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Mammographic Breast Density and Serum Phytoestrogen Levels

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Abstract

Some forms of estrogen are associated with breast cancer risk as well as with mammographic density (MD), a strong marker of breast cancer risk. Whether phytoestrogen intake affects breast density, however, remains unclear. We evaluated the association between serum levels of phytoestrogens and MD in postmenopausal women. We enrolled 269 women, ages 55–70 yr, who received a screening mammogram and had no history of postmenopausal hormone use. Subjects completed a survey on diet and factors related to MD and provided a blood sample for analysis of 3 phytoestrogens: genistein, daidzein, and coumestrol. We examined whether mean percent MD was related to serum level of phytoestrogens, adjusting for age and body mass index. Genistein and daidzein levels correlated with self-reported soy consumption. Mean percent MD did not differ across women with different phytoestrogen levels. For example, women with nondetectable genistein levels had mean density of 11.0% [95% confidence intervals (CI) = 9.9–12.4], compared to 10.5% (95% CI = 8.0–13.7) and 11.2% (95% CI = 8.7–14.6) for < and median detectable levels, respectively. In a population with relatively low soy intake, serum phytoestrogens were not associated with mammographic density. Additional studies are needed to determine effects of higher levels, particularly given patterns of increasing phytoestrogen intake.

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Notes:

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Keywords

phytoestrogen; isoflavone; mammographic density; breast cancer

INTRODUCTION

Mammographic density (MD) is a strong marker of breast cancer risk. Women with 75% MD are nearly 5 times as likely to develop breast cancer as women with <5% MD, according to a recent meta-analysis [relative risk (RR) = 4.6, 95% confidence intervals (CI) = 3.6–5.9] (1). Percent MD is the proportion of the total breast area on a mammogram that appears light and is thought to represent dense, fibroglandular tissue (in contrast with fat tissue, which appears dark) (2). It is not clear why MD is associated with breast cancer risk, but MD may reflect cumulative exposure to estrogens and other hormones which may increase both MD and breast cancer risk (3). There is evidence that estrogen hormone therapy may increase MD, either when combined with progesterone (4) or alone (5), but it is unclear whether other estrogenic compounds, including dietary phytoestrogens, influence MD.

Isoflavones, a type of phytoestrogen, are consumed in relatively high amounts in Asian countries (25–50 mg/day) (6), primarily in foods made from minimally processed soy (7). In Western populations, isoflavone intake is much lower (<1–3 mg/day) (6), and major dietary sources include legumes, certain vegetables, and processed foods (8–10) that contain purified or extracted soy (7). Isoflavones, including genistein and daidzein, have been shown to exhibit both estrogenic and antiestrogenic properties (11). Genistein appears to bind to both the alpha and beta isoforms of the estrogen receptor (ER α and ER β). ER α promotes epithelial proliferation in the breast, and ER β inhibits the action of ER α (12). Coumestrol, another type of phytoestrogen, is primarily found in clover and alfalfa sprouts and legumes (13). Though less well studied, coumestrol has also been shown to have potent estrogenic activity (14,15).

Cross-sectional studies of self-reported isoflavone intake and MD have yielded mixed findings (16) that may be explained by differences in subjects' age, menopausal status, geography, culture, and race, and differences in phytoestrogen dose (16), lifetime patterns of intake (17), and level of processing (which may be positively associated with the degree of estrogenicity) (7,10). Self-reported intake does not account for differences in metabolism, which could influence the effective biological dose; it also fails to capture phytoestrogen exposure via foods not known to contain added soy, which would likely constitute a particularly high proportion of the total exposure in a population that does not knowingly consume many soy products (20). These studies are also unable to account for differences in isoflavone content due to seasonal variations in isoflavone production in the plant (18).

Eight randomized trials of the effect of phytoestrogen ingestion on change in MD have also yielded inconclusive results (16). Although these studies generally did not observe statistically significant differences between the intervention and control arms, half observed that phytoestrogen intake was associated with a slower decrease in MD over time with aging. The remaining studies reported either a positive association between phytoestrogen

intake and MD, different associations by subgroup, or no difference between intervention and control arms. These studies used varying forms and amounts of isoflavones for the intervention, and enrolled different populations (16). Most trials in postmenopausal women did not assess the effects of other phytoestrogens such as coumestrol (19).

The present study is of interest because the study subjects are likely representative of postmenopausal women with typical (low-soy) Western diets, and because measurement of serum phytoestrogen levels may provide complementary information to previous studies of self-reported phytoestrogen intake. No previous studies have directly examined serum phytoestrogen concentrations in relation to MD. We examined the relationship between serum measurements of 3 common phytoestrogens (genistein, daidzein, and coumestrol) and MD in a population of healthy postmenopausal women.

METHODS

Setting and Population

The study setting and population recruitment methods have been described previously (21). Study methods were approved by the University of Wisconsin Health Sciences Institutional Review Board. Briefly, invitation letters for this study were mailed to women aged 55–70 yr who were due for a routine screening mammogram at 2 clinic sites in Madison, Wisconsin. Women were excluded if they were not postmenopausal (defined as 1 menstrual cycle within the past 12 mo), had ever used postmenopausal hormones or tamoxifen, had breast implants, or had a personal history of breast cancer. A total of 269 women were recruited between June 2008 and July 2009.

Questionnaire

After undergoing a mammogram and providing informed consent to participate in the study, subjects provided a blood sample and completed a survey on diet as well as factors potentially related to MD, including age, weight, height, smoking status, parity, physical activity, and first- and second-degree family history of breast cancer. To assess self-reported phytoestrogen (primarily isoflavone) intake, we asked subjects, “On average, how often do you eat a serving of soy products (tofu, soymilk, etc.)?” We asked them to choose from the following responses: “Never or <1 serving/month,” “1–3 servings/month,” “1 serving/week,” “2–4 servings/week,” “5–6 servings/week,” “1 serving/day,” “2–3 servings/day,” or “4+ servings/day.” We calculated a range for the mean self-reported intake of soy foods by summing the lowest value of each possible response (e.g., 2/wk, for the response, “2–3 servings/week,”) multiplied by the proportion of women who provided each response (e.g., 6.3%), and then repeating for the highest possible value for each response.

Blood Analyses

A 3 0-ml whole blood sample was collected via venipuncture in 3 uncoated glass 10-ml red top vacutainer tubes (no anticoagulant; Fisher Scientific, Pittsburgh, PA). Blood samples were allowed to clot for 30 min and then spun down in a centrifuge for 20 min at 2,500 rpm. Serum was aliquoted in 2-ml aliquots and frozen at –70°C. Phytoestrogen levels (genistein, daidzein, and coumestrol) were evaluated using solid phase extraction (Strata-X;

Phenomenex, Torrance, CA) (22) and isotope dilution high-performance liquid chromatography (Agilent 1100; Agilent Technologies, Waldbronn, Germany) with tandem mass spectrometry (API4000; AB/SCIEX, Framingham, MA) with APCI negative ionization (23,24). We selected phytoestrogens that were expected to be common exposures with the highest estrogenic potency and which could be accurately measured. Analytical quality assurance parameters included reagent and method blanks, calibration check standards, and double charcoal-treated human serum matrix control spikes at low (1 ng/ml) and mid (5 and 10 ng/ml) calibration curve levels. Lower limits of detection were based on observed 3:1 signal-to-noise ratios. Analytical quality assurance results were as follows: blanks were nondetected for all analytes; correlation coefficients for calibration curves were all greater than 0.9939; mean (SD) results ($n = 31$) for calibration checks were 102% (7.4), 102% (5.0), and 102% (6.9) for genistein, daidzein, and coumestrol, respectively. Mean (SD) results for low matrix spikes ($n = 12$) were 94.5% (13.1), 99.3% (11.3), and 110% (17.2), and for mid-curve level matrix spikes ($n = 31$) they were 101% (6.0), 102% (4.2), and 101% (7.4) for genistein, daidzein, and coumestrol, respectively.

Mammographic Density (MD)

Subjects underwent routine screening mammograms, carried out on either a Clearview CSm2 CR (Fujifilm Corporation, Tokyo, Japan) or a Senographe 2000D (GE Medical Systems, Chalfont St Giles, Buckinghamshire, United Kingdom) machine. Cumulus software was used to measure dense area and total breast area of digitized left-breast craniocaudal mammograms by computer-assisted thresholding (25). We calculated percent MD as dense area divided by total area. All density measurements were performed by the same reader (Erin J. Aiello Bowles). Ten percent of mammograms were reread for quality-control purposes. Reliability was high, with an intraclass correlation coefficient of 0.98 for percent MD, and a lower confidence limit of 0.96.

Statistical Analyses

Five women were missing phytoestrogen measurements and were excluded, leaving a total of 264 subjects for analysis. Percent MD and phytoestrogen levels were log-transformed to improve the normality of the data. Spearman correlation coefficients were calculated to describe the associations between phytoestrogens and other factors. We used linear regression to estimate mean percent MD adjusted for age (continuous) and body mass index (BMI) for 3 phytoestrogen exposure categories (nondetectable, below and above the median among detectable levels). The detection limits for genistein, daidzein, and coumestrol were 0.0031, 0.0084, and 0.0985 ng/ml, respectively. Age and BMI were selected a priori as potential confounders because they are known to be associated with MD and potentially with serum phytoestrogen levels. Inclusion of additional potential confounding factors in the model (including soy intake and physical activity) did not substantially change the results. Test for trends were conducted by the inclusion of an ordinal term representing the category of phytoestrogen level (nondetectable, below and above the median detectable level). All analyses were performed using Stata 11 (StataCorp, College Station, TX).

RESULTS

The average age of study subjects was 60.6 yr (SD = 4.4). Approximately 31% were overweight (BMI between 25–30 kg/m²) and 36% were obese (BMI ≥ 30 kg/m²) (Table 1). Mean percent MD was 15.6% (SD = 13.1, median = 10.9, range = 0.4–71.2). The mean intake of soy based on self-reported levels was between 5.3 and 7.8 servings of soy foods per month. Sixty-four percent of participants reported consuming soy foods “never or <1 serving per month” (Table 1), whereas only 10% of women reported consuming soy products every day (not shown).

Phytoestrogen serum levels were low, with most women having nondetectable levels (Table 1). Genistein and daidzein levels were positively correlated with self-reported soy consumption. Of women reporting <1 serving of soy per month, 19% had detectable serum levels of genistein vs. 56% of women who reported 5 or more servings per wk. Women who consumed <1 serving of soy per mo were less likely to have detectable levels of daidzein than those reporting >4 servings per wk (51% vs. 78%). This trend was not observed for coumestrol (22% vs. 21%; Table 1).

The proportion of women with detectable phytoestrogen levels decreased with increasing BMI; for coumestrol, this proportion also decreased with older age and with lower physical activity levels (Table 1). Median detectable serum levels did not differ by age, BMI, or physical activity (Table 1). Daidzein levels were positively correlated with genistein and coumestrol (Spearman $r = 0.40$ and 0.21 , respectively), and genistein and daidzein levels were positively correlated with self-reported habitual soy consumption ($r = 0.30$ and 0.28 , respectively).

After adjusting for age and BMI, the mean percent MD of women with the highest phytoestrogen levels differed by less than 1 percentage point from that of women with nondetectable levels, for each phytoestrogen (Table 2). For example, women with nondetectable serum levels of genistein had a mean density of 11.0% (95% CI = 9.9–12.4), compared to 10.5% (95% CI = 8.0–13.7) and 11.2% (95% CI = 8.7–14.6) for women below and above median detectable levels, respectively. Similarly, there were no statistically significant differences in mean MD between women with detectable vs. nondetectable serum phytoestrogen levels. Finally, no trends were detected in MD according to increasing category of phytoestrogen exposure (all P values for trend > 0.6).

DISCUSSION

Serum phytoestrogens were not associated with MD in a sample of healthy postmenopausal women recruited at breast screening mammography clinics in Wisconsin. The levels of self-reported soy intake observed in this study were comparable to other studies of women with Western diets (26). The mean amount of isoflavones consumed per day can be estimated by considering that the mean daily intake of soy foods reported fell between 0.18 and 0.26 servings per day, based on the lower and upper bounds of each categorical response, respectively, and the proportion of women who selected each response. Assuming 112 g of “soy food” in a typical 4 oz serving of tofu (27) yields a range of 20–29 g of soy food per

day, which is approximately equivalent to 4–6 mg isoflavones based on conversions used in a previous study (16). This is comparable to the average isoflavone intake in white women in Hawaii in 2001 (4 mg/day) (26). However, the distribution of self-reported soy intake was skewed and over half of women reported <1 serving per mo.

Our interest in the potential effect of phytoestrogens on MD is based in part on the potential for phytoestrogens to influence breast cancer risk. Evidence on this question has been inconsistent. A pooled analysis of 18 observational studies suggested that higher soy intake was associated with a modest decrease in breast cancer risk (OR = 0.86, 95% CI = 0.75–0.99) (28). A second meta-analysis, restricted to 8 studies that more thoroughly assessed phytoestrogen exposure and potential confounders, reported an inverse association in Asians (OR = 0.71, 95% CI = 0.60–0.85, for 20 mg isoflavones/day vs. <5 mg) and no association in low-soy-consuming Western populations, overall or when limited to postmenopausal women (29). Inconsistencies may reflect differences in the amount, timing, and/or metabolism of soy intake across different populations (30).

Our study had several limitations. The low levels of soy intake in our study limit the extrapolation of our results to populations with higher soy consumption. A high percentage of women had nondetectable phytoestrogen levels. However, for each phytoestrogen, at least 65 women had detectable levels, which allowed us to assess potential trends among such women (Table 2), who are likely to be representative of a large portion of postmenopausal women with Western diets. We asked only 1 question about dietary phytoestrogen intake. By measuring serum levels directly we captured all sources of recent exposure to the 3 measured phytoestrogens and incorporated interindividual variation in phytoestrogen metabolism. We were unable to assess interpersonal differences in ability to produce equol, a more biologically active metabolite of daidzein associated with a lower risk of some cancers (31,32). One study observed that neither dietary soy nor equol producer status was independently associated with MD, but there was a statistically significant interaction between these 2 factors (33). Among equol producers, those who consumed soy products weekly had a lower percent MD, whereas among equol nonproducers, percent MD was higher for weekly soy consumption. Another study observed that among overweight, postmenopausal women, percent MD was relatively 39% lower in equol producers compared to nonproducers though the absolute difference in percent MD was only 1.6% (34). Notably, we would not expect a high proportion of equol users in our study population, as they tend to be more frequent in Asian Americans than Caucasian Americans (51% vs. 36%) (35), and vegetarians than nonvegetarians (59% vs. 25%) (36).

We also cannot address the effects of early-life patterns of exposures to phytoestrogens (30). Serum phytoestrogen levels reflect recent intake, whereas the survey question on soy consumption referred to habitual intake. However, serum levels have previously been shown to correlate with dietary intake (37,38) and we did observe modest correlations between self-reported habitual soy intake and serum isoflavones (except coumestrol, which is derived primarily from other food sources).

Our study suggests that at the levels of phytoestrogen intake typical of the Western diet and Caucasian postmenopausal women in the United States, serum phytoestrogen levels do not

appear to influence MD. However, phytoestrogen intake among postmenopausal women in the United States is increasing, both as a substitute for postmenopausal hormone therapy and for other health and dietary reasons (39,40). We were unable to assess the relationship at higher serum levels of phytoestrogens, such as those that might be observed with traditional Asian diets, or with regular use of phytoestrogen supplements. Future studies may benefit from targeting women with higher levels of phytoestrogen intake or evaluating phytoestrogen exposure levels in conjunction with equol producer status.

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Table 1

Characteristics of the study population, Wisconsin Breast Density Study, 2008–2009

	Genistein			Daidzein		Coumestrol	
	N (%)	Percent detectable (%)	Median of detectable, ng/ml (95% CI)	Percent detectable (%)	Median of detectable, ng/ml (95% CI)	Percent detectable (%)	Median of detectable, ng/ml (95% CI)
Overall	264 (100)	27	0.33 (0.29-0.38)	54	0.37 (0.32-0.45)	24	0.29 (0.25-0.32)
Age (years)							
55-59	139 (53)	27	0.33 (0.24-0.47)	54	0.38 (0.32-0.48)	26	0.30 (0.21-0.33)
60-64	72 (27)	27	0.36 (0.29-0.58)	55	0.41 (0.24-0.50)	23	0.30 (0.20-0.47)
65-70	53 (20)	26	0.30 (0.23-0.48)	54	0.36 (0.25-0.46)	20	0.25 (0.17-0.43)
BMI (kg/m²)							
<25	85 (32)	31	0.28 (0.19-0.51)	58	0.43 (0.30-0.59)	29	0.24 (0.16-0.29)
25-30	81 (31)	27	0.33 (0.21-0.52)	55	0.34 (0.28-0.46)	22	0.37 (0.23-0.67)
>30	96 (36)	24	0.37 (0.32-0.69)	51	0.35 (0.29-0.48)	22	0.33 (0.24-0.51)
Missing	2 (1)						
Dietary intake of soy products (number of servings per month)							
<1	168 (64)	19	0.31 (0.24-0.36)	51	0.30 (0.25-0.36)	21	0.26 (0.20-0.33)
1 - 16	63 (24)	34	0.29 (0.23-0.45)	53	0.35 (0.29-0.48)	32	0.30 (0.26-0.50)
>16	32 (12)	56	0.55 (0.31-0.86)	78	0.75 (0.60-1.46)	22	0.17 (0.12-0.78)
Missing	1 (0.4)						
Smoked 100 cigarettes							
Yes	105 (40)	31	0.32 (0.28-0.47)	52	0.32 (0.28-0.43)	22	0.33 (0.19-0.56)
No	159 (60)	25	0.35 (0.24-0.48)	56	0.41 (0.33-0.48)	26	0.28 (0.24-0.30)
First-degree family history of breast cancer							
No	148 (56)	26	0.34 (0.25-0.45)	53	0.34 (0.30-0.45)	24	0.30 (0.25-0.33)
Yes	112 (42)	27	0.34 (0.26-0.57)	58	0.42 (0.33-0.49)	25	0.25 (0.16-0.41)
Unknown	4 (2)						
Parity							
Nulliparous	67 (25)	28	0.34 (0.22-0.70)	52	0.43 (0.31-0.52)	21	0.27 (0.19-0.52)
Parous	197 (75)	27	0.32 (0.29-0.38)	55	0.34 (0.30-0.45)	25	0.30 (0.25-0.33)
Vigorous physical activity (hours/week)							
1	79 (30)	29	0.48 (0.31-0.71)	54	0.38 (0.29-0.49)	12	0.22 (0.15-0.32)
>1 to 4	91 (34)	24	0.33 (0.25-0.45)	54	0.39 (0.27-0.48)	26	0.32 (0.26-0.50)
>4	94 (36)	28	0.29 (0.19-0.39)	54	0.35 (0.30-0.55)	32	0.26 (0.18-0.39)

Percents may not sum to 100 due to rounding

Table 2

Percent mammographic density by serum level of phytoestrogens.

	N	Percent Density: Geometric Mean (95% CI) ^c	P-value for trend
Genistein ^a			
Non-detectable level (<0.0031 ng/ml) ^b	191	11.0 (9.9-12.4)	
Detectable level (0.0031 ng/ml)	73	10.9 (9.0-13.1)	
<Median of detectable levels (0.0031 – 0.326 ng/ml)	36	10.5 (8.0-13.7)	0.99
Median of detectable levels (0.326 – 1.74 ng/ml)	37	11.2 (8.7-14.6)	
Daidzein ^a			
Non-detectable level (<0.0084 ng/ml) ^b	118	11.5 (9.9-13.3)	
Detectable level (0.0084 ng/ml)	146	10.6 (9.3-12.1)	
<Median of detectable levels (0.0084 – 0.3675 ng/ml)	73	10.3 (8.6-12.4)	0.62
Median of detectable levels (0.3675 – 4.36 ng/ml)	73	10.9 (9.1-13.1)	
Coumestrol ^a			
Non-detectable level (<0.0985 ng/ml) ^b	199	10.8 (9.6-12.0)	
Detectable level (0.0985 ng/ml)	65	11.7 (9.6-14.2)	
<Median of detectable levels (0.0985 – 0.287 ng/ml)	32	13.4 (10.2-17.7)	0.87
Median of detectable levels (0.287 – 1.21 ng/ml)	33	10.2 (7.8-13.4)	

^aWe had 83%, 92%, and 85% power to detect a 5% difference in mean percent mammographic density (MD) between women with detectable vs. non-detectable levels of genistein, daidzein, and coumestrol, respectively, based on group size and variation in percent MD.

^bSerum levels were below the level of detection, which is specified for each phytoestrogen.

^cAdjusted for age and BMI.