

Mammographic Density in Relation to Daidzein-Metabolizing Phenotypes in Overweight, Postmenopausal Women

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Abstract

Circulating hormones are associated with mammographic density, an intermediate marker of breast cancer risk. Differences in circulating hormones, including estrone and testosterone, have been observed in premenopausal women based on their capacity to metabolize daidzein, an isoflavone found predominantly in soybeans. Equol and *O*-desmethylangolensin (*O*-DMA) are products of intestinal bacterial metabolism of daidzein. There is interindividual variability in the capacity to produce daidzein metabolites; individuals can be equol producers or non-producers and *O*-DMA producers or non-producers. We tested the hypothesis that daidzein-metabolizing phenotypes are associated with mammographic density. Participants were recruited from among 92 sedentary, postmenopausal women, ages 50 to 75 years, who participated in a 1-year physical activity intervention. Pre-intervention mammographic density was determined using a computer-assisted, gray-scale thresholding technique. Fifty-five of these women consumed supplemental soy

protein (>10 mg daidzein/d) for 3 days and collected a first-void urine sample on the fourth day to determine daidzein-metabolizing phenotypes. Equol and *O*-DMA concentrations were measured using gas chromatography-mass spectrometry. Associations between daidzein-metabolizing phenotypes and percent mammographic density were adjusted for age, maximum adult weight, gravidity, family history of breast cancer, and serum follicle-stimulating hormone and free testosterone concentrations. Mammographic density was 39% lower in equol producers compared with non-producers ($P = 0.04$). *O*-DMA producers had mammographic density 69% greater than non-producers ($P = 0.05$). These results suggest that particular intestinal bacterial profiles are associated with postmenopausal mammographic density, and these associations are not entirely explained by differences in reproductive or anthropometric characteristics or circulating hormones. (Cancer Epidemiol Biomarkers Prev 2004;13(7):1156–62)

Introduction

Mammographic density has been positively associated with breast cancer risk (1-3). Although the mechanism underlying the relationship between mammographic density and risk of breast cancer has not been fully explained, several hormone-related factors have been associated with mammographic density changes, including decreased density with contraceptive hormone use (4) and use of the anti-estrogen tamoxifen (5-7), and increased density with non-contraceptive exogenous sex hormone use (8-10). Mammographic tissue has been observed to be less radiologically dense during the follicular phase of the menstrual cycle (11), a period characterized by increasing estrogen secretion (12). In postmenopausal

women, percent mammographic density was positively associated with sex hormone binding globulin and progesterone and inversely associated with free estradiol (13). Nulliparous women and women with a later age at first birth were observed to have denser breasts than women who had children at a young age (14-19), a difference found even among postmenopausal women (14).

Intestinal bacteria are important in the metabolism of reproductive hormones (20), but whether particular bacterial profiles are associated with mammographic density has not been studied. Particular intestinal bacteria are capable of metabolizing the soy isoflavone daidzein to equol or *O*-desmethylangolensin (*O*-DMA). Humans are considered equol producers if they harbor bacteria capable of metabolizing daidzein to equol, and similarly *O*-DMA producers are individuals who harbor bacteria that can metabolize daidzein to *O*-DMA. Although the intestinal bacteria that can metabolize daidzein to equol or to *O*-DMA have not yet been identified, the presence or absence of these metabolites in urine can serve as markers of particular intestinal bacteria. Animal studies suggest that equol-producing capacity is present in all members of other mammalian species (21-23). In contrast, the prevalence of equol

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producers is approximately 30% to 50% in human populations (24-27). The prevalence of *O*-DMA producers is approximately 80% to 90% among humans (24, 28). Results from a study of premenopausal women suggest that equol-producing women have lower circulating concentrations of estrogens and androgens (29). On the basis of these observations, we tested the hypothesis that percent mammographic density, a hormone-associated intermediate marker of breast cancer risk, is influenced by the equol-producer phenotype. Although relationship between steroid hormones and the *O*-DMA-producer phenotype has not been reported, we also examined the association between mammographic density and *O*-DMA-producer phenotype.

Materials and Methods

Subjects and Experimental Design. Participants were recruited from women who participated in the Physical Activity for Total Health Study (parent study), a study designed to examine the effect of adopting a moderate intensity exercise program (compared with a stretching program) on the reproductive hormone profile in overweight [body mass index (BMI) ≥ 25 or BMI between 24 and 25 if body fat $>33\%$], sedentary, postmenopausal [having no menstrual periods in prior year or follicle-stimulating hormone (FSH) > 30 mIU/mL for women who had hysterectomy] women. Details about study procedures and inclusion and exclusion criteria are published elsewhere (30). Briefly, women eligible for participation in the parent study were non-smokers; moderate or non-drinkers (two or fewer alcoholic drinks per day); physically able to undertake a moderate physical activity program; not using any exogenous hormone medications; free from diabetes mellitus or significant endocrine abnormalities; and free of prevalent cancers. Before randomization, data about demographics and reproductive history information were collected via self-administered questionnaire, and urine and blood samples were obtained.

Of the 173 women who participated in the parent study, 92 women participated in an ancillary mammographic density study; of these 92 women, 34 women did not participate in the present study (daidzein-metabolizing phenotyping) for the following reasons: unable to locate ($n = 10$), consented but did not complete study ($n = 4$), indicated willingness to participate during telephone call but did not return consent ($n = 5$), lack of interest ($n = 5$), and declined but no reason given ($n = 10$). Fifty-eight women completed the daidzein-metabolizing phenotyping.

Mammographic density was determined from screening mammograms taken in the year before randomization ($n = 42$) or within 1 month post-randomization ($n = 16$) in the parent study. An additional exclusion criterion for the mammographic density study was hormone use in the year before randomization in the parent study. There was an approximately 2 to 3 years time difference between baseline data collection (including mammographic density) for the parent study and collection of urine for daidzein-metabolizing phenotyping. Exclusion criteria for participation in the equol/*O*-DMA-producer phenotyping were known allergy to soy and chronic antibiotic

therapy. Women who were taking acute antibiotic therapy were scheduled for phenotyping 3 months after the completion of antibiotic therapy. The Institutional Review Board at the Fred Hutchinson Cancer Research Center approved all procedures and written, informed consent was obtained from all participants.

Study Procedures. Women were mailed three soy protein bars and a urine collection kit. On each of three consecutive days, each woman supplemented her usual diet with either one soy protein bar (Revival Soy, Physicians Laboratories, Winston-Salem, NC, ~ 83 mg daidzein per day) or one third of a package of soy nuts (GeniSoy, Fairfield, CA, ~ 10 mg daidzein per day). On the morning of the fourth day, the woman collected a sample (50 to 80 mL) of first-void urine, and mailed the sample to the Fred Hutchinson Cancer Research Center. Urinary equol and *O*-DMA concentrations are stable at room temperature for 14 days.⁷

Mammographic Density. Mammographic images were digitized for percent density, using a computerized interactive density thresholding technique with a graphics overlay (31, 32). One trained technician (E.A.), blinded to daidzein-metabolizing phenotypes, determined mammographic density for all women in the parent study. A 10% random, blinded sample of mammographic images was re-read to evaluate reliability. The intraclass correlation for repeated mammographic percent density determinations was 0.88. A gray area was selected as a threshold to separate the outside edge of the breast from the background, which was used to determine the total breast area. The back of the breast was defined by outlining any pectoral muscle, which was then excluded from the final calculation. The edges of the regions that are representative of radiologically dense tissue in the image are identified by the selection of a second threshold, the sum of which provides the area of density in the breast. Dense tissue is typically brighter than fat, which shows up as a darker gray or black. Percent density was calculated as the proportion of the area of density in the breast relative to the total breast area.

Daidzein-Metabolizing Phenotypes. Urinary equol and *O*-DMA were analyzed using a variation of the ether extraction method of Heinonen et al. (33). Urine samples, frozen and stored at -70°C until analysis, were ether-extracted and analyzed by gas chromatography-mass spectrometry as described elsewhere.⁷ Quality control urine samples were included in each batch. The mean intra-assay coefficient of variations for equol and *O*-DMA in the quality control urine samples, measured in duplicate in each batch, were $<7\%$. Given the sensitivity of the assay, urine concentrations less than 182 nmol/L (44 ng/mL in urine) of equol and 170 nmol/L (44 ng/mL in urine) of *O*-DMA were considered below detectable limit. Equol/*O*-DMA producers were defined as individuals with any detectable concentration of equol/*O*-DMA. All women had detectable concentrations (>44 ng/mL in urine) of genistein

⁷ CL Frankenfeld, C Atkinson, WK Thomas, E Goode, A Gonzalez, T Jokela, K Wähälä, SM Schwartz, SS Li, JW Lampe. Familial correlations, segregation analysis and non-genetic correlates of soy isoflavone-metabolizing phenotypes, submitted for publication.

(minimum: 100 ng/mL) and of daidzein (minimum: 90 ng/mL), indicating compliance with the soy consumption protocol in all women.

Statistical Analysis. Three women were excluded from analysis because of poor mammogram image quality for both breasts, leaving a final sample size of 55 for analysis. Mean percent breast density of left and right breasts was used as a summary measure of percent breast density in 50 women. The value of left breast ($n = 1$) or the right breast ($n = 4$) only was used for women whose mammogram image quality was considered poor for the contralateral breast. Before analysis, natural logarithmic transformations were done for all variables that appeared to be skewed, including percent mammographic density. Linear regression was used to model associations between covariates and mammographic density. Linear regression was used to estimate differences in percent mammographic density by equol/*O*-DMA-producer phenotypes separately and combined using six models for the separate phenotypes and five models for the combined phenotype: (1) unadjusted; (2) adjusted for age; (3) adjusted for age and the other daidzein-metabolizing phenotype (not in the model for combined phenotype); (4) adjusted for age and serum hormone concentrations; (5) adjusted for age and reproductive and anthropometric factors; and (6) adjusted for age, reproductive and anthropometric factors, and serum hormone concentrations. Combined phenotypes were classified as equol non-producer/*O*-DMA non-producer (equol−/*O*-DMA−), equol non-producer/*O*-DMA producer (equol−/*O*-DMA+), equol producer/*O*-DMA non-producer (equol+/*O*-DMA−), and equol producer/*O*-DMA producer (equol+/*O*-DMA+). Variables for adjustment were chosen from selected anthropometric, lifestyle, and reproductive characteristics and circulating hormone and sex hormone binding globulin concentrations (SHBG) concentrations (measured at baseline of parent study) using a best-fit model procedure based on forward stepwise regression. Specifically, univariate regression models were fit for each of these variables with percent mammographic density as the outcome. Variables with a model P value < 0.3 were included in a forward stepwise regression [P value for entry (pe) = 0.1] by category (anthropometric, lifestyle, hormonal, and reproductive). For each category, the best-fit model was identified. Variables from each of the category models were included together for an overall multivariate adjustment of each phenotype on percent mammographic density using linear regression. The specific variables considered were BMI, clinic-measured current weight, DEXA-measured percent body fat, intentional loss of >10 pounds in past 20 years, reported maximum adult height, reported maximum adult weight, circulating estrone, FSH, testosterone, free testosterone, androstenedione, estradiol, free estradiol, DHEA, DHEA-sulfate and SHBG, urinary 2-hydroxyestrone:16 α -hydroxyestrone, percent dietary calories from fat, smoking status (never/former), ever/never use of non-contraceptive exogenous estrogen, years of exogenous estrogen use, ever/never oral contraceptive use, years of oral contraceptive use, gravidity, age at first pregnancy, previous hysterectomy, previous oophorectomy, type of menopause (surgical, natural, or other), ever breastfed for more than 1 month, age at first menses,

menstrual regularity in premenopausal period, and family history of breast cancer (mother, sister, aunt, or daughter). Measurement of these variables is reported elsewhere (30). Analyses were done using Stata 7.0 (Stata Corporation, College Station, TX).

Results

Twelve women were classified as equol-producers (25%) and 45 women were classified as *O*-DMA producers (82%). There was no apparent relationship between equol-producer and *O*-DMA-producer phenotype classifications in this sample of women ($\chi^2 P$ value = 0.73): 10 (18%) individuals were equol producers as well as *O*-DMA producers (equol+/*O*-DMA+), 8 (15%) equol non-producer plus *O*-DMA non-producer (equol−/*O*-DMA−), 2 (4%) equol+/*O*-DMA−, and 35 (64%) equol−/*O*-DMA+. Eighty-three percent of equol producers and 81% of equol non-producers were *O*-DMA producers.

Participant demographic, anthropometric, and reproductive characteristics by daidzein-metabolizing phenotypes are presented in Table 1. Women were, on average, 60 years of age, were well educated ($n = 28$ having postgraduate education), and most women indicated their racial background to be Caucasian ($n = 51$). No differences in age, education, or racial affiliation were observed between producers and non-producers within either daidzein-metabolizing phenotype. Twenty-nine (53%) women were former smokers. Former smokers were approximately three times as likely as never smokers to be equol producers than equol non-producers [odds ratio = 3.5, 95% confidence interval (CI): 0.7-22.1]. Similarly, former smokers were approximately six times as likely to be *O*-DMA producers than *O*-DMA non-producers (odds ratio = 6.0, 95% CI: 1.0-62.0).

The geometric mean percent mammographic density in all women was 4.0% (95% CI: 3.1-5.2). The geometric mean absolute dense area was 11.5 cm² (95% CI: 9.0-14.7). Percent mammographic density and absolute dense area were strongly correlated. Age-adjusted correlation was 0.98 when both measures were log-transformed ($P < 0.001$) and the untransformed correlation was 0.93 ($P < 0.001$).

Inverse associations with percent mammographic density were observed with anthropometric characteristics BMI [β -coefficient (β) = −0.10 (95% CI: −0.16 to −0.04)], log-transformed maximum adult weight [β = −3.04 (95% CI: −4.36 to −1.72)], and current weight [β = −0.04 (95% CI: −0.05 to −0.02)], and with previous oophorectomy [β = −0.84 (95% CI: −1.64 to −0.04)]. Women with a family history of breast cancer in a first- or second-degree female relative had greater percent mammographic density [β = 0.53 (95% CI: 0.01-1.04)] than women without such family history. Age at first menstrual period [β = 0.04 (95% CI: −0.07 to 15.3)], being nulligravid [β = 0.45 (95% CI: −0.23 to 1.13)], and natural menopause (versus surgical) [β = 0.29 (95% CI: −0.29 to 0.88)] were non-statistically significantly positively associated with percent mammographic density. Ever use of oral contraceptives [β = −0.42 (95% CI: −0.99 to 0.15)], ever use of non-contraceptive exogenous estrogen [β = −0.33 (95% CI: −0.84 to 0.18)] and previous

Table 1. Demographic, anthropometric, and reproductive characteristics of 55 postmenopausal women by daidzein-metabolizing phenotypes

	Equol producers (n = 12), n (%)	Equol non-producers (n = 43), n (%)	O-DMA producers (n = 45), n (%)	O-DMA non-producers (n = 10), n (%)
Age (y)				
50-59	7 (58)	25 (58)	26 (58)	6 (60)
60-69	4 (33)	12 (28)	12 (27)	4 (40)
70-75	1 (8)	6 (14)	7 (16)	0 (0)
Education				
High school or some college	5 (42)	19 (44)	20 (44)	4 (40)
College graduate	7 (58)	24 (56)	25 (56)	6 (60)
Race				
Asian or Pacific Islander	0 (0)	1 (2)	0 (0)	1 (10)
African-American	0 (0)	3 (7)	3 (7)	0 (0)
Caucasian	12 (100)	39 (91)	42 (93)	9 (90)
Smoking status				
Former	9 (75)	20 (47)	27 (60)	2 (20)
Never	3 (25)	23 (53)	18 (40)	8 (80)
BMI (kg/m ²)				
25-29.9	7 (58)	22 (51)	25 (56)	4 (40)
30-34.9	3 (25)	15 (35)	14 (31)	4 (40)
35-40.0	2 (17)	6 (14)	6 (13)	2 (20)
Age at first menstrual period (y)				
9-11	3 (25)	9 (21)	11 (24)	1 (10)
12-13	5 (42)	26 (60)	24 (53)	7 (70)
14-21	4 (33)	8 (19)	10 (22)	2 (20)
Regular menstrual periods during most of life (vs no or sometimes)	10 (83)	33 (77)	35 (78)	8 (80)
Nulligravid*	3 (25)	6 (14)	7 (16)	2 (20)
Ever breastfed more than 1 month (among parous)	7 (58)	22 (51)	24 (53)	5 (50)
Menopause occurred naturally (vs surgical)	9 (75)	32 (74)	33 (73)	8 (80)
Previous hysterectomy	2 (17)	10 (23)	11 (24)	1 (10)
Previous oophorectomy	1 (8)	5 (12)	4 (9)	2 (20)
Ever used oral contraceptives	10 (83)	27 (63)	31 (69)	6 (60)
Ever used estrogen (non-contraceptive)	8 (67)	21 (49)	22 (49)	7 (70)
Ever used herbal estrogen	1 (8)	3 (7)	4 (9)	0 (0)
Family history of breast cancer in first- or second-degree female relative	3 (25)	16 (37)	17 (38)	2 (20)

*Nulligravid: no pregnancies that lasted at least 6 months.

hysterectomy [$\beta = -0.14$ (95% CI: -0.76 to 0.49)] were non-statistically significantly inversely associated with percent mammographic density. No association between smoking status (former/never) with mammographic density was observed [$\beta = 0.003$ (95% CI: -0.52 to 0.52)].

Unadjusted geometric mean mammographic density was lower in equol producers than non-producers (Table 2), and this was independent of age and O-DMA-producer phenotype. Adjustment for gravidity, maximum adult weight ($r = 0.92$ correlation with current

Table 2. Unadjusted and adjusted percent mammographic density and percent difference in 55 postmenopausal women by equol-producer phenotype

Equol-producer phenotype, adjusted for:	Producers (n = 12)	Non-producers (n = 43)	% Difference*	Absolute difference [†] (95% CI)	P value
	Geometric mean (95% CI [‡])	Geometric mean (95% CI [‡])			
No other covariates	2.4 (1.4-4.2)	4.5 (3.4-6.1)	-48%	-1.9 (-3.5 to -1.0)	0.04
Age	2.4 (1.4-4.1)	4.6 (3.5-6.1)	-46%	-1.9 (-3.4 to -1.0)	0.04
Age + O-DMA-producer phenotype	2.4 (1.4-4.1)	4.6 (3.5-6.0)	-48%	-1.9 (-3.4 to -1.1)	0.03
Age + FSH + free testosterone	2.3 (1.4-3.8)	4.6 (3.6-6.0)	-50%	-2.0 (-3.5 to -1.1)	0.02
Age + maximum adult weight + gravidity + family history of breast cancer [§]	2.8 (1.9-4.3)	4.4 (3.5-5.4)	-36%	-1.6 (-2.5 to -1.0)	0.07
All covariates in previous models	2.7 (1.8-4.0)	4.4 (3.6-5.5)	-39%	-1.6 (-2.6 to -1.0)	0.04

*Producers relative to non-producers = (mean value of producers - mean value of non-producers)/mean value of non-producers.

[†]Difference in geometric means, calculated from regression models, of producers minus non-producers.

[‡]CI: confidence interval; 95% geometric mean CI may overlap regardless of a significant (<0.05) P value from linear regression using log-transformed mammographic density.

[§]Gravidity: number of pregnancies that lasted at least 6 months; family history of breast cancer: first- or second-degree female relative.

Table 3. Unadjusted and adjusted percent mammographic density and percent difference in 55 postmenopausal women O-DMA-producer phenotype

O-DMA-producer phenotype, adjusted for:	Producers (n = 45)	Non-producers (n = 10)	% Difference*	Absolute difference† (95% CI)	P value
	Geometric mean (95% CI‡)	Geometric mean (95% CI‡)			
No other covariates	4.3 (3.3-5.8)	2.7 (1.5-4.9)	+60%	1.6 (-1.2 to 3.1)	0.17
Age	4.3 (3.3-5.8)	2.6 (1.5-4.7)	+66%	1.7 (-1.2 to 3.2)	0.12
Age + equol-producer phenotype	4.4 (3.3-5.7)	2.6 (1.5-4.6)	+69%	1.7 (-0.1 to 3.2)	0.10
Age + FSH + free testosterone	4.3 (3.2-5.6)	3.0 (1.7-5.3)	+43%	1.4 (-1.3 to 2.8)	0.28
Age + maximum adult weight + gravidity + family history of breast cancer§	4.4 (3.5-5.4)	2.6 (1.7-4.1)	+76%	1.7 (1.0 to 2.8)	0.04
All covariates in previous models	4.4 (3.6-5.4)	2.6 (1.7-4.1)	+69%	1.7 (-1.0 to 2.8)	0.05

*Producers relative to non-producers = (mean value of producers – mean value of non-producers)/mean value of non-producers.

†Difference in geometric means, calculated from regression models, of producers minus non-producers.

‡CI: confidence interval; 95% geometric mean CI may overlap regardless a significant (<0.05) P value from linear regression using log-transformed mammographic density.

§Gravidity: number of pregnancies that lasted at least 6 months; family history of breast cancer: first- or second-degree female relative.

weight), and family history of breast cancer attenuated this difference. In contrast, after adjusting for FSH and free testosterone (the hormones identified as the strongest predictors of mammographic density from the best-fit model procedure), a larger difference between equol producers and equol non-producers was observed.

Unadjusted geometric mean mammographic density was greater in O-DMA producers than non-producers (Table 3), and this was independent of age and equol-producer phenotype. After adjustment for gravidity, maximum adult weight, and family history of breast cancer, a larger difference in mammographic density between O-DMA producers and O-DMA non-producers was observed, whereas adjustment for serum FSH and free testosterone attenuated the difference between O-DMA producers and O-DMA non-producers.

Results of exploratory analysis of the combined daidzein-metabolizing phenotypes and mammographic density are presented in Table 4. Equol producers had lower mammographic density regardless of O-DMA-producer phenotype, and O-DMA producers had higher mammographic density regardless of equol-producer phenotype.

Discussion

We observed that two daidzein-metabolizing phenotypes were associated with mammographic density in this

group of postmenopausal women. Equol-producing women had, on average, 39% lower adjusted percent mammographic density than equol non-producing women. Our work was motivated by the study of Duncan et al. (29), in which the equol-producer phenotype was associated with circulating reproductive hormones in premenopausal women; five equol-producing premenopausal women had lower circulating concentrations of several estrogens and androgens, including estrone, testosterone, and DHEA, and higher progesterone concentrations compared with nine equol non-producing women.

Because endogenous hormones have been associated with mammographic density in pre- and postmenopausal women (13), we hypothesized that mammographic density differences between equol-producing and non-producing women may be mediated by hormonal differences. However, adjustment for FSH and free testosterone concentrations (stronger than circulating estrogens and SHBG as statistical predictors of mammographic density in this study) did not attenuate the association between equol-producer phenotype and mammographic density. Thus, the association we observed seems not to be mediated through circulating concentrations of these hormones.

In our analysis, O-DMA-producing women had adjusted mammographic density approximately 69% greater than that of O-DMA non-producing women.

Table 4. Unadjusted and adjusted percent mammographic density (geometric means and 95% CIs) in relation to combined daidzein-metabolizing phenotypes in 55 postmenopausal women

Combined phenotypes, adjusted for:	Equol-/O-DMA- (n = 8)	Equol-/O-DMA+ (n = 35)	Equol+/O-DMA- (n = 2)*	Equol+/O-DMA+ (n = 10)
No other covariates	3.4 (1.8-6.6)	4.9 (3.6-6.7)	1.1 (0.3-4.1)	2.8 (1.6-5.1)
Age	3.3 (1.8-6.3)	4.9 (3.6-6.6)	1.0 (0.3-3.7)	2.9 (1.6-5.1)
Age + FSH + free testosterone	3.7 (2.0-6.8)	4.9 (3.6-6.6)	1.2 (0.3-3.9)	2.6 (1.5-4.6)
Age + maximum adult weight + gravidity + family history of breast cancer†	2.8 (1.7-4.6)	4.9 (3.8-6.1)	1.8 (0.7-5.0)	3.1 (2.0-4.7)
All covariates in previous models	2.9 (1.7-4.9)	4.9 (3.8-6.2)	1.8 (0.7-5.0)	2.9 (1.9-4.6)

*Results presented for Equol+/O-DMA- group presented for completeness; caution should be used in interpretation of results because of small sample number.

†Gravidity: number of pregnancies that lasted at least 6 months; family history of breast cancer: first- or second-degree female relative.

Little is known about the physiologic effects associated with the *O*-DMA-producer phenotype, and we are unaware of other studies that have specifically evaluated mammographic density in relation to *O*-DMA-producer phenotype. We observed that adjustment for selected circulating hormone concentrations markedly attenuated the association between the *O*-DMA-producer phenotype and mammographic density, suggesting that endogenous hormones, in part, modulate the association.

These daidzein-metabolizing phenotypes are markers of the presence of particular intestinal bacteria profiles capable of metabolizing daidzein to equol/*O*-DMA. Although the mechanism by which the intestinal bacteria, and specifically the daidzein-metabolizing phenotypes, could influence mammographic density is not known, we propose several hypotheses. One hypothesis is that circulating equol and *O*-DMA, which can bind to estrogen receptors α and β (34, 35), may influence mammographic density. However, this hypothesized mechanism may not be physiologically relevant in low-soy consuming populations, such as postmenopausal women in the United States (36, 37). Whether exposure to low levels of daidzein metabolites would result in clinically relevant physiologic effects remains to be established. A second hypothesis is that these daidzein-metabolizing phenotypes may arise as a result of exogenous factors that concurrently and separately influence intestinal environment and mammographic density. Similarly, a third hypothesis is that these daidzein-metabolizing phenotypes may be markers of a genetic profile that is associated with intestinal environment and mammographic density. In these latter two hypotheses, the daidzein-metabolizing phenotypes serve as surrogate markers of other factors influencing mammographic density. A fourth hypothesis is that there are differences in metabolic capacities of equol/*O*-DMA-producing and non-producing bacteria. Bacteria responsible for daidzein-metabolizing phenotypes in humans have not been definitively established. However, strain-specific metabolism of reproductive hormones has been observed between other bacteria (38). Differences in intestinal bacterial populations may result in different reproductive hormone metabolite profiles, and, thus, differing concentrations of metabolites available for reabsorption, an effect that could influence circulating concentrations of some hormones within the human host. A final hypothesis is that the presence of daidzein-metabolizing bacteria is correlated with the presence of other bacteria that also thrive in a particular intestinal environment or that the presence of daidzein-metabolizing bacteria may influence the capacity for other bacteria to colonize the intestinal tract. If this other (not daidzein-metabolizing) bacteria were to influence mammographic density through some mechanism, the daidzein-metabolizing bacteria could be indirectly associated with mammographic density. These hypothesized mechanisms are not mutually exclusive, and it is possible that intestinal bacteria may influence mammographic density through several mechanisms. Also, the mechanisms by which each of the daidzein-metabolizing phenotypes is associated with mammographic density may be different.

The overall average percent mammographic density in this group of women was 4%. This value is lower than reported in other studies. However, this study population is very different from many of the populations

reported in the literature. Vachon et al. (19) observed a mean of 32% (SD: 14) mammographic density in first-degree and second-degree female relatives of breast cancer cases, Maskarinec et al. (39) observed a mean of 28% (SD: 20) in premenopausal and postmenopausal Caucasian women, Ursin et al. (40) observed a median percent mammographic density of 20% (interquartile range: 8 to 38) in women 50 years or age and older and without family history of breast cancer, and Gapstur et al. (41) observed mean percent density of 15% (SD: 9) in postmenopausal Hispanic women. All women in our study were postmenopausal and overweight or obese, with an average BMI of 30, and did not use hormone treatment in the 6 months before randomization. Hormone therapy regimens and lower BMI are factors that have been associated with greater postmenopausal mammographic density (8, 14, 41); thus, women in our study would be expected to have low mammographic density, compared with women in these other studies. Despite the low values, percent mammographic density was associated with several characteristics as expected. Notably, percent mammographic density was positively associated with family history of breast cancer and being nulligravid and was inversely associated with BMI and current weight, which has been observed in other studies (14, 19, 42). Similar to our finding, former hormone use, compared with never use, has not been a strong determinant of postmenopausal mammographic density in other studies (14, 19, 41). Although not statistically significant, we observed that age at menarche and surgical menopause were both inversely associated with percent mammographic density, which is the same direction of the association observed in other studies (14, 41). Overall, this suggests that, despite the low values, the percent mammographic density measure in our study was valid.

Our study has some limitations. One, given the small sample of women, we were not able to estimate precisely the magnitude of association between daidzein-metabolizing phenotypes and mammographic density and we also may have failed to identify some true associations. However, we know of no other studies to date that have evaluated the association between mammographic density and daidzein-metabolizing phenotypes. This preliminary work provides a foundation for further study in which to confirm and more precisely estimate the magnitude of association between daidzein-metabolizing phenotypes and mammographic density. Two, the parent study was a randomized trial, and the women were selected for the parent study based on particular selection criteria, which may have also reduced variability in our outcomes and decreased the power to detect associations in this ancillary study.

In summary, we observed that postmenopausal percent mammographic density, an intermediate marker of breast cancer risk, was lower in equol producers compared with non-producers and was higher in *O*-DMA producers than non-producers. Further adjustment for covariates suggested that the equol-producer phenotype may influence mammographic density, in part, through anthropometric and/or reproductive characteristics and that the *O*-DMA-producer phenotype may influence mammographic density, in part, through circulating FSH and free testosterone concentrations. Additionally, the association of each daidzein-metabolizing phenotype

with mammographic density appeared to be independent of the other phenotype. These results should be confirmed with a larger sample size, and evaluation in other age groups and populations is also warranted.

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