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Research Article

Equol-Producing Status, Isoflavone Intake, and Breast Density in a Sample of U.S. Chinese Women

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Abstract

Background: Differences in ability to metabolize daidzein to equol might help explain inconsistent findings about isoflavones and breast cancer. We examined equol-producing status in relation to breast density, a marker of breast cancer risk, and evaluated whether an association of isoflavone intake with breast density differs by equol-producing status in a sample of Chinese immigrant women.

Methods: Participants were 224 women, ages 36 to 58 years, enrolled in a study on diet and breast density. All women completed dietary recall interviews, underwent a soy challenge to assess equol-producing status, and received a mammogram assessed for breast density using a computer-assisted method.

Results: In our sample, 30% were classified as equol producers. In adjusted linear regression models, equol producers had significantly lower mean dense tissue area (32.8 vs. 37.7 cm^2, $P = 0.03$) and lower mean percent breast density (32% vs. 35%, $P = 0.03$) than nonproducers. Significant inverse associations of isoflavone intake with dense area and percent density were apparent, but only in equol producers (interaction $P = 0.05$ for both).

Conclusions: These results support the possibility that equol-producing status affects breast density and that effects of isoflavones on breast density depend on ability to metabolize daidzein to equol.

Impact: Although these findings warrant confirmation in a larger sample, they offer a possible explanation for the inconsistent findings about soy intake and breast density and possibly breast cancer risk as well. The findings further suggest the importance of identifying factors that influence equol-producing status and exploring appropriate targeting of interventions. *Cancer Epidemiol Biomarkers Prev; 22(11); 1975–83. ©2013 AACR.*

Introduction

The isoflavones genistein and daidzein are key biologically relevant factors in soy that are thought to reduce breast cancer risk through their estrogenic and anticarcinogenic properties (1, 2). However, epidemiologic evidence for a protective effect of isoflavones on breast cancer risk remains relatively weak and inconclusive (2). Previous studies may have underestimated the effects of soy if its effects depended on differences in its metabolism. Between 20% and 60% of individuals possess intestinal bacteria capable of metabolizing the isoflavone daidzein to equol. Equol seems to have higher estrogenic activity than its precursor. A greater proportion of it circulates in free or unbound form, and it shows the greatest antioxidant activity of all the isoflavones (3). Because of these properties, the ability to produce equol from daidzein may be a major determinant of the impact of soy.

Because of its strong association with breast cancer risk (4, 5), breast density, or the portion of total breast area with a mammographically dense appearance, is a useful marker for breast cancer risk in epidemiologic studies (5, 6). Notably, one study of 55 postmenopausal women found that mean breast density was 39% lower in equol producers than in nonproducers (7). Another study (8) found that soy intake was associated with lower percent density only among equol producers. An investigation of the importance of equol-producing status in Asian women could help answer the question of whether higher equol-producing prevalence in Asian women might partly account for their lower breast cancer risk.

The objectives of this study were 2-fold, i.e., (i) to examine the association of equol-producing status with breast density in a sample of Chinese immigrant women and (ii) to determine if equol-producing status modifies the association of isoflavone intake with breast density in this sample. We hypothesized that breast density would be lower in equol producers than nonproducers, consistent with previous work (7). We further hypothesized that soy isoflavones would be associated with lower breast density only among equol producers.

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Materials and Methods

Study population

Study participants were recruited from among a cohort of 436 women enrolled in a longitudinal study of diet and mammographic breast density (9). The original cohort participants were recruited between November 2005 and April 2008, through community organizations, local medical practices, newspaper advertisements, and other contacts within the Chinese community in the Philadelphia region. Equol substudy participants were recruited between September 2008 and October 2009, as part of follow-up efforts for the longitudinal study. Research staff contacted each cohort participant approximately 2 weeks in advance of her follow-up mammogram to explain the study. If the participant expressed interest, then she was screened for eligibility. Eligibility criteria for the longitudinal study included Chinese heritage, migration from Asia 20 or more years ago, and being of mammography screening age. Exclusion criteria were as follows: postmenopausal status; history of breast augmentation/reduction, prophylactic mastectomy, or any cancer except nonmelanoma skin cancer; current pregnancy; current breastfeeding or breastfeeding within last 9 months; or symptoms of new breast problem, such as palpable lump, skin changes, or nipple discharge. For the cross-sectional equol substudy, use of antibiotics in the previous 3 months was an additional exclusion criterion because of its impact on the presence of intestinal bacteria. Of 436 cohort members, 271 were recontacted for follow-up during the recruitment period. Of these, 232 expressed interest in the study. Two were deemed ineligible because they reported use of antibiotics, resulting in a total of 230 members fully enrolled in the study. Of these, 6 women were subsequently excluded because mammographic images could not be obtained ($n = 3$), urine samples were not located ($n = 2$), or because of pregnancy at the time of the study ($n = 1$), leaving a sample of 224 women for this analysis.

The study was approved by the Institutional Review Board at the Fox Chase Cancer Center, and all participants provided their written informed consent to participate.

Study procedures

As part of the longitudinal study, participants received annual mammograms at no cost through the Fox Chase Cancer Center or its mobile mammography unit. Interviewers conducted detailed health interviews before mammographic screening and two 48-hour dietary recall interviews within a month of screening. Health interviews elicited baseline and updated information on reproductive history, including age at menarche, pregnancy history, and oral contraceptive or other hormone use; family history of breast cancer; and level of acculturation as indicated by the General Ethnicity Questionnaire–American. Acculturation score was calculated as the average over 11 items in the General Ethnicity Questionnaire, which dealt with exposure to or familiarity with American people, culture, and activities (e.g., "Now, I am exposed to American culture," "I go to places where people are American," and "I celebrate American holidays"). The 11 items showed high internal reliability in our sample (Cronbach $\alpha = 0.91$). Trained research staff also followed an established protocol to measure weight, standing height, and waist circumference; all measures were taken in duplicate by the same interviewer, with the mean used in analyses.

We followed a procedure similar to that used by Frankenfeld and colleagues (7) to assess participants' equol-producing status. Before her scheduled mammographic examination, each participant received a 6.5-oz container (with 1 g of ascorbic or boric acid to prevent microbial contamination and oxidative degradation) and three 8-fluid ounce containers of soymilk (Nature Soy) containing approximately 25 to 30 mg of isoflavones (sum of genistein, daidzein, and glycitein) per container. Participants were instructed to consume 1 container of soymilk in the late afternoon or evening on each of 3 consecutive days before her scheduled mammogram, and to bring a first-void urine sample on the day of her appointment. At the appointment, the participant was asked how much of the soymilk she consumed in the 3 days, as well as whether she consumed other soy foods in those days.

Dietary data

Trained interviewers followed a standardized protocol for conducting two 48-hour dietary recall interviews for each participant, within 2 weeks of each other, with a target of at least 1 weekend day among the 4 days. We used 48-hour recalls rather than 24-hour recalls to balance the need to capture greater intraindividual variability in dietary intake with the logistic difficulty of trying to reach participants for interviews on multiple days. Additional analyses comparing the distributions of several nutrient variables based on days 2 and 4 of the 4 dietary recall days (the equivalent of two regular 24-hour recalls) with distributions based on all 4 days from the two 48-hour recalls showed comparable means and the expected increase in variance of means based on only 2 rather than 4 days of recalls.

Interviewers also followed a standardized protocol for entering responses into the Nutrition Data System for Research [version 2008; Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN]. The NCC database includes 160 nutrients, nutrient ratios, and other food components and incorporates nutrient values from the USDA National Nutrient Database for Standard Reference, including its tables on isoflavone content of foods. Isoflavones included in these analyses were genistein, daidzein, glycitein, formonetin, and biochanin A. Foods not found in the nutrient database were added by creating recipes for new mixed dishes or by the NCC, which bases nutrient values on information from food manufacturers, foreign food composition tables, the scientific literature, and other available databases.

In addition to providing estimates of nutrient intake, the software assigns each food item to 1 of 166 possible

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food subgroups and estimates serving counts for each food subgroup. Food items are counted at the whole food level when appropriate (e.g., bread, apple pie, and French fries) or at the component/ingredient level (e.g., lasagna, soup, fruit salad, and sandwiches) to capture intake of ingredients. Food subgroup definitions and serving sizes were based primarily on recommendations from the 2005 Dietary Guidelines for Americans (10) and the Food Guide Pyramid (11). U.S. Food and Drug Administration serving sizes (12) were used for foods not included among current recommendations, such as cookies and fruit drinks. Estimates of nutrient and food intake were averaged over the 4 days to obtain an estimate of mean intake for each participant.

Measurement of urinary equol

Urine samples were transported to the Fox Chase Cancer Center Protocol Support Laboratory Facility within 8 hours of collection, and then aliquoted and stored at −20°C until shipped to the laboratory of Dr. M. Kurzer at the University of Minnesota. Urinary phytoestrogens were analyzed in the Kurzer laboratory by an established liquid chromatography–mass spectrometry (LC–MS) method (13), following hydrolysis and ether extraction. Intra-assay variabilities were 6.2%, 2.7%, and 2.4%, and interassay variabilities were 16% for both equol and daidzein. To ensure sufficient concentration of the urine samples (>80 mg or 0.71 mmol creatinine/L), urinary creatinine concentrations were measured with a VITROS Clinical Chemistry analyzer (Ortho-Clinical Diagnostics) using freeze-dried reagent standards (Johnson & Johnson Clinical Diagnostics).

All women had detectable concentrations of genistein (minimum 4.3 ng/mL) and daidzein (minimum 5.1 ng/mL) in their urine, suggesting good compliance with the specified protocol. In additional analyses, we used a daidzein concentration cutoff point of <44 ng/mL to define noncompliance (7). Of 2 women identified as possible noncompliers using this criterion, only 1 was classified as a nonproducer of equol; excluding this participant had little effect on final estimates, and results presented include this participant. No participants had urinary creatinine concentrations <80 mg/L, indicating insufficiently concentrated urine.

Participants with urinary equol concentration of at least 30 ng/mL were considered to be producers. This cutoff point was selected on the basis of the frequency distribution of $\log_{10}$-transformed equol concentrations, which showed a bimodal distribution with a break at approximately $\log_{10}$ equol of 1.5 (~30 ng/mL; Fig. 1), excluding 38 women with concentrations that were less than the detectable limit (urine concentrations <0.47 ng/mL). We also compared results using alternative methods to define equol-producing status. First, a frequency distribution of $\log_{10}$-transformed equol:daidzein ratios (14) produced a bimodal distribution with a break at approximately −2.2, and classification of 224 women according to the two methods agreed in all but 2 women. Second, a frequency distribution of $\log_{10}$-transformed equol adjusted for creatinine (ng/mg creatinine) produced a bimodal distribution with a break at approximately 0.5, and classification according to this distribution agreed with classification based on a cutoff point of 30 ng/mL in all but 3 women. Results are presented using a cutoff point of 30 ng/mL equol concentration.

Assessment of breast density

For most participants ($n = 219$), processed and full-field digital images were directly available through use of digital mammography equipment at the Fox Chase

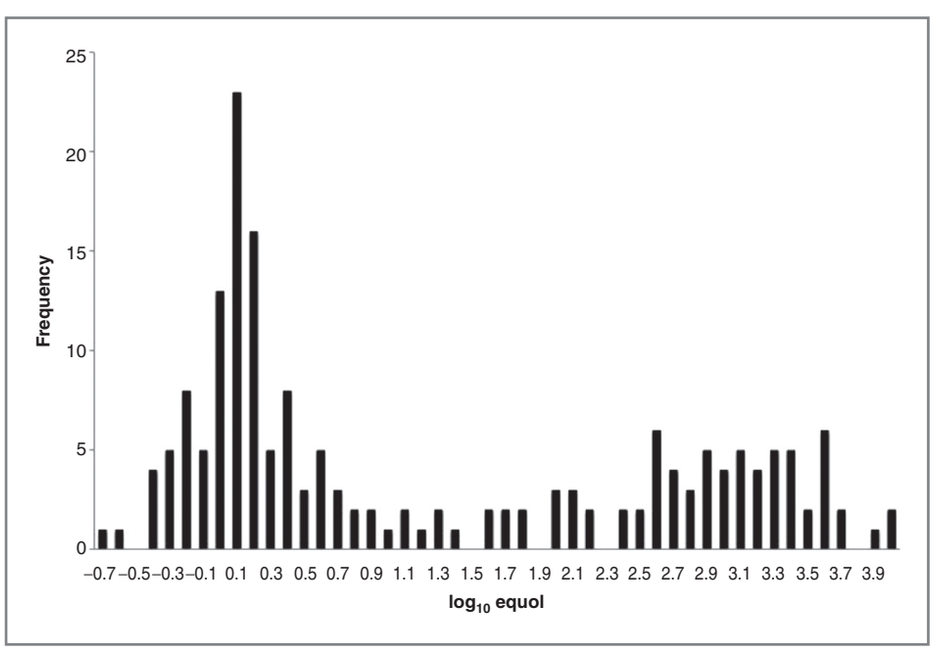

Figure 1. Frequency distribution for $\log_{10}$ equol concentrations in urine.

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Cancer Center. Breast density assessed from processed images has been shown to be similar to raw digital images in predicting breast cancer risk (15). For 5 participants whose mammograms were conducted on the Fox Chase Cancer Center mobile mammography unit or van, craniocaudal mammographic films were digitized using a Kodak LS-85 laser film scanner at a resolution of 100 pixels/cm. Breast density was assessed using a highly reproducible computer-assisted method (16–18). In 10% reproducibility samples conducted among baseline images for the cohort, intrabatch and interbatch intraclass correlation coefficients were all more than 0.94, indicating excellent reproducibility.

Statistical analyses

Equol producers and nonproducers were compared with respect to sociodemographic, reproductive, and dietary characteristics using *t* tests for continuous variables and χ^2 test statistics for categorical variables. Univariate analyses indicated that distributions for dense and nondense breast tissue areas (cm^2) and percent breast density were not highly skewed, and these were subsequently modeled, untransformed, in linear regression analyses with equol-producing status as the primary predictor of interest. All linear regression models were adjusted at a minimum, for age (years) and mammographic image modality (digitized film or digital). Variables were included in multivariate models as potential confounders if they were associated with at least one of the three outcomes of interest (dense and nondense tissue areas or percent density) and if removal from a model including all these variables changed the estimate for equol-producing status by more than 10%. Variables included in the fully adjusted model were body mass index (BMI; kg/m^2), waist circumference (cm), a combined, six-category variable representing number of live births (0–1, 2, and 3+) and age at first live birth (<25 or ≥25 years), total duration of breastfeeding (none, ≤1 year, >1–2 years, and >2 years), and level of education (≤8 years, 9–12 years or technical school, at least some college). The fully adjusted model included 221 participants, excluding 3 women missing data on at least one covariate. Other variables thought to be risk factors for breast cancer were evaluated as potential confounders but not included in fully adjusted models; these were age at menarche, having a first or second degree relative with breast cancer, having ever used oral contraceptives or hormones, menopausal stage, and level of acculturation. None of the participants reported having ever smoked, and alcohol use was also very low in the sample; thus, these were not evaluated further as potential confounders.

Isoflavone intake was both modeled in the full study sample and stratified on equol-producing status. Isoflavone intake was adjusted for energy intake using the residual method (19) and modeled as both a continuous variable (mg) and, because of its skewed distribution, as a categorical variable. Different categorization schemes used were quartiles, tertiles, quartiles with two middle categories collapsed, and dichotomizing at the median. We also cross-classified participants on the basis of equol-producing status and isoflavone intake dichotomized at the median. Fully adjusted models included the same covariates as earlier, but with additional adjustment for energy intake (continuous kcal). We estimated *P* values for the interaction between isoflavone intake and equol-producing status in models including all women, with a cross-product term representing either continuous or categorical energy-adjusted isoflavone intake × equol-producing status (yes/no). *P* values less than 0.05 were considered statistically significant. All statistical analyses were conducted using SAS (version 9.2, 2008; SAS Institute).

Results

Mean age of the study sample was 46.4 (SD 4.7) years and ranged from 36 to 58 years (Table 1). Most women were premenopausal at the time of the substudy, whereas 21% were perimenopausal and 10% had transitioned to menopause. The women had been in the United States for an average of 10.2 (SD 4.7) years. Although length of U.S. residence ranged from less than 1 to almost 24 years, level of acculturation was low overall; mean acculturation score was 2.2 out of a possible maximum score of 5.0. Most participants (72%) reported speaking no English at home. With respect to daidzein metabolism, 30% were classified as equol producers (urinary concentration ≥30 ng/mL). A comparison of equol producers and nonproducers showed no significant differences between groups with respect to sociodemographic characteristics or dietary intake (Table 1).

In linear regression analyses, women classified as equol producers had significantly lower mean dense tissue area and percent density than women classified as nonproducers (Table 2). In fully adjusted models, equol producers had a mean dense tissue area of 32.8 cm^2 and mean percent density of 31.8% compared with 37.7 cm^2 and 35.3%, respectively, in nonproducers. Equol-producing status was not associated with nondense breast tissue area in our sample. Results were similar when 5 participants with film images ($n = 3$ equol producers, $n = 2$ nonproducers) were excluded. In additional analyses, we explored whether equol excretion was associated with breast density in a dose–response manner. Nonproducers had the highest dense tissue area and percent density, but we did not see a linear decrease in breast density across categories of increasing urinary equol concentration (not shown).

In analyses examining the association of isoflavone intake with breast density measures, no significant or meaningful associations were evident in the full sample (Table 3). When we stratified on equol-producing status, isoflavone intake, modeled as a continuous variable, was significantly inversely associated with dense area and percent density in equol producers, but not in nonproducers (Table 3). The interaction between isoflavone intake and equol-producing status was statistically significant

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Table 1. Descriptive characteristics of study sample ($n = 224$)

	All participants ($n = 224$)	Equol nonproducer ($n = 149$)	Equol producer ($n = 75$)	P[a]
		Mean (SD)		
Age (y)	46.4 (4.7)	46.5 (4.6)	46.2 (5.0)	0.63
Length of U.S. residence (y)	10.2 (4.7)	10.4 (4.7)	9.8 (4.8)	0.34
General Ethnicity Questionnaire – American version score	2.2 (0.6)	2.2 (0.6)	2.2 (0.7)	0.38
BMI (kg/m^2)[b]	23.7 (2.9)	23.6 (3.0)	23.8 (2.9)	0.71
Age at menarche (y)	14.9 (1.6)	15.0 (1.5)	14.6 (1.8)	0.06
Number of live births	2.0 (1.0)	2.1 (0.9)	1.9 (1.0)	0.15
Age at first live birth (y)[b]	25.4 (4.8)	25.3 (4.7)	25.6 (5.2)	0.74
Diet				
Mean (SD) amount per day				
Energy (kcals)	1,478 (366)	1,481 (376)	1,472 (348)	0.87
Energy from fat (%)	25.1 (5.7)	24.8 (5.6)	25.8 (5.8)	0.19
Fiber (g)	13.7 (4.7)	13.7 (4.7)	13.8 (4.7)	0.93
Isoflavones (mg)	10.4 (16.5)	10.6 (16.6)	9.9 (16.4)	0.75
Mean (SD) servings/wk				
Red meat	11.8 (9.6)	11.7 (9.8)	11.9 (9.3)	0.87
Dairy	4.1 (5.0)	4.0 (5.0)	4.4 (5.1)	0.61
Fruit	10.1 (7.3)	10.7 (7.3)	8.9 (7.3)	0.08
Vegetables	25.0 (11.6)	25.3 (12.9)	24.3 (8.6)	0.54
Tofu	4.8 (9.1)	4.7 (8.4)	4.8 (10.5)	0.98
		%		
Education				0.14
≤8 y	53	56	48	
9–12 y/technical school	34	35	33	
At least some college	13	9	19	
Speak English at home				0.53
Not at all	72	72	72	
A little	20	21	17	
Somewhat, much, or very much	8	7	11	
First or second degree relative with breast cancer	0.9	0.7	1.3	0.62
Perimenopausal stage[b]				0.35
Premenopausal	69	69	69	
Early perimenopausal	15	16	12	
Late perimenopausal	6	7	4	
Postmenopausal	10	8	15	
Total duration of breastfeeding				0.40
None	20	19	23	
≤1 y	42	42	40	
>1–2 y	26	29	21	
>2 y	12	10	16	
Ever used oral contraceptives	12	11	13	0.57
Ever used female hormones	1.8	2.0	1.3	0.72

NOTE: Correlates of equol-producing status in sample of Chinese immigrant women ($n = 224$).

[a]P comparing equol producers and nonproducers using t tests for continuous variables and χ^2 test statistics for categorical variables.

[b]Because of missing values, sample size was $n = 223$ for BMI, $n = 217$ for age at first live birth, and $n = 221$ for perimenopausal stage.

with dense breast tissue area as the outcome (interaction $P = 0.046$). A similar pattern of findings emerged when we modeled isoflavone intake as a categorical variable, whether in quartiles, in tertiles, or dichotomous, although P values for interaction were not statistically significant; women who were equol producers and had higher intake

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Table 2. Mean (95% confidence interval) dense area, nondense area, and percent breast density by equol-producing status in a sample of Chinese immigrant women ($n = 221$)

	Nonproducer ($n = 147$)	Producer ($n = 74$)	*P*
Dense area (cm^2)			
Minimally adjusted[a]	**38.0 (31.1–44.9)**	**33.8 (26.8–40.8)**	**0.047**
Fully adjusted[b]	**37.7 (30.4–44.9)**	**32.8 (25.6–40.1)**	**0.03**
Nondense area (cm^2)			
Minimally adjusted	63.0 (51.2–74.9)	60.2 (48.2–72.2)	0.44
Fully adjusted	65.2 (54.9–75.5)	61.9 (51.7–72.2)	0.29
Percent density			
Minimally adjusted	36.6 (31.0–42.2)	33.3 (27.7–39.0)	0.06
Fully adjusted	**35.3 (29.9–40.7)**	**31.8 (26.4–37.2)**	**0.03**

[a]Linear regression models adjusted for age (y) and image modality (digital or film).

[b]Linear regression models adjusted for age (y), image modality (digital or film), education (≤8 y, 9–12 y, at least some college), total duration of breastfeeding (none, ≤1 y, >1–2 y, >2 y), BMI (kg/m^2), waist circumference (cm), and a combined variable representing number of live births (0–1, 2, 3+) and age at first live birth (<25 or ≥25 y).

of isoflavones had the lowest mean dense tissue area and percent density. As an example, Table 3 shows adjusted mean dense and nondense tissue areas and percent density by equol-producing status and isoflavone intake dichotomized at the median. Median isoflavone intake for women in the lower and higher intake categories was 0.16 and 14.3 mg/day, respectively. In these analyses, lower mean dense tissue area and percent density with higher isoflavone intake were apparent only among equol producers. Conversely, equol-producing status was associated with lower dense tissue area and percent density only among women with higher (above median) isoflavone intake, but not among women with lower isoflavone intake. Again, results were not meaningfully different when we excluded 5 women with film images.

Discussion

Two notable findings emerged from this study. First, women classified as equol producers in our sample had significantly lower mean dense tissue area and percent breast density than nonproducers. Second, an inverse association of isoflavone intake with breast density was evident only among equol producers, or conversely, equol-producing status was inversely associated with breast density only among women with higher isoflavone intake.

The 30% prevalence of equol production in this sample was lower than what we had expected. Previous studies estimated prevalence of 40% to 60% in Asian samples, compared with 20% to 40% in Caucasians (3, 20). Although other studies noted differences in the macronutrient and fiber composition of the diet between equol producers and nonproducers (21), we saw no differences between the two groups with respect to any sociodemographic characteristics or dietary variables that we examined, including overall level of acculturation. Overall, the extent to which equol-producing status is inducible remains unclear, and correlates of equol-producing status have not been consistently identified.

With respect to the association between equol production and mammographic density, our findings were similar to those of a previous study (7) of 55 participants, in which women identified as equol producers had 39% lower mammographic density than nonproducers when comparing geometric means. Another study showed no association between equol production and mammographic density (7, 8, 22), and studies on equol and breast cancer are inconsistent (23–30). In our study, (arithmetic) mean percent density was 10% lower than the mean in nonproducers, with an absolute difference of 3.5%. The clinical significance of a difference of 3.5% in percent density or a 4.9-cm^2 difference in dense breast tissue area on a mammogram (37.7 vs. 32.8 cm^2) is unclear. However, existing evidence consistently shows a trend of increasing breast cancer risk with increasing breast density (31), and an ecologic analysis showed a strong linear correlation ($r = 0.93$) between age-adjusted dense tissue area and breast cancer incidence across 7 populations with mean dense areas ranging from 28.3 to 42.8 cm^2 (32).

Equol seems to have higher estrogenic and antioxidant activity than its precursor, and a greater proportion of it circulates in free or unbound form (3). Associations attributed to equol could also be due to genetic or other factors that determine equol-producing status, although we were unable to identify any factors to distinguish equol producers. Equol-producing status might also be a phenotypic marker of the activity of daidzein-metabolizing intestinal bacteria (21, 33). The fact that equol concentration was not associated with breast density in a dose–response manner suggests that the effect is not due to equol itself but to another attribute or due to other unmeasured factors related to the intestinal bacterial profile that produces a daidzein-metabolizing phenotype (33).

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Table 3. Adjusted[a] β-coefficients for continuous, energy-adjusted isoflavone intake (mg) and adjusted means by isoflavone intake category[b] for dense area, nondense area, and percent breast density, in all participants and stratified on equol-producing status among Chinese immigrant women ($n = 221$)

	All participants ($n = 221$)	Equol nonproducer ($n = 147$)		Equol producer ($n = 74$)	Interaction *P*
Dense area (cm^2)					
β (SE)	−0.01 (0.06)	0.06 (0.08)		**−0.24 (0.10)**	**0.046**
P	0.81	0.45		**0.02**	
Mean (95% CI)					
Low isoflavone intake	35.8 (28.3–43.3)	37.7 (29.8–45.5)		35.5 (27.4–43.6)	0.18
High isoflavone intake	35.0 (27.8–42.1)	38.3 (30.8–45.9)		30.4 (22.4–38.4)	
P	0.68		0.07[c]		
Nondense area (cm^2)					
β (SE)	0.09 (0.09)	0.06 (0.11)		0.16 (0.14)	0.67
P	0.33	0.62		0.28	
Mean (95% CI)					
Low isoflavone intake	62.6 (52.2–73.1)	65.4 (54.4–76.6)		58.8 (47.3–70.3)	0.21
High isoflavone intake	64.2 (54.1–74.2)	64.3 (53.6–75.0)		65.2 (53.9–76.6)	
P	0.59		0.41		
Percent density					
β (SE)	−0.03 (0.05)	0.03 (0.06)		**−0.22 (0.09)**	0.05
P	0.47	0.65		**0.02**	
Mean (95% CI)					
Low isoflavone intake	34.2 (28.6–39.8)	35.3 (29.5–41.1)		34.5 (28.5–40.5)	0.07
High isoflavone intake	33.2 (27.8–38.5)	35.9 (30.3–41.5)		29.3 (23.4–35.2)	
P	0.51		**0.04**		

[a]Linear regression models adjusted for age, image modality (digital or film), energy intake (kcal), education category, months of breastfeeding, BMI, waist circumference, and a combined variable representing number of live births and age at first live birth.
[b]Energy-adjusted isoflavone intake dichotomized at median.
[c]Represents *P* for four-category variables representing cross-classification by equol-producing status and dichotomized isoflavone intake.

Other findings in our sample, however, support the opposite view. If the effect of equol were due to a daidzein-metabolizing phenotype, then we would expect to see an association between equol-producing status and breast density regardless of the level of isoflavone intake. Instead, we observed little difference in breast density among low consumers and a substantial difference with greater isoflavone intake. In other words, equol producers seemed to have lower breast density only in the presence of isoflavones. Conversely, soy isoflavones had no effect on breast density except among women with the ability to metabolize daidzein to equol. These results are consistent with findings of Fuhrman and colleagues, who found that soy intake was associated with lower percent density only among equol producers (8).

Evidence that any physiologic effects of soy are dependent on daidzein-metabolizing status has important implications for prevention. Three cross-sectional studies, all conducted among Asian women, suggest an inverse association between soy intake and breast density (34–36), but others, including studies in Asian populations, have shown no association (37–41). Furthermore, despite strong evidence for anticarcinogenic properties of genistein from *in vitro* and animal studies (1, 2), interventions lasting 6 months to 3 years show no effect on breast density overall, and even a possible increase among premenopausal women (42). In the only one of these intervention studies that stratified on equol-producing status, Verheus and colleagues (43) found no effect of a 1-year, 99 mg/day isoflavone supplement on mammographic density and no difference in effect by equol-producing status. The analysis, however, was based on only 126 of the 175 participants originally enrolled in the study. Stratified analyses were further limited to 108 participants whose equol-producing status was assessed, and power to detect a difference in effect might have been limited. As a next step to clarify any potential effects of isoflavones on breast cancer risk, future studies, whether observational or isoflavone/soy intervention studies, should assess equol-producing status and be designed with adequate power to detect an interaction. Ideally, such studies should also assess equol-producing status at different time points given the potential mutability of equol-producing status over time (44, 45). If an effect of

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isoflavones depends on equol-producing status, then further studies on determinants of equol-producing status and appropriate targeting of interventions are warranted.

A limitation of the current study is its relatively small sample size, although our sample size is comparable with those of previous studies on this topic that used a soy challenge and urine sample to characterize equol-producing status. The size of our sample further allowed for the collection of detailed, quantitative dietary data. Misclassification of equol-producing status is also possible. We used different methods to determine equol-producing status, including different cutoff points for equol excretion and the ratio of equol to daidzein, and considering the sufficiency of daidzein and creatinine concentrations in the urine samples collected. Recent work shows that equol-producing status might change over time in some women (44, 45). Such misclassification should attenuate estimates of association between equol-producing status and breast density, however, unless there is reason to expect differential misclassification of equol-producing status depending on breast-density measures. Finally, also unresolved from this study is whether the effect for equol is due to equol itself, whether it is due to unmeasured confounders such as those occurring earlier in life, or whether equol is a marker for a different factor of physiologic importance such as intestinal bacteria.

Our study provides further support for the possibility that an effect of soy isoflavones on mammographic density is dependent on a person's ability to metabolize daidzein to equol. It also provides evidence of an association between equol-producing status and lower mammographic density. Given the implications of this for understanding the effect of soy isoflavones on breast cancer risk, replication of these results is warranted, in Asian as well as non-Asian samples and in experimental settings.

Disclosure of Potential Conflicts of Interest

M.S. Kurzer is a consultant/advisory board member of the Soy Nutrition Institute. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Tseng, C. Byrne
Development of methodology: M. Tseng, C. Byrne, M.S. Kurzer
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Byrne, C.Y. Fang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Tseng, C. Byrne, M.S. Kurzer
Writing, review, and/or revision of the manuscript: M. Tseng, C. Byrne, M.S. Kurzer, C.Y. Fang
Study supervision: M. Tseng, C.Y. Fang

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