women were classified as premenopausal. Therefore, perimenopausal women or women who entered menopause recently ( $< 12$  months) were classified as premenopausal. All statistical analyses were done using SAS programs (19).

## Results

Tables 1 and 2 show the means or percentages of the selected nondietary factors among subjects according to their menopausal status. The arithmetic means and SD of total breast area, dense area, and percent breast density were 71.2 cm<sup>2</sup> (28.5 cm<sup>2</sup>), 26.5 cm<sup>2</sup> (21.4 cm<sup>2</sup>), and 39.2% (27.6%), respectively, in premenopausal women. The corresponding values were 77.1 cm<sup>2</sup> (28.1 cm<sup>2</sup>), 12.5 cm<sup>2</sup> (14.5 cm<sup>2</sup>), and 18.2% (20.3%), respectively, for postmenopausal women. The means of percent breast density as well as dense area were greater in premenopausal women than those in postmenopausal women. The mean of total breast area was greater in postmenopausal women than that in premenopausal women.

Table 3 presents the associations of selected nutrients with percent breast density. In premenopausal women, none of the listed nutrients showed significant associations with percent breast density. In postmenopausal women, protein, total fat, and saturated fat were significantly positively associated with percent breast density after controlling for the covariates. Carbohydrate intake was inversely associated with percent breast density.

The associations of dietary factors with dense area were very similar to those with percent density; intakes of protein, total fat, and saturated fat were significantly positively associated with dense area and intake of carbohydrate was significantly inversely associated with dense area in postmenopausal women.

There were no significant associations of total breast area with dietary factors except for polyunsaturated fat in postmenopausal women; the means of total breast area for the lowest to the highest quartile of polyunsaturated intakes were 67.9, 76.4, 79.4, and 74.2 cm<sup>2</sup>, respectively, after controlling for covariate ( $P_{\text{trend}} = 0.02$ ).

**Table 1. Distribution of nondietary factors among study subjects according to menopausal status**

Variables	Premenopausal (n = 348)	Postmenopausal (n = 253)
Age (y)	42.6 (5.8)	57.8 (6.1)
BMI (kg/m <sup>2</sup> )	22.1 (2.9)	23.0 (3.0)
Education (y)	2.7 (1.8)	11.1 (2.1)
Age at menarche (y)	12.8 (1.3)	14.1 (1.7)
Age at first birth (y)	25.3 (2.7)	24.8 (2.9)
Age at menopause (y)	—	49.2 (4.2)
Parity	2.2 (0.8)	2.3 (0.8)
Alcohol intake (mL/d)	6.6 (17.1)	4.7 (13.1)
Exercise (MET h/wk)	31.0 (39.8)	30.0 (44.1)
Current smokers (%)	9.2	4.1
Ex-smokers (%)	5.2	2.8
Family history of breast cancer		
Among first-degree relatives (%)	4.6	4.0
Breast feeding (%)	90.1	90.0

NOTE: Values are means (SD) or percentages. Breast feeding: self-reported, ever/never.

Abbreviation: MET, metabolic equivalents.

**Table 2. Means (SD) of daily dietary intake of selected nutrients according to menopausal status**

Variables	Premenopausal (n = 348)	Postmenopausal (n = 253)
Total energy (kcal)	2,277 (816)	2,280 (907)
Protein (g)	90.5 (35.7)	96.5 (43.9)
Carbohydrate (g)	315 (112)	319 (122)
Total fat (g)	67.6 (30.2)	65.6 (32.0)
Saturated fat (g)	19.2 (9.3)	17.8 (9.3)
Monounsaturated fat (g)	23.2 (10.5)	22.0 (11.3)
Polyunsaturated fat (g)	17.0 (7.4)	17.9 (9.3)
Long n-3 fatty acids (mg)	765 (507)	934 (687)
Dietary fiber (g)	17.2 (8.9)	21.6 (10.9)
Soy isoflavones (mg)	42.4 (28.8)	57.3 (37.6)

## Discussion

We found that intakes of total fat, saturated fat, and protein were associated with percent breast density in postmenopausal women but not in premenopausal women. Few studies have examined the association between diet and percent density or a parenchymal pattern. The results regarding fat intake have not been conclusive. An early study among participants in the Canadian National Breast Screening Study (5) found that saturated fat intake was significantly positively associated with the percentage of the breast showing nodular or homogeneous densities. There were no significant association between polyunsaturated fat intake and mammographic features in their study. However, in a study reported by Vachon et al. (8), saturated fat intake was significantly inversely associated with percent breast density, whereas polyunsaturated fat and the ratio of polyunsaturated to saturated fat were significantly positively associated with percent breast density in premenopausal women. Another observational study reported no significant associations between fat intake and the type of fat with high-risk parenchymal patterns (P2 and DY, increasing ductal prominence and dysplasia; ref. 9). In an intervention study among women with a high percent density (The Canadian Diet and Breast Cancer Prevention Study), a low-fat, high-carbohydrate diet for 2 years reduced the total breast and dense areas but not the percentage of dense area (6). There has been a hypothesis that dietary fat increases the risk of breast cancer (20). We should keep in mind that results from cohort studies found null or a slightly positive association between fat intake and types of fat and breast cancer risk (21). Saturated fat intake may be associated with percent density but not with a risk of breast cancer. It is also possible that dietary associations might be able to be detected with a quantitative trait with greater power than breast cancer. There has been no prospective study on fat intake and the risk of breast cancer among Japanese women. We cannot deny the possibility that total fat, saturated fat, as well as total protein, which were associated with percent density in the present study, may have implications for breast cancer risk among Japanese women.

If dietary factors could affect percent density through their effect on sex hormones, the influence might differ by menopausal status. Some studies have suggested that the association between diet and breast density may differ by menopausal status. However, their findings were not consistent with our results. In the intervention study reported by Boyd et al. (6), the low-fat, high-carbohydrate diet for 2 years reduced dense area of density in women who went through menopause but not in women who were postmenopausal at entry. In the study reported by Vachon et al. (8), saturated fat and polyunsaturated fat were significantly associated with percent density in premenopausal women but not in postmenopausal women.

Sala et al. (9) found a significant positive association between protein intake and high-risk (P2 and DY) mammographic parenchymal patterns, supporting our results. However, they observed a significant positive association between high-risk mammographic parenchymal patterns and carbohydrate intake.

**Table 3. Adjusted means with 95% confidence interval of percent breast density according to quartiles of dietary variables**

	n	Premenopausal	n	Postmenopausal
<b>Total energy (kcal)</b>				
Q1: <1,697	85	29.8 (24.5-35.6)	66	11.2 (7.6-15.4)
Q2: 1,697-2,062	84	28.1 (22.9-33.8)	66	10.0 (6.6-14.2)
Q3: 2,063-2,697	88	34.6 (29.0-40.8)	62	11.2 (7.5-15.7)
Q4: >2,697	91	34.8 (29.3-40.9)	59	12.2 (8.2-17.0)
<i>P</i> <sub>trend</sub>		0.50		0.59
<b>Protein (g)</b>				
Q1: <78.6	102	33.9 (28.7-39.5)	48	6.7 (3.6-10.7)
Q2: 78.6-85.8	95	28.7 (23.8-34.1)	55	11.8 (7.8-16.6)
Q3: 85.9-93.3	85	32.4 (26.8-38.5)	66	10.8 (7.3-14.9)
Q4: >93.3	66	32.6 (26.3-39.6)	84	13.9 (10.4-18.0)
<i>P</i> <sub>trend</sub>		0.97		0.02
<b>Carbohydrate (g)</b>				
Q1: <277.3	93	32.7 (27.3-38.5)	58	15.6 (11.1-20.9)
Q2: 277.3-297.1	87	31.7 (26.2-37.7)	63	10.4 (6.9-14.6)
Q3: 297.2-319.5	95	31.3 (26.1-37.0)	56	9.8 (6.3-14.2)
Q4: >319.5	73	31.7 (25.6-38.3)	76	9.6 (6.5-13.2)
<i>P</i> <sub>trend</sub>		0.95		0.03
<b>Total fat</b>				
Q1: <54.9	74	30.9 (25.0-37.4)	77	9.9 (6.8-13.7)
Q2: 54.9-61.4	84	31.9 (26.3-38.0)	65	8.9 (5.7-12.8)
Q3: 61.5-67.7	93	32.6 (27.2-38.4)	58	11.6 (7.7-16.2)
Q4: >67.7	97	31.8 (26.7-37.5)	53	15.5 (10.8-21.2)
<i>P</i> <sub>trend</sub>		0.69		0.04
<b>Saturated fat (g)</b>				
Q1: <14.5	71	29.3 (23.5-35.8)	80	7.3 (4.7-10.4)
Q2: 14.5-16.8	85	34.8 (29.0-41.1)	64	11.3 (7.7-15.6)
Q3: 16.9-19.4	89	29.4 (24.2-35.1)	62	12.8 (8.9-17.4)
Q4: >19.4	103	33.4 (28.3-39.0)	47	16.5 (11.3-22.6)
<i>P</i> <sub>trend</sub>		0.93		0.02
<b>Monounsaturated fat (g)</b>				
Q1: <18.2	67	30.8 (24.8-37.5)	84	8.8 (6.0-12.2)
Q2: 18.2-20.7	86	36.1 (30.3-42.5)	63	11.1 (7.4-15.5)
Q3: 20.8-23.4	93	27.9 (23.0-33.4)	58	13.1 (8.9-18.0)
Q4: >23.4	102	32.7 (27.6-38.2)	48	13.2 (8.7-18.7)
<i>P</i> <sub>trend</sub>		0.63		0.11
<b>Polyunsaturated fat (g)</b>				
Q1: <14.0	98	31.3 (26.1-36.9)	52	12.7 (8.5-17.8)
Q2: 14.0-16.1	91	33.0 (27.6-38.9)	59	6.9 (4.0-10.5)
Q3: 16.2-18.0	84	30.5 (25.1-36.6)	67	11.8 (8.1-16.1)
Q4: >18.0	75	32.6 (26.7-39.1)	75	13.3 (9.6-17.5)
<i>P</i> <sub>trend</sub>		0.79		0.30
<b>Long n-3 fatty acids (mg)</b>				
Q1: <498	100	34.1 (28.8-39.9)	51	11.0 (7.0-15.8)
Q2: 498-661	94	28.2 (23.2-33.6)	55	10.5 (6.7-15.2)
Q3: 662-897	82	30.8 (25.3-36.8)	69	9.4 (6.2-13.3)
Q4: >897	72	34.9 (28.6-41.8)	78	13.3 (9.6-17.5)
<i>P</i> <sub>trend</sub>		0.61		0.49
<b>Dietary fiber (g)</b>				
Q1: <14.0	123	30.9 (26.4-35.8)	28	9.7 (4.9-16.1)
Q2: 14.0-16.7	101	34.6 (29.4-40.3)	48	8.4 (4.2-13.0)
Q3: 16.8-20.4	74	31.9 (26.1-38.4)	77	12.0 (8.5-16.0)
Q4: >20.4	50	28.6 (21.8-36.2)	100	12.3 (9.2-15.9)
<i>P</i> <sub>trend</sub>		0.59		0.12
<b>Soy isoflavones (mg)</b>				
Q1: <28.6	116	30.2 (25.6-35.2)	34	9.7 (5.3-15.5)
Q2: 28.6-40.2	99	32.0 (26.9-37.6)	51	12.1 (7.8-17.3)
Q3: 40.3-56.7	72	29.5 (23.8-35.8)	79	9.1 (6.1-12.5)
Q4: >56.7	61	37.8 (30.7-45.6)	89	13.1 (9.7-17.0)
<i>P</i> <sub>trend</sub>		0.28		0.33

NOTE: Data are adjusted for age (continuous), BMI (continuous), smoking status (current, ex-smokers, or never smokers), number of births (none, 1-2, or >2), and history of breast feeding (ever or never) for premenopausal women, and for age, BMI, number of births (none, 1-2, or >2), years of education (<12, 12-13, or >13 years), and age at menopause (<47, 47-49, 50-52, or >52 years) for postmenopausal women. Nutrient intakes were adjusted for total energy.

Inconsistent results among studies may be related to difference in the evaluation of mammographic features. Considering that the risk of breast cancer has been more strongly associated with percent density or dense area measured with computer-based quantitative methods than with mammographic features qualitatively assessed, quantitative measurements of the dense area in breasts may be valuable in gaining insight into the effects of diet on breast cancer risk. The mean intakes of protein, total fat, and saturated fat relative to total energy seemed to be lower in our study subjects than those previously reported in the Western populations (5-7). In addition, nutrient intakes are likely to come from different kinds of foods in Japanese populations versus Western populations. These differences in diet may also explain partially the differences in findings among studies.

We failed to find a significant association between soy isoflavone intake and percent density. Our results were consistent with those from previous studies (22, 23). Soy intake was positively associated with percent density among Caucasian and Native Hawaiian women but not in the Chinese and Japanese women living in Hawaii (22). A 2-year soy intervention did not change mammographic densities in a multiethnic population from Hawaii (23). Limitations of our nutrient database precluded analysis of type of protein with percent density.

Although we presented the mean values of dietary intake, some of them may have been overestimated by our questionnaire. The means estimated from the questionnaire were generally higher than those estimated from 12 daily diet records. Previous studies also reported that amounts of nutrient intakes estimated from food-frequency questionnaire tended to yield higher intakes than those estimated from dietary records (24, 25). However, it is unlikely that such measurement errors were dependent on percent breast density and, thus, would attenuate associations.

We used mammograms taken in the mediolateral oblique direction because the screening system in Japan has adopted the mediolateral oblique view. Most of the previous studies conducted in other countries have used the craniocaudal direction. High correlation between the mediolateral oblique and craniocaudal views for dense area was reported by Byng et al. (26), although the estimates of breast density from the mediolateral oblique view are systematically lower than those from the craniocaudal view.

As is the nature of cross-sectional study, we cannot ascertain the temporal relationship between change in diet and breast density. Because of the potential importance of diet in the etiology of breast cancer, the association of diet with breast density deserves further studies.

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