Equol Status Modifies the Association of Soy Intake and Mammographic Density in a Sample of Postmenopausal Women

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Abstract

Only 30% to 50% of people produce the daidzein-metabolite equol after eating soy. We conducted a cross-sectional study of the associations between equol status, intake of soy foods, and mammographic density in a sample of postmenopausal women recruited at a radiology clinic near Buffalo, New York. Participants were 48 to 82 years old, had no history of cancer or breast reduction/augmentation, and no recent use of antibiotics or hormones. Percent density was measured by computer-assisted analysis of digitized images of craniocaudal films. Equol status was assessed using a soy-challenge protocol and usual soy intake by questionnaire. General linear models were used to assess independent and joint effects of equol status and intake of soy on multivariate adjusted percent density (covariates included age, body mass index, parity, age at first birth, and ever use of combined hormone therapy). Of 325 enrolled, 232 (71%) participants completed study assessments and are included in the present analysis. Mean percent density was 34% (±18%). Seventy-five (30%) participants were producers of equol. Forty-three (19%) participants reported regularly eating >1 soy food or supplement/wk. There were no significant independent associations of equol status or soy intake with percent density, but the interaction between these factors was significant ($P < 0.01$). Among equol producers, those with weekly soy intake had lower percent density (30.7% in weekly consumers of soy versus 38.9% in others; $P = 0.08$); among nonproducers, weekly soy intake was associated with higher percent density (37.5% in weekly soy consumers versus 30.7% in others; $P = 0.03$). Results suggest that equol producers and nonproducers may experience different effects of dietary soy on breast tissue. (Cancer Epidemiol Biomarkers Prev 2008;17(1):33–42)

Introduction

Relatively low breast cancer incidence in Asian countries (1) and increasing risk among Asian migrants to Western countries (2-4) led investigators to hypothesize that soy, a dietary staple in many Asian nations, may be protective against breast cancer (5, 6). Over the past 15 years, many epidemiologic and clinical studies have been conducted to test the hypothesis that dietary soy can prevent breast cancer. *In vitro* studies have shown biological properties of soy isoflavones that could underlie an anticancer effect (7). Experimental evidence and observational studies consistently suggest that exposure to dietary soy early in life can result in lasting protection from subsequent breast cancer (8, 9). In contrast, effects of exposure to soy later in life remain controversial (10); the observational literature in this area is characterized by mixed findings, although pooled estimates suggest a small reduction in risk associated with increasing soy intake (11).

In 2002, Setchell et al. (12) suggested that interindividual differences observed in metabolism of soy isoflavones may result in differential health effects of the exposure. Equol is a metabolite of the soy isoflavone daidzein, which can only be produced through metabolic processing by bacteria residing in the human intestinal tract. Following consumption of soy, 30% to 50% of subjects are observed to excrete equol (13). Equol is more bioavailable than other soy isoflavones; in addition, it has a unique ability to bind dihydrotestosterone and, when in combination with genistein, causes the synergistic inhibition of estrone sulfation, which could result in increased exposure of breast tissues to unbound estrogens (14).

Mammographic density is a measure of the extent of radiodensity present on the breast image on mammogram.

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Percent mammographic density is a ratio of dense area to total area of the breast image; this then represents an aggregate measure that reflects breast tissue composition. Studies have consistently shown that mammographic density is associated with risk of subsequent breast cancer (15-19); mammographic density is also associated with many known breast cancer risk factors and can be modified by interventions known to affect disease risk, such as hormone replacement therapy and tamoxifen use (20). Mammographic density may be best understood as an intermediate marker of disease risk (21) and recently has been applied frequently as such.

Several observational studies and intervention trials have investigated the association of soy intake and mammographic density, with inconsistent results (22-26). However, no studies have previously considered joint effects of equol status and soy intake on mammographic density or on breast cancer risk. The present study investigates independent and joint associations of equol-producer status and dietary soy intake with mammographic density in a sample of postmenopausal women.

Materials and Methods

The Biomarkers for Breast Cancer Prevention Study (B4BCP) was a cross-sectional study of postmenopausal women attending Windsong Radiology in Williamsville, New York, to undergo mammographic assessment. The study protocol was approved by the Institutional Review Board of the University at Buffalo, and informed consent was obtained from all participants. To join the study, mammography clients had to be willing to undergo film screen rather than digital mammography, to be at least 45 years old, and postmenopausal as of the date of study entry (defined as having last menstrual period more than 12 months ago, or, for women whose menses ceased due to partial hysterectomy, as having last menstrual period more than 12 months prior and age >51 years). Exclusion criteria included history of any cancer other than non-melanoma skin cancer, use of hormone replacement therapy within the month before mammography, history of breast augmentation or breast reduction surgery, and known allergy to soy or peanuts.

Anthropometric Measures. Height was self-reported whereas weight was assessed using a Tanita Scale plus Body Fat (Tanita Corporation of America, Inc.). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Repeated measures of both self-reported height and measured weight were conducted in a subsample of participants, with intraclass correlation coefficients of 99.1% for self-reported height, 99.9% for measured weight, and 99.8% for calculated BMI.

Dietary Assessments. A self-given questionnaire was used to assess usual dietary intake of soy foods and of other foods and dietary supplements containing soy isoflavones. Queried items were selected based on observations of available soy foods at two local grocery stores and included tofu, tempeh, miso soup, soy milk, soy burgers and hotdogs, soy-based ''deli meats,'' soy protein powders and shakes, and soy-based protein bars. The questionnaire queried frequency and serving size for each item. Participants were also queried about use of dietary or ''hormonal'' supplements containing soy isoflavones. Similar approaches have been used by other investigators (27, 28). For the purposes of this study, responses on this questionnaire were used to classify participants with regard to frequency of intake of soy foods and/or supplements (<1 versus ≥1 soy foods or supplements/wk). These categories were selected after assessing variability in frequency of intake among study participants.

For descriptive purposes, we also estimated isoflavone intake in a quantitative fashion. Among participants reporting intake of at least 1 soy food/wk, isoflavone intake was calculated using soy questionnaire data on intake of soy foods and soy supplements, including their frequency of intake, number of servings per unit time, and reported serving size. Roughly 20% of those who reported regular soy food intake indicated a unit of time (daily, weekly, monthly, less than once per month) but failed to provide the number of servings per unit time. For this subset of participants, we imputed servings per unit time using the median number of servings for that food and unit of time. Estimates of isoflavone content in each food came from published tables (29, 30); where published values were not available, estimates from food manufacturers were used.

All participants completed Block 1998 questionnaires to report on usual diet. Among participants who reported intake of <1 soy food or supplement/wk, we used estimates of isoflavone intake based on this questionnaire, which queries respondents on 109 food items accounting for 90% of intakes of each of the nutrients in the National Health and Nutrition Examination Survey III database for African Americans, Whites, and Hispanics. Berkeley Nutrition Services calculated nutrient levels based on their own food tables, which have been updated to include levels of isoflavones in the limited set of queried soy foods and in processed and other foods that contain isoflavones (31).

Soy Challenge. To assess equol status, study participants underwent a soy challenge according to the procedure described and used by Lampe et al. for the same purpose (32, 33). Participants received a set of three Revival Soy Bars, which each contain a standardized dose of 160-mg soy isoflavones including genistein, daidzein, and glycetein. They were instructed to eat one soy bar per day for each of 3 days before a scheduled appointment. On the appointed day, participants collected and delivered a first morning urine sample to the study clinic. Participants with recent antibiotic use had their soy challenge scheduled to occur at least 1 month after discontinuation of antibiotic therapy.

Collection, Transport, and Processing of Urine Specimens. Urine samples were transported by study investigators to the University of Buffalo where each specimen was filtered and aliquoted into labeled 1.8-mL cryovials, to be stored in a −70°C freezer. For logistical reasons, samples were transferred on dry ice to a −80°C So-Low Freezer in the Biological Specimen Bank in 232 Farber Hall on a monthly basis.

Measurement of Daidzein and Equol in Urine. Measurements of daidzein and equol levels in urine samples were conducted using gas chromatography

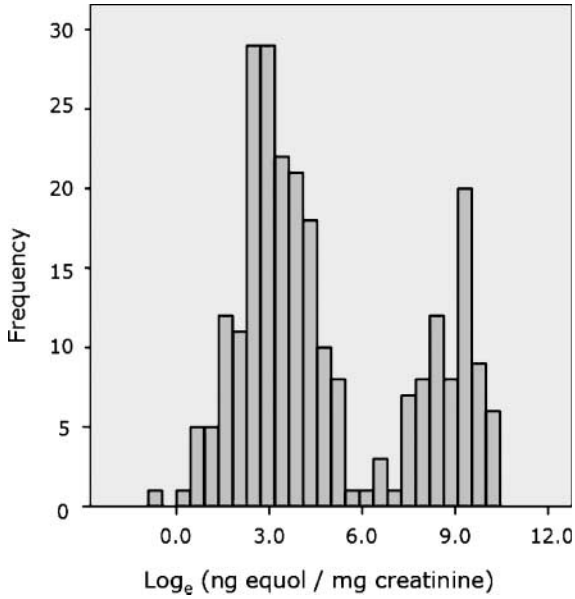

Figure 1. Distribution of $\log_e$ (creatinine-adjusted urinary equol) among 232 postmenopausal women, March to August 2005, Buffalo, New York.

following extraction of phytoestrogens by enzymatic hydrolysis, solid-phase extraction, and high-performance liquid chromatography purification according to the method developed for this purpose (34). Twenty-four quality control samples were included across 12 batches, with a coefficient of variation of 13.1% for daidzein and 7.1% for equol. Urinary isoflavone measures were creatinine adjusted. Participants with urinary equol concentrations of at least 400 ng/mg creatinine were considered to be equol producers. This cutoff point was selected based on the observed bimodal distribution of the log-transformed equol measure (Fig. 1). Five participants had both low urinary levels of daidzein (<600 ng/mg creatinine) and low levels of equol (<400 ng/mg creatinine) and thus were considered as potential noncompliers. These participants were classified as nonproducers of equol and included in study analyses; the potential effect of this decision was assessed in sensitivity analyses, as discussed below.

Mammographic Density. For each participant, a right and left craniocaudal view from the current mammographic assessment was selected for measurement. Mammographic films were digitized at 100 pixels/cm with a Kodak Lumysis 85 laser film scanner, which covers an absorbance range of 0 to 4.0 absorbance. Calibration of the scanner was checked and judged adequate before scanning each of three batches. Identifiers were removed from the mammographic image to ensure blinding of the reader. A single reader conducted the measurements of mammographic density of each image using Cumulus software (15, 35). Right and left films were read together; the order of films for reading was randomized by subject and side. Films were read in batches of 100 views, and a final review of images was conducted for quality assurance after completion of all measurements. Mean percent density was calculated from measures of right and left breasts. To assess reliability, three replicates for each of 39 randomly selected films were placed in the queue for reading. The intraclass correlation coefficient for percent breast density measurements was 0.95. The between-batch coefficient of variation for percent density was 8.5%, and the intraclass correlation coefficient for variability between sides was 0.94.

Statistical Methods. All analyses were conducted using the Statistical Package for Social Sciences (SPSS version 12). Distributions for all variables of interest were examined using histograms and descriptive statistics. Associations of participant characteristics with equol status were assessed using Student's t tests for continuous variables and χ^2 tests for categorical variables. Associations of participant characteristics with measures of mammographic density were assessed using linear regression models.

Covariates were selected for inclusion in multivariate models by the following process: (*a*) A list of potential covariates was created using a priori knowledge of factors associated with mammographic density and breast cancer risk. (*b*) All variables observed to be associated with equol status, soy intake, or percent density ($P \leq 0.20$) were tested in linear regression models. (*c*) Factors whose inclusion in linear regression models either led to a change of at least 10% in the β coefficients associated with exposures under study or were statistically significant independent predictors of percent density were selected. Participants with missing data items for variables included in final regression models were not included in this study, whereas those who were missing other data items were simply omitted, as noted, from individual analyses based on these variables.

General linear models were used to test for differences in mammographic density by equol status and frequency of soy intake while adjusting for the selected covariates and to assess the interaction of these factors. Finally, in exploratory analyses, logistic regression models were run to assess the joint effects of equol status and soy intake on odds of being in the top tertile of percent density (>42.5%).

Results

Between March and August 2005, 325 participants enrolled in the study. Of these, 24 were excluded as follows: digital mammogram (n = 10), uncertain menopausal status (n = 9), current hormone use (n = 2), and cancer diagnoses resulting from radiologic assessments (n = 3). Of those remaining, 69 were excluded from the present analysis due to unavailable data because participants did not complete study assessments (n = 42) or were unable to identify which exogenous hormones they had used postmenopausally (n = 16), because mammographic films could not be obtained (n = 1), or because technical problems were encountered in laboratory processing or assays (n = 10). Thus, 232 participants are included in the present analysis.

Most study participants were White (98%) and non-Hispanic (96%) and had a mean age of 59.6 years (SD,

Table 1. Characteristics of B4BCP study participants (232 postmenopausal women) by equol producer status, March to August 2005, Buffalo, New York

	Equol nonproducers			Equol producers			*P**
	n	Mean/%	SD	*n*	Mean/%	SD	
Age (y)	157	60.1	6.3	75	58.4	6.1	0.07
BMI (kg/m^2)	157	29.3	6.5	75	27.5	5.7	0.03
Education (*n*, %)							
High school	33	21.0		15	20.0		0.71
Some college/technical school	51	32.5		21	28.0		
Completed college	73	46.5		39	52.0		
Age at menarche (y) †	155	12.5	1.5	74	12.5	1.5	0.91
Age at natural menopause (y)	122	50.4	4.2	62	50.1	4.4	0.60
Age at surgical menopause (y)	35	40.5	6.4	13	45.8	4.9	0.01
Surgical menopause (*n*, %)	35	22.3		13	17.3		0.24
Parous (*n*, %)	136	86.6		61	81.3		0.33
Full-term pregnancies ‡	136	2.5	1.2	61	2.5	1.0	0.81
Age at first birth (y) ‡	136	24.5	4.5	61	25.0	5.1	0.49
Family history of breast cancer (*n*, %)§	32	22.2		15	21.4		0.52
History of hormone therapy use (*n*, column %)							<0.01
Never used postmenopausal hormone therapy	55	35.0		47	62.7		
Unopposed ERT only (*n*, %)	43	27.4		7	9.3		
CHT only (*n*, %)	49	31.2		19	25.3		
History of both ERT and CHT use (*n*, %)	10	6.4		2	2.7		
Percent density (%)	157	32.1	17.9	75	37.6	17.1	0.02

Abbreviations: ERT, estrogen replacement therapy; CHT, combined hormone therapy.
*For continuous covariates, *P* values were obtained using *t* tests and reflect significance of mean differences between equol producers and nonproducers. For categorical covariates, *P* values were obtained using χ^2 tests and reflect differences in distribution.
† Two missing or unknown.
‡ Among parous only.
§Nineteen participants were adopted or had unknown family histories.

6.3) and a mean BMI of 28.7 (SD, 6.3). Mean percent density was 33.9% (SD, 17.8). Seventy-five (30%) participants were classified as equol producers. Forty-eight (21%) participants reported a history of surgical menopause. Among participants reporting past use of hormone replacement therapy, median duration of use was 6.5 years (range, 0-42 years; SD, 3.8 years) and median lag time from last use to the time of the study mammogram was 3 years (range, 1 month-31 years; SD, 6.7 years).

Demographic and other characteristics for equol producers and nonproducers are shown in Table 1. Compared with nonproducers, equol producers were younger ($P = 0.07$) and had lower BMI ($P = 0.03$). Equol producers were significantly less likely than nonproducers to have a history of unopposed estrogen use ($P < 0.01$). Among participants with a history of surgical menopause, equol producers were older at menopause compared with nonproducers ($P = 0.01$).

As shown in Table 2, frequencies of soy food intake and use of soy supplements did not vary significantly by equol status. There were also no significant differences in types of soy foods consumed by equol status. However, small numbers of soy consumers have resulted in limited power to detect differences. Estimates of weekly isoflavone intake for participants with and without regular weekly intake of soy foods are presented in Table 3. There were no significant differences overall, or in any subgroup, by equol status.

Table 2. Intake of soy foods and isoflavone-containing dietary supplements by equol status among B4BCP study participants (232 postmenopausal women)

	Equol nonproducers, *n* = 157		Equol producers, *n* = 75		*P**
	n	Column %	*n*	Column %	
Current Intake of soy foods					
At least 1 soy food/d	13	8.3	3	4.0	0.18
At least 1 soy food/wk	29	18.5	12	16.0	0.40
Current use of isoflavone-containing dietary supplements					
At least 1 soy supplement/d	3	1.9	1	1.3	0.61
Current intake of at least 1 soy food or supplement/wk	31	19.7	12	16.0	0.31
History of hormone replacement therapy use and soy intake					
Never hormone users who consume <1 soy food or supplement/wk	38	24.2	35	46.7	<0.01
Never hormone users who consume ≥1 soy food(s) or supplement(s)/wk	11	7.0	7	9.3	
Ever hormone users who consume <1 soy food or supplement/wk	88	56.1	28	37.3	
Ever hormone users who consume ≥1 soy food(s) or supplement(s)/wk	20	12.7	5	6.7	

*P values were obtained using Fisher's exact tests.

Table 3. Total weekly isoflavone intake (mg/wk) by equol status among B4BCP study participants (232 postmenopausal women)

	Equol nonproducers				Equol producers				P*
	n	Median	IQR	Mean (SD)	n	Median	IQR	Mean (SD)	
Total isoflavones (mg/wk) from soy foods, among those reporting intake of ≥1 soy food/wk †	29	30.9	64.1	61.9 (79.6)	12	43.2	103.2	65.6 (77.4)	0.94
Total isoflavones (mg/wk) from soy foods and supplements, among those reporting intake of ≥1 soy food or supplement/wk †	31	41.4	90.5	79.5 (106.1)	12	56.9	198.9	129.8 (175.7)	0.51
Total isoflavones (mg/wk) among those reporting intake of <1 soy food or supplement/wk ‡	126	6.7	5.7	7.1 (4.4)	63	6.3	7.0	7.7 (6.3)	0.94

Abbreviation: IQR, interquartile range.
*P values were obtained using Mann-Whitney U tests.
† Estimates based on self-reported intake of soy foods and isoflavone-containing dietary supplements.
‡ Estimates based on foods queried in Block 1998 questionnaire.

We also assessed associations of participant characteristics with frequency of soy intake (data not shown). Participants who reported eating ≥1 soy foods or supplements weekly were more likely to have completed post-secondary education than those without weekly soy intake ($P = 0.03$). No significant associations were found between soy intake and other study variables.

Associations of mammographic density with participant characteristics were also assessed (Tables 4 and 5). After mutual adjustment, age ($r = -0.16$, $P = 0.01$) and BMI ($r = -0.49$, $P < 0.01$) were each significantly inversely correlated with percent density. Among parous women, age- and BMI-adjusted percent density was correlated with age at first birth ($r = 0.13$, $P = 0.06$). On average, parous women had lower percent density than nulliparous women ($P = 0.04$). Participants reporting ever use of combined hormone therapy had higher adjusted percent density compared with those without such a history ($P < 0.01$). In contrast, we found no significant difference in percent density by history of unopposed estrogen use. These associations are consistent with the findings of previous studies of this marker (21). There were no significant associations of percent density with duration of combined hormone therapy use or with duration of unopposed estrogen use. Age, BMI, parity, age at first birth, and ever use of combined hormone therapy were selected for inclusion in multivariate regression models based on the criteria described above.

Independent associations of equol status and soy intake with percent density were assessed using crude and adjusted general linear models (data not shown). Whereas percent density was significantly higher in equol producers as compared with nonproducers, the difference was no longer statistically significant after adjustment for age, BMI, parity, age at first birth, and ever use of combined hormone therapy (adjusted mean, 35.2% versus 32.2%; $P = 0.21$). Differences in percent density between weekly soy consumers and those with less than weekly intake of soy were not statistically significant (34.5% versus 32.9%; $P = 0.55$).

Effects of equol status and soy intake on percent density were examined in stratified samples (Table 6). Among equol producers, those reporting intake of at least 1 soy food or supplement/wk had lower percent

Table 4. Correlations between mammographic density (%) and selected covariates among B4BCP study participants (232 postmenopausal women)

	Bivariate correlation		Partial correlation*	
	Pearson correlation	Sig. (two tailed)	Pearson correlation	Sig. (two tailed)
Age	−0.12	0.07	−0.16	0.01
BMI	−0.48	<0.01	−0.49	<0.01
Full-term pregnancies	−0.10	0.13	−0.09	0.21
Age at first birth †	0.21	<0.01	0.13	0.06
Age at menarche ‡	−0.01	0.93	−0.04	0.57
Age at natural menopause§	−0.06	0.43	−0.03	0.73
Age at surgical menopause‖	0.02	0.92	−0.07	0.59
Duration of CHT (y)¶	−0.16	0.19	−0.14	0.26
Duration of unopposed estrogen therapy (y)**	−0.01	0.95	0.01	0.95

*Partial correlations adjusted for age and BMI. Partial correlation for age is adjusted for BMI, and partial correlation for BMI is adjusted for age.
† Among parous only; $n = 197$.
‡ Three missing or unknown; $n = 229$.
§Among participants with natural menopause; $n = 184$.
‖Among participants with surgical menopause; $n = 48$.
¶Among participants ever using combined hormone replacement therapy and able to report duration of use; $n = 71$.
**Among participants ever using unopposed estrogen therapy, and able to report duration of use; $n = 55$.

Table 5. Adjusted mean percent density (%) by categorical covariates among B4BCP study participants (232 postmenopausal women)

	n	Mean (SE)	*P**
Level of education			
Attended or graduated high school	48	37.0 (2.3)	0.29
Some college or technical school	72	32.7 (1.8)	
Graduated college	112	33.3 (1.5)	
Parity			
Nulliparous	35	38.8 (2.6)	0.04
Parous	197	33.0 (1.1)	
Reason periods stopped			
Natural menopause	184	34.6 (1.1)	0.17
Surgical menopause	48	31.1 (2.2)	
Use of combined hormone therapy			
Never	152	31.9 (1.2)	<0.01
Ever	80	37.7 (1.7)	
Use of estrogen replacement therapy			
Never	170	34.6 (1.2)	0.24
Ever	62	31.8 (2.0)	
Breast cancer among first-degree relatives*			
No	167	34.0 (1.2)	0.33
Yes	47	31.5 (2.3)	
Frequency of soy food or supplement intake			
<1/wk	189	33.1 (1.3)	0.19
≥1/wk	43	37.0 (2.7)	
Equol producer			
No	157	33.1 (1.2)	0.27
Yes	75	35.5 (1.8)	

NOTE: Data were adjusted for age and BMI.
*P values were obtained using one-way ANOVA and reflect differences between groups.

density compared with those with less frequent soy intake after adjustment for covariates (30.7% versus 38.9%; $P = 0.08$). Among nonproducers, those reporting intake of at least 1 soy food/wk had higher percent density compared with those with less frequent soy intake (37.5% versus 30.7%; $P = 0.03$). Among participants reporting intake of <1 soy food/wk, equol producers had higher percent density than nonproducers and the effect remained statistically significant after adjustment for covariates (36.9% versus 31.4%; $P = 0.03$). Among participants with intake of ≤1 soy food or supplement/wk, equol producers had significantly lower percent density than nonproducers (28.8% versus 40.3%, $P < 0.01$).

Table 7 shows both adjusted mean percent density and odds of membership in the top percent density tertile for participants grouped by equol status and frequency of soy intake. In these unstratified analyses, nonproducers with less than weekly soy intake are the reference category. In multivariate linear analyses adjusting for age, BMI, parity, age at first birth, and ever combined hormone therapy use, the interaction term for equol status × weekly intake of soy was statistically significant ($P_{\text{interaction}} = 0.01$) as a predictor of percent density. Results of adjusted logistic models suggest that equol producers who did not consume soy on a weekly basis were more likely (odds ratio, 1.99; 95% confidence interval, 0.98-4.06), whereas those with regular exposure to soy were less likely (odds ratio, 0.14; 95% confidence interval, 0.02-1.01), to fall into the top tertile of percent density when compared with the reference group. In contrast, the risk of falling into the top percent density tertile did not seem to differ by soy intake among nonproducers of equol.

In sensitivity analyses, we assessed the potential effects of decisions made during data analysis on our findings. Neither exclusion of five potential noncompliers in the soy challenge nor exclusion of two participants with exceptionally long duration of hormone therapy use modified the direction and/or magnitude of associations seen in this study. Likewise, when participants reporting use of soy-based hormonal supplements but no regular intake of soy foods ($n = 4$) were reclassified in the nonexposed group, there was no meaningful change in study findings. Inclusion of duration of combined and/or estrogen-only hormonal therapies in multivariate models also did not modify study findings in any meaningful way.

Table 6. Crude and adjusted mean differences in mammographic density (%) by soy intake and by equol status in selected strata

	<1 soy food or supplement/wk		≥1 soy foods or supplement/wk		Mean difference (SE)	*P*
	n	Mean (SE)	*n*	Mean (SE)		
Equol nonproducers						
Crude model	126	29.9 (1.6)	31	40.7 (3.1)	−10.8 (3.5)	<0.01
Adjusted model*	126	30.7 (1.3)	31	37.5 (2.8)	−6.7 (3.1)	0.03
Equol producers						
Crude model	63	39.5 (2.1)	12	27.5 (4.8)	12.0 (5.2)	0.03
Adjusted model*	63	38.9 (1.8)	12	30.7 (4.3)	8.2 (4.7)	0.08
	Equol nonproducers		Equol producers		Mean difference (SE)	*P*
	n	Mean (SE)	*n*	Mean (SE)		
<1 soy food or supplement/wk						
Crude model	126	30.1 (1.6)	63	39.5 (2.2)	−9.4 (2.7)	<0.01
Adjusted model*	126	31.4 (1.4)	63	36.9 (2.0)	−5.5 (2.5)	0.03
≥1 soy food or supplement/wk						
Crude model	31	40.7 (2.7)	12	27.5 (4.3)	13.2 (5.1)	0.01
Adjusted model*	31	40.3 (2.0)	12	28.8 (3.4)	11.5 (4.1)	<0.01

*Adjusted for age, BMI, parity, age at first birth, and ever use of combination hormone (estrogen + progesterone) therapy.

Table 7. Adjusted odds of high mammographic density (top tertile, percent density >42.5%) and adjusted mean percent density (%) in groups defined by equol status and weekly intake of soy foods

	Equol nonproducers			Equol producers		
	n	Mean PD (SE)	OR (95% CI)	*n*	Mean PD (SE)	OR (95% CI)
<1 soy food or supplement weekly	126	31.8 (1.3)	1.00 (reference)	63	37.1 (1.9)	1.99 (0.98-4.06)
>1 soy food or supplement weekly	31	38.0 (2.7)	1.24 (0.49-3.15)	12	28.2 (4.3)	0.14 (0.02-1.01)

NOTE: Logistic model was adjusted for age, BMI, parity, age at first birth, and ever use of combined hormone therapy; $P_{interaction} = 0.05$. General linear model was adjusted for age, BMI, parity, age at first birth, and ever use of combined hormone therapy; $P_{interaction} < 0.01$.
Abbreviations: PD, percent density; OR, odds ratio; 95% CI, 95% confidence interval.

Discussion

In this cross-sectional study of postmenopausal women without cancer, neither equol status nor habitual soy intake was independently associated with mammographic density after adjustment for potential confounders; however, there was a significant interaction between these factors in predicting percent density. Stratified analyses show distinct differences among groups defined by both equol status and soy intake, suggesting that these factors may have joint effects on mammographic density that would be undetectable without consideration of both factors.

Among equol producers, those with weekly intake of ≥1 soy foods and/or supplements had lower adjusted percent density compared with those with less frequent soy intake, with an adjusted mean difference of 9.7 percentage points. Among women with at least weekly intake of soy, equol producers had significantly lower percent density compared with nonproducers. Whereas adjusted mean percent density was not significantly different between equol producers who regularly consumed soy and nonproducers of equol without significant soy intake, the odds of membership in the top percent density tertile was significantly reduced in the equol-exposed group. Together, these findings provide support for our a priori hypothesis that repeated exposures to equol can result in favorable changes in mammographic density. However, the pattern of differences among groups defined by both equol status and soy intake cannot be fully explained as effects of exposure to equol.

Among nonproducers of equol, regular intake of soy foods/supplements is associated with significantly higher percent density but not with significantly different probability of falling in the top percent density tertile. Investigators have long hypothesized that exposure to isoflavones found in dietary soy might lead to increased mammographic density through agonist interactions with estrogen receptors in breast tissue. This association has not been found in soy studies conducted to date (22-26) but a study of another diet-derived phytoestrogen, enterolactone, also found a weak but statistically significant direct association with mammographic density (36). The magnitude of the difference in percent density observed between these subgroups (5.5 percentage points) is not negligible and should be investigated further.

Among participants without regular intake of soy, equol producers had significantly higher percent density than nonproducers and were roughly twice as likely to fall into the top tertile of high percent density. This finding could suggest that equol status is serving as a surrogate for some other physiologic characteristic associated with mammographic density and/or breast cancer risk, or it could suggest a nonlinear dose response relationship between equol and percent density. This finding should be interpreted cautiously, however, because the observed difference in percent density is due almost entirely to significant differences in total area of the breast on mammogram rather than to differences in dense area (data not shown). Densities on mammogram are understood to represent epithelial and stromal tissues, those at greatest risk of carcinogenic transformation. In contrast, nondense area is fatty breast tissue, whose role in breast cancer etiology is not clear. The causal relationship underlying the predictive association of percent density with risk of breast cancer is not well understood, and thus Haars et al. (37) suggest that inferences about breast cancer etiology based on findings of associations with percent density, particularly in the absence of an association with dense area, may be problematic.

The epidemiologic literature on the relationship between soy intake and breast cancer risk can be characterized as mixed (38), likely due to a number of methodologic issues, including the lack of variability in soy intake within many study populations, a hypothesized threshold in the effects of soy on breast cancer risk (39), and the possibility that the effect of soy intake on breast cancer risk may vary across the life span, with adolescence as the period at which it may have its greatest effect on risk (9). Our results suggest that an additional reason for mixed results may be heterogeneity in the effects of soy intake on breast cancer risk among metabolic subgroups. To these authors' knowledge, this is the first published study with assessment of both dietary soy intake and equol status and their independent and joint associations with mammographic density. Due to the observational and cross-sectional design of this study, the nature of the underlying causal mechanisms cannot be determined; however, our findings should motivate further study.

Observational and intervention trials of soy and breast health have been done with mammographic density and change in mammographic density, respectively, as outcomes. An observational study by Jakes et al. (23) on Singapore Chinese women found that favorable parenchymal patterns were more prevalent among those with higher soy intake. In contrast, in their study of premenopausal women in Hawaii, Maskarinec and Meng (22) found that higher levels of dietary soy were associated with significantly higher percent density; however, this was due to lower breast area in this group

rather than increased dense area. This finding was echoed in a year-long intervention trial of an isoflavone supplement, also conducted by Maskarinec et al. (24), in which the intervention arm experienced a nonsignificant decline in breast area. Two other randomized trials, a 2-year-long soy food intervention and a year-long trial of isoflavones derived from red clover, were unable to show significant differences between intervention and control groups in change in mammographic density (25, 26). In a study of the cross-sectional association between equol status and mammographic density among overweight postmenopausal women, Frankenfeld et al. (40) found that equol producers had significantly lower percent density compared with nonproducers.

Our study findings suggest that studies of soy intake and mammographic density that fail to account for equol status may have null findings because the effects of soy intake on percent density in one subgroup (equol producers) will be balanced by the effects of soy intake on percent density in the other larger subgroup (nonproducers of equol). This may also be true by inference in studies of soy intake and breast cancer risk.

Two recent studies have investigated the effects of soy or isoflavone supplements on breast cancer–related hormones in participants by equol status. These could suggest potential mechanisms by which exposure to equol could reduce mammographic density and/or breast cancer risk.

The first, a small controlled intervention trial (n = 34 postmenopausal women) by Nettleton et al. (41), showed that soy protein supplementation resulted in increased urinary 2-hydroxy estrogens and an increased ratio of excreted 2:16 hydroxy-estrogens in the subgroup of participants who excreted high levels of equol. Whereas prospective studies have not consistently supported an association of circulating levels of these particular estrogen metabolites or their ratio with breast cancer risk (42-44), a growing body of research supports a role in breast carcinogenesis for the quinones of catechol estrogens, which are unstable and therefore difficult to measure directly in an epidemiologic setting (45-47). Effects of equol on estrogen metabolism could potentially modify production of genotoxic quinones, resulting in reduced breast cancer risk.

An intervention trial of soy supplementation in a sample of 37 men at high risk of colorectal cancer showed that changes in serum levels of insulin-like growth factor I following the intervention were inversely associated with serum equol concentrations (48). The insulin-like growth factor pathway modulates proliferation and survival of many cell types and plays a role both in normal breast growth and development and in the biology of breast tumors. Among premenopausal women, circulating levels of insulin-like growth factor I have consistently been found to be associated with mammographic density (49, 50) and, in some but not all prospective studies, with breast cancer risk (51-53). Whereas the same associations were not found among postmenopausal women, this could reflect a premenopausal window of susceptibility to insulin-like growth factor I or differences between premenopausal and postmenopausal women in the association between circulating levels and breast tissue levels of this growth factor. If exposure to equol does result in lower levels of insulin-like growth factor I in breast tissue, this could result in reduced mammographic density and perhaps also in reduced breast cancer risk.

It must be considered that the findings presented here may be, in part or in whole, a result of noncausal associations. In our study population of mostly Caucasian, postmenopausal women, 19% reported consuming at least 1 soy food or supplement/wk; regular intake of soy is a highly self-selected activity, associated with a higher level of education and perhaps also with other health habits and lifestyle factors. At least one study has found a cross-sectional association between soy intake and vasomotor symptoms in perimenopausal and postmenopausal women (54); unfortunately, this factor was not measured in the present study.

Determinants of equol status are poorly understood, and the finding that it is associated with history of postmenopausal hormone use suggests that equol status and mammographic density may share some common causes. A recent article by Setchell and Cole (55) suggested that vegetarians may be more likely than others to be equol producers, independent of their dietary soy intake. A number of studies have looked at dietary components associated with equol status, and identified correlates have been suggested by the results of individual studies, but none is consistently found across studies (32, 56, 57). In the B4BCP study sample, there were few vegetarians (n = 5); further, in analyses conducted to date on this data, no significant associations of dietary macronutrients with equol status have been identified.[10]

This study has several strengths, particularly the careful assessment of equol status based on a soy-challenge protocol and use of a highly sensitive and reliable assay for equol, and the quantitative and reliable measurement of our outcome, mammographic density. This study also has some limitations. The cross-sectional design does not allow assessment of temporal or causal relationships. However, the joint association of dietary soy intake and equol status with percent density represents an innovative finding and a potential source of scientific hypotheses to be tested in further studies. Some limitations in generalizability might derive from having recruited participants at a single clinic; in comparison with the general population, our study sample underrepresents racial and ethnic minorities and overrepresents women at high risk of breast cancer. However, this sample is representative of women seeking mammographic assessment in many community-based screening settings.

Other limitations stem from the nature of the considered exposure in that soy intake is a highly self-selected activity associated with a higher level of education and also, perhaps, with a healthier diet or lifestyle. The small number of regular soy consumers resulted in limited power to detect significant associations in subgroups. Finally, the use of a surrogate measure of breast cancer risk limits our ability to definitively state whether the same associations would hold for the clinically significant end point (breast cancer

[10] Fuhrman B, Teter B, Horvath P, Muti P. Biomarkers for Breast Cancer Prevention (B4BCP) Study, Buffalo, New York; 2006. Unpublished data.

incidence). However, because mammographic screening is less effective in women with high levels of mammographic density, factors that lower mammographic density are of interest even if their etiologic implications are unclear.

The results of this study suggest that equol status should be considered as a potential modifying factor when assessing the effects of soy on mammographic density, and perhaps also on breast cancer risk. There is much concern and few answers about the effects on breast cancer risk of eating soy in adulthood, particularly in postmenopause. Because there is significant interindividual variability in the metabolism of soy isoflavones and because equol may have uniquely potent health effects, ascertainment of equol status may be the key to understanding the true health effects of eating soy. Randomized intervention trials will be particularly important to reduce confounding by factors associated with regular intake of soy foods in populations.

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References

1. Cancer incidence in five continents. Parkin D, Whelan S, Ferlay J, Raymond L, Young J, editors. Vol. VII. Lyon, France: IARC Sci Publ; 1997.
2. Buell P. Changing incidence of breast cancer in Japanese American Women. J Natl Cancer Inst 1973;51:1479–83.
3. Schimizu H, Ross RK, Bernstein L. Cancers of the prostate and breast among Japanese and White immigrants in Los Angeles County. Br J Cancer 1991;63:963–6.
4. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 1993; 85:1819–27.
5. Messina M, Barnes S. The role of soy products in reducing risk of cancer. J Natl Cancer Inst 1991;83:541–6.
6. Adlercreutz H, Mousavi Y, Clark J, et al. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. J Steroid Biochem Mol Biol 1992;41:331–7.
7. Kurzer MS, Xu X. Dietary phytoestrogens. Annu Rev Nutr 1997;17: 353–81.
8. Shu XO, Jin F, Dai Q, et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. Cancer Epidemiol Biomarkers Prev 2001;10:483–8.
9. Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC. Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. Carcinogenesis 2002;23:1491–6.
10. Messina M, McCaskill-Stevens W, Lampe JW. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. J Natl Cancer Inst 2006;98:1275–84.
11. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta analysis of soy intake and breast cancer risk. J Natl Cancer Inst 2006;98:459–71.
12. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. J Nutr 2002;132:3577–84.
13. Atkinson C, Frankenfeld CL, Lampe JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp Biol Med (Maywood) 2005;230:155–70.
14. Setchell KD, Clerici C, Lephart ED, et al. S-equol, a potent ligand for estrogen receptor β, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. Am J Clin Nutr 2005;81:1072–9.
15. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995; 87:670–5.
16. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J Natl Cancer Inst 1995;87:1622–9.
17. Wolfe JN. Risk for breast cancer development determined by mammographic parenchymal pattern. Cancer 1976;37:2486–92.
18. Byng JW, Yaffe MJ, Jong RA, et al. Analysis of mammographic density and breast cancer risk from digitized mammograms. Radiographics 1998;18:1587–98.
19. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1159–69.
20. Rutter CM, Mandelson MT, Laya MB, Seger DJ, Taplin S. Changes in breast density associated with initiation, discontinuation, and continuing use of hormone replacement therapy. JAMA 2001;285: 171–6.
21. Boyd N, Rommens JH, Vogt K, et al. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6: 798–808.
22. Maskarinec G, Meng L. An investigation of soy intake and mammographic characteristics in Hawaii. Breast Cancer Res 2001;3: 134–41.
23. Jakes RW, Duffy SW, Ng FC, et al. Mammographic parenchymal patterns and self-reported soy intake in Singapore Chinese women. Cancer Epidemiol Biomarkers Prev 2002;11:608–13.
24. Maskarinec G, Williams AE, Carlin L. Mammographic densities in a one-year isoflavone intervention. Eur J Cancer Prev 2003;12: 165–9.
25. Atkinson C, Warren RM, Sala E, et al. Red-clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial [ISRCTN42940165]. Breast Cancer Res 2004;6: R170–9.
26. Maskarinec G, Takata Y, Franke AA, Williams AE, Murphy SP. A 2-year soy intervention in premenopausal women does not change mammographic densities. J Nutr 2004;134:3089–94.
27. Kirk P, Patterson RE, Lampe J. Development of a soy food frequency questionnaire to estimate isoflavone consumption in US adults. J Am Diet Assoc 1999;99:558–63.
28. Williams AE, Maskarinec G, Hebshi S, Oshiro C, Murphy S, Franke AA. Validation of a soy questionnaire with repeated dietary recalls and urinary isoflavone assessments over one year. Nutr Cancer 2003; 47:118–25.
29. Umphress ST, Murphy SP, Franke AA, Custer LJ, Blitz CL. Isoflavone content of foods with soy additives. J Food Comp Anal 2005;18:533–50.
30. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr Cancer 2006;54:184–201.
31. Horn-Ross PL, Barnes S, Lee M, et al. Assessing phytoestrogen exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control 2000;11:289–98.
32. Lampe JW, Karr SC, Hutchins AM, Slavin JL. Urinary equol excretion with a soy challenge: influence of habitual diet. Proc Soc Exp Biol Med 1998;217:335–9.
33. Frankenfeld CL, Atkinson C, Thomas WK, et al. High concordance of daidzein-metabolizing phenotypes in individuals measured 1 to 3 years apart. Br J Nutr 2005;94:873–6.
34. Venturelli E, Rinaldi S, Cambie M, Cavalleri A, Secreto G. Quantitative analysis of urinary daidzein and equol by gas chromatography after solid-phase extraction and high-performance liquid chromatography. Int J Biol Markers 2002;17:182–8.
35. Cumulus3 program. Sunnybrook and Women's College Health Sciences Centre; 2006.
36. Stuedal A, Gram IT, Bremnes Y, Adlercreutz H, Veierod MB, Ursin G. Plasma levels of enterolactone and percentage mammographic density among postmenopausal women. Cancer Epidemiol Biomarkers Prev 2005;14:2154–9.
37. Haars G, van Noord PA, van Gils CH, Grobbee DE, Peeters PH. Measurements of breast density: no ratio for a ratio. Cancer Epidemiol Biomarkers Prev 2005;14:2634–40.
38. Martinez ME. Soy and breast cancer: the controversy continues. J Natl Cancer Inst 2006;98:430–1.
39. Messina M. Western soy intake is too low to produce health effects. Am J Clin Nutr 2004;80:528–9; author reply 529–30.
40. Frankenfeld CL, McTiernan A, Aiello EJ, et al. Mammographic density in relation to daidzein-metabolizing phenotypes in overweight, postmenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:1156–62.

41. Nettleton JA, Greany KA, Thomas W, Wangen KE, Adlercreutz H, Kurzer MS. The effect of soy consumption on the urinary 2:16-hydroxyestrone ratio in postmenopausal women depends on equol production status but is not influenced by probiotic consumption. J Nutr 2005;135:603–8.
42. Muti P, Bradlow HL, Micheli A, et al. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16α-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology 2000;11:635–40.
43. Cauley JA, Zmuda JM, Danielson ME, et al. Estrogen metabolites and the risk of breast cancer in older women. Epidemiology 2003; 14:740–4.
44. Wellejus A, Olsen A, Tjonneland A, Thomsen BL, Overvad K, Loft S. Urinary hydroxyestrogens and breast cancer risk among postmenopausal women: a prospective study. Cancer Epidemiol Biomarkers Prev 2005;14:2137–42.
45. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents-DNA adducts and mutations. J Natl Cancer Inst Monogr 2000;(27):75–93.
46. Crooke PS, Ritchie MD, Hachey DL, Dawling S, Roodi N, Parl FF. Estrogens, enzyme variants, and breast cancer: a risk model. Cancer Epidemiol Biomarkers Prev 2006;15:1620–9.
47. Rogan EG, Badawi AF, Devanesan PD, et al. Relative imbalances in estrogen metabolism and conjugation in breast tissue of women with carcinoma: potential biomarkers of susceptibility to cancer. Carcinogenesis 2003;24:697–702.
48. Vrieling A, Rookus MA, Kampman E, et al. Isolated isoflavones do not affect the circulating insulin-like growth factor system in men at increased colorectal cancer risk. J Nutr 2007;137:379–83.
49. Boyd NF, Stone J, Martin LJ, et al. The association of breast mitogens with mammographic densities. Br J Cancer 2002;87:876–82.
50. Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankinson SE. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000;60:3744–8.
51. Schernhammer ES, Holly JM, Hunter DJ, Pollak MN, Hankinson SE. Insulin-like growth factor-I, its binding proteins (IGFBP-1 and IGFBP-3), and growth hormone and breast cancer risk in The Nurses Health Study II. Endocr Relat Cancer 2006;13:583–92.
52. Schernhammer ES, Holly JM, Pollak MN, Hankinson SE. Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:699–704.
53. Renehan AG, Harvie M, Howell A. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and breast cancer risk: eight years on. Endocr Relat Cancer 2006;13:273–8.
54. Gold EB, Bair Y, Zhang G, et al. Cross-sectional analysis of specific complementary and alternative medicine (CAM) use by racial/ethnic group and menopausal status: the Study of Women's Health Across the Nation (SWAN). Menopause 2007;14:612–23.
55. Setchell KD, Cole SJ. Method of defining equol-producer status and its frequency among vegetarians. J Nutr 2006;136:2188–93.
56. Adlercreutz H, Honjo H, Higashi A, et al. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr 1991;54:1093–100.
57. Rowland IR, Wiseman H, Sanders TA, Adlercreutz H, Bowey EA. Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. Nutr Cancer 2000;36:27–32.