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S-(–)equol producing status not associated with breast cancer risk among low isoflavone consuming US postmenopausal women undergoing a physician recommended breast biopsy

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Abstract

Soy foods are the richest sources of isoflavones, mainly daidzein and genistein. Soy isoflavones are structurally similar to the steroid hormone 17 β -estradiol and may protect against breast cancer. S-(–)equol, a metabolite of the soy isoflavone daidzein, has a higher bioavailability and greater affinity for estrogen receptor- β than daidzein. About one third of the Western population is able to produce S-(–)equol, and the ability is linked to certain gut microbes. We hypothesized that the prevalence of breast cancer, ductal hyperplasia, and overall breast pathology will be lower among S-(–)equol producing, as compared to non-producing, postmenopausal women undergoing a breast biopsy. We tested our hypothesis using a cross-sectional study design. Usual diets of the participants were supplemented with one soy bar per day for three consecutive days. Liquid chromatography-multiple reaction ion monitoring mass spectrometry analysis of urine from 143 subjects revealed 25 (17.5%) as S-(–)equol producers. We found no statistically significant associations between S-(–)equol producing status and overall breast pathology (OR 0.68; 95% CI 0.23 – 1.89), ductal hyperplasia (OR 0.84; 95% CI 0.20 – 3.41), or breast cancer (OR 0.56; 95% CI 0.16 – 1.87). However, the mean dietary isoflavones intake was much lower (0.3 mg/day) than in previous reports. Given that the amount of S-(–)equol produced in the gut depends on the amount of daidzein exposure, the low soy intake coupled with lower prevalence of S-(–)equol producing status in the study population favors towards null associations. Findings from our study could be used for further investigations on S-(–)equol producing status and disease risk.

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Keywords

Dietary soy isoflavones; S-(–)equol status; postmenopausal women; breast biopsy; ductal hyperplasia; breast cancer

1. INTRODUCTION

Epidemiologic studies suggest that breast cancer rates are low in populations that consume soy [1–3]. Overall, soy intake among Asian populations has been associated with lower breast cancer risk [4–7], whereas inconsistent results have been reported among non-Asian populations [8–13]. The timing of soy exposure and the amount of soy consumed have been studied in-depth for breast cancer prevention [2,14–18]. However, individual variation in isoflavone metabolism is not well characterized. Isoflavones are one of the three main classes of the phytoestrogens and have structural similarity to the hormone 17 β -estradiol [19,20]. Isoflavones in most soy foods are conjugated to sugars and exist as both simple and complex β -D-glycosides [21]. After ingestion, isoflavones undergo hydrolysis with the action of brush border and bacterial β -glucosidases to remove the sugar moiety; the isoflavone aglycone is released and is either absorbed or undergoes further metabolism by intestinal bacteria in the large bowel. In the case of the isoflavone daidzein, it is usually metabolized to dihydrodaidzein or O-desmethylangolensin (O-DMA) [22–25]. In a smaller group of subjects, it is also metabolized to S-(–)equol.

Equol lacks a double bond in the heterocyclic ring and exists in two stereoisomers. The two isomers R-(+) and S-(–) differ significantly from each other in terms of their binding affinities with ER [26]. S-(–)equol has a higher affinity for ER β as compared to R-(+) (0.73 nmol/L vs. 15.4 nmol/L) [27]. However, depending on cell line, no ER subtype preference in terms of the transcriptional potency ($\beta/\alpha = 1.3$) in HEC-1 cells, or significantly higher ER α transcriptional activity through AF-1 has been observed in HepG2 cells [28]. Human gut microflora exclusively metabolize daidzein to produce the S-(–)equol isomer [27]. On average, only about a third of a general Western population challenged with soy is able to metabolize daidzein to produce S-(–)equol [29–34]. A higher prevalence (60%) of S-(–)equol producers has been reported among native Asians [35]. Similar to the geographic disparity, ethnic/racial differences exists within the US; a higher prevalence of S-(–)equol producing phenotype has been reported among Korean American girls as compared to Caucasian girls (51% vs. 36%; $p = 0.015$) [36].

The role of gut microflora in the formation of S-(–)equol originated from experiments with germ-free rats challenged with daidzein, which did not produce S-(–)equol [37]. This was further verified by the findings that germ-free rats infected with fecal-flora from S-(–)equol producers were capable of producing S-(–)equol, whereas no S-(–)equol was detected either from germ-free rats, or those infected with micro-flora of non-producers [22]. A number of bacterial species capable of converting daidzein to S-(–)equol in-vitro have been isolated from both food and human intestine, *Lactococcus garviae* from Italian cheese [38], six strains of bacteria belonging to the Coriobacteriaceae family from Taiwanese stinky tofu brine [39], *Eggerthella* sp. strain YY791 [40,41] and *Slackia isoflavoniconvertens* [42] from the human intestine. Presence of urinary S-(–)equol following the soy-challenge suggests the

presence of certain gut-microflora. In addition to facilitating the daidzein metabolism, it is plausible that these bacteria are potentially involved in metabolic conversion of a variety of other dietary components important for health and overall well being.

It has been proposed that S-(–)equol producers may have improved disease risk patterns as compared to non-producers [43–48]. Collective evidence suggest S-(–)equol producers have a hormone profile favorable for lower breast cancer risk as compared with non-producers [49], with a high urinary estrogen metabolite 2-hydroxyestrone to 16 α -hydroxyestrone ratio [50–52]. The ratio is inversely associated with breast cancer risk, and a higher ratio is considered protective.

The prevalence of S-(–)equol producing status among postmenopausal women with abnormal mammogram undergoing a physician-recommended breast biopsy is unknown, including whether being capable of producing S-(–)equol would confer any benefit for the biopsy outcome or breast cancer risk than in non-producers. We hypothesized that prevalence of ductal hyperplasia, overall breast pathology, or breast cancer will be lower among S-(–)equol producing as compared to non-producing postmenopausal women with abnormal mammogram undergoing physician-recommended breast biopsy. The specific objectives of this cross-sectional study were to: (1) determine the prevalence of S-(–)equol producing status among postmenopausal women with abnormal mammogram undergoing a physician-recommended breast biopsy, and (2) evaluate the associations between S-(–)equol producing status and breast biopsy outcome: ductal hyperplasia, breast cancer, or overall breast pathology (hyperplasia + breast cancer). The study objectives were tested using a cross-sectional study design to avoid any potential reporting biases or potential changes in lifestyle or dietary behaviors associated with the positive biopsy outcome for cancer. We administered the demographic, and soy screen questionnaires and started the soy challenge on the day of the biopsy using a cross-sectional study design.

2. METHODS AND MATERIALS

2.1. Study Design

We conducted a cross-sectional study to test the hypothesis. Participants were postmenopausal women with abnormal mammogram receiving a physician-recommended breast biopsy at the Mammography Department at the University of Alabama at Birmingham, and written consent was obtained prior to study enrollment. Eligible participants were 45 years or older, postmenopausal (defined as having no menstrual periods within the previous 12 months), and had no personal history of breast cancer, no known soy allergy, and no antibiotic therapy within the previous three months. We screened 392 women that underwent a physician-recommended breast biopsy from June 2009 to May 2011 (Fig. 1).

All study protocols were approved by the Institutional Review Board at the University of Alabama at Birmingham (UAB), and the Clinical Trial Review Committee at the UAB Comprehensive Cancer Center.

2.2. Study Procedures

Patients with abnormal mammogram undergoing a physician recommended breast biopsy at the Radiology Department, Kirklin Clinic, UAB, were informed about the study on the day of their biopsy, and were screened for eligibility. The study procedures were explained in detail, and written consents were obtained and three-digit codes were assigned to those who met study criteria and were interested in volunteering. Height (cm) and weight (kg) were measured using a standardized scale (model number WB3007301 Tanita Corporation of America, INC.) on the day of the biopsy. Participants were given study related supplies: three Revival (Kernersville, NC) soy bars, a urine collection kit, Exact pack human specimen shipping box, freezer gel packs, and FedEx return label for completing the three-day soy challenge at home and returning their urine samples. Written instructions for soy bar supplementation for three consecutive days, urine collection on the fourth day morning, packing and shipping details, along with a contact e-mail and phone number for any further questions were provided. Usual dietary intakes of regular food items were estimated using the semi-quantitative Block 2005 Food Frequency Questionnaires (FFQ) [53], and habitual soy intake was assessed using the Block Soy Screen Questionnaire (SSQ) [54,55]. In addition, data on age, age at menarche, age at first pregnancy, number of children, breastfeeding, length of breast feeding, family history of breast cancer in first degree relatives, and any current or past hormone therapy were collected using an interview based demographic questionnaire.

2.3. Breast Biopsy

All study participants enrolled in the study underwent a breast biopsy as a part of their routine medical care. The pathologists analyzing the biopsy tissue slides were blinded to the recruitment status of our study participants. Biopsy pathology reports were obtained from the clinic. Biopsy outcomes were categorized and coded as: 0 if normal tissue; 1 if mild, florid or atypical hyperplasia (ductal or lobular); and 2 if carcinoma *in-situ* (ductal carcinoma *in-situ* or lobular carcinoma *in-situ*) or cancer.

2.4. Sample sizes for various analyses

Of the 392 women, 228 (58.2%) women met our eligibility criteria. Of these, 28 were not interested, and the remaining 200 women were enrolled in our study (125 Caucasian (CA), 74 African American (AA), and 1 Asian (AS). Of the 200 women who participated in the study, 56 (28%) subjects were non-compliant and did not return their urine samples. A total of 144 subjects (92 CA; 51 AA; and 1 AS) provided their urine samples and completed the Block SSQ. Urinary samples for S-(–)equol levels were analyzed from 144 subjects. For the logistic regression model building, the only subject belonging to the AS racial group was excluded. Thus, the final sample size for S-(–)equol producing phenotype and the initial models for associations with biopsy outcome included 143 participants (92 CA; 51 AA).

Usual dietary intakes were assessed using the Block FFQ. Out of 143 subjects that were included for the urinary S-(–)equol analyses, 16 did not return their FFQ, leaving a sample size of 127 subjects for the dietary analyses. We used *a priori* energy intake cut-offs of 500 kcal or 5000 kcal per day. Using this criterion, we excluded one subject for too low (353 kcal/d), and one for too high (5612 kcal/d) energy intakes. Hence, the final sample size

for the FFQ dietary analyses, and the final logistic regression models of S-(–)equol status by biopsy outcomes were thus restricted to 125 subjects (81 CA; 44 AA).

2.5. Isoflavones composition of the Soy Bar

One Revival soy bar was crushed and three 200 mg aliquots were extracted in 80% aqueous methanol at 4°C for 2 hours. Isoflavones in aliquots (10 µl) of the clarified extracts were analyzed by LC on an Agilent HP 1100 instrument. The column (2.1 mm i.d. × 25 cm C₁₈ reverse-phase) was equilibrated in 10 mM ammonium acetate in 30% acetonitrile. The compounds were eluted with a 40 min 30–100% linear gradient of acetonitrile in 10 mM ammonium acetate at 0.2 ml/min. Eluted compounds were detected by their UV absorption using a diode array detector. Known amounts of the isoflavone β-glucosides (daidzin, genistin and glycitin) and the aglycones, daidzein, genistein and glycitein were used to establish standard curves. Peaks due to the malonyl- and acetyl β-glucosides were expressed in aglycone units.

Further validation was carried out by reverse-phase liquid chromatography-mass spectrometry, as described previously [56], on an AB Sciex (Concord, Ontario, Canada) 4000 triple quadrupole mass spectrometer. Selected ion chromatograms were created using the following: malonyldaidzin *m/z* 503; acetyldaidzin *m/z* 459; daidzin *m/z* 417; malonylgenistin *m/z* 519; acetylgenistin *m/z* 475; and genistin *m/z* 433. In LC-tandem MS experiments, the chromatography was repeated and ions corresponding to different isoflavone β-glucoside conjugates were selected for collision-induced dissociation. From these, ion chromatograms were developed using the transitions, malonyldaidzin *m/z* 503 to *m/z* 255; acetyldaidzin *m/z* 459 to *m/z* 255; daidzin *m/z* 417 to *m/z* 255; malonylgenistin *m/z* 519 to *m/z* 271; acetylgenistin *m/z* 475 to *m/z* 271; and genistin *m/z* 433 to *m/z* 271.

2.6 S-(–)equol Phenotyping

Urine samples were delivered to the lab by overnight delivery. Samples were aliquoted at the UAB Mass spectrometry lab and stored at –40°F. The details of sample preparation and HPLC mass spectrometry are published elsewhere [57]. Hydrolyzed extracts were analyzed by reverse-phase liquid chromatography-multiple reaction ion monitoring mass spectrometry (LC-MRM-MS) on an AB Sciex 4000 triple quadrupole mass spectrometer. All samples were extracted and run in duplicate, and quality control samples were included in each batch for analysis. The mean intra-assay coefficient of variation for S-(–)equol measured in duplicate in each batch was < 3%. The sensitivity and limit of quantification of the assay was set at < 5 nmol/L, and if the mass spectrometer reported no detectable levels of S-(–)equol, 4.9 nmol/L was recorded for that individual. All women had detectable concentrations of daidzein, genistein, and glycitein indicating compliance with eating the soy bars.

2.7 Statistical Analyses

Urinary isoflavones and S-(–)equol concentrations were expressed in nmol/L. As previously described [64], log₁₀ transformed product/precursor ratios (log₁₀ S-(–)equol/daidzein) were used for defining the S-(–)equol producing status. Logistic regression models were built to determine the association between S-(–)equol producing status and the breast biopsy

outcome: normal vs. overall pathology (coded as 0 vs. 1+2); normal vs. ductal hyperplasia (coded as 0 vs. 1); and normal vs. breast cancer (coded as 0 vs. 2), and were expressed as an odds ratio and its 95% confidence intervals (CI). Both unadjusted and adjusted models were analyzed. The fully adjusted models were controlled for a number of confounding variables which included age, race, body mass index (BMI), smoking, age at menarche, age at first pregnancy, age at menopause, family history of breast cancer among first degree relatives, and energy intake. A chi-square test for the frequency variables (parity, hysterectomy, hormone usage, current diuretic usage, family history of breast cancer in the first degree family relative, and biopsy outcome) was used. P-values are reported for the chi square test. In cases where the cell frequency dropped below 5, Fisher's exact test was used. Data from the SSQ were analyzed for median isoflavone intakes and were reported along with the 25th and 75th percentile. The FFQ dietary data were analyzed by S-(–)equol status, and were reported as means $\pm$ SD, and resulting p-values from the t-tests. The α was set at 0.05 for determining the level of significance for all analyses. All data were analyzed using SAS (version 9.3; SAS Institute, Cary, NC), and SPSS (version 20; IBM statistics, Armonk, NY).

3. RESULTS

3.1. Composition of the Soy Bar

LC-MS and MRM-MS confirmed that the soy bar contained malonyl and acetyl β -glucosides of daidzein and genistein, as well as non-esterified β -glucosides. HPLC-UV-diode array analyses revealed that the ratios of the β -glucoside conjugates were 1:10:5 (for malonyl β -glucosides, acetyl β -glucosides and β -glucosides, respectively) for daidzein and 1:10:3 for genistein. The total isoflavone content was 160 mg/bar.

3.2. Prevalence of S-(–)equol Producers

The LC-MRM-MS urinary analyses of 143 subjects revealed measurable S-(–)equol levels among 130 study participants (90.91%), and no values were reported for 13 (9.09%). For these 13 subjects, a threshold value of 4.9 nmol/L (based on limit of quantitation < 5 nmol/L) was used for statistical purposes. The urinary isoflavone data for S-(–)equol were expressed as log₁₀ S-(–)equol/daidzein ratio, and a cut-off at -1.60 represented a separation between the two groups (Fig. 2). Any subjects with values above -1.60 were considered as S-(–)equol producers, and those with values below -1.60 were considered as non-producers. Using this criterion, 25 subjects were classified as S-(–)equol producers (17.5%) and 118 as non-producers (82.5%) (Fig. 3). No statistical differences were observed for the demographic, anthropometric, or reproductive variables by S-(–)equol producing status (Table 1). The prevalence of S-(–)equol producing status was lower among AA as compared to CA, but this difference was not statistically significant (AA 13.72 % vs. CA 19.56 %; $p=0.34$).

3.3. Breast biopsy results

The biopsy pathology reports of 143 subjects revealed a total of 45 (31.5%) subjects with breast cancer, 33 (23.1%) with hyperplasia, and 65 (45.4%) as normal tissue. To evaluate any potential bias introduced by the biopsy results (Table 2), we accessed biopsy pathology

reports for all 200 subjects and analyzed those by urine sample availability (compliant: n = 144 vs. non-compliant: n = 56). The chi-square test (1.69; p = 0.43) revealed no influence of the biopsy status for those who were compliant as compared to those who did not provide their urine sample.

The estrogen (ER +/-) and progesterone (PR +/-) receptors data were available for 40 breast cancer patients. Analyses of the receptor status by S(-)equol producing status revealed that none of the S(-)equol producers had ER -ve receptors, all 8 were ER +ve, however, the differences were not statistically significant (Fisher's exact 2-sided p = 0.31) (Table 3).

3.4. Dietary Data

Usual isoflavone intake assessment using the Block SSQ for the 143 subjects analyzed as $\mu\text{g/d}$ (median and its associated 25th and 75th percentiles) was 220 $\mu\text{g/d}$; 0.00 – 1235 $\mu\text{g/d}$. Overall, the usual intakes of soy protein and isoflavones were very low in this population, and the distributions appeared similar for S(-)equol producers and non-producers (Table 4).

Overall, the mean total intake of energy for the study population (n = 125) was 1565 ± 648 kcal/d, and was not different when analyzed by S(-)equol producing status. However, the total dietary protein (50.2 ± 18.7 g/d vs. 62.7 ± 27.5 g/d, p = 0.05) and trans fat (1.80 ± 0.52 g/d vs. 2.21 ± 1.25 g/d, p = 0.005) intakes were significantly lower among S(-)equol producers as compared to non-producers, respectively.

3.5. Logistic Regression Models

Two main models were formulated for the logistic regression analyses: an unadjusted initial model for evaluating the association of S(-)equol status on breast pathology (hyperplasia + cancer), hyperplasia only, and cancer only (Table 5). There were no statistically significant associations between S(-)equol status and overall breast pathology (OR 0.76; 95% CI 0.32 – 1.84), hyperplasia (OR 0.82; 95% CI 0.27 – 2.5), or breast cancer (OR 0.73; 95% CI 0.27 – 1.97). The model was then adjusted for known risk factors for breast cancer such as: age, race, BMI, menarche, parity, and family history of breast cancer in the first degree relative, smoking, hormone usage, and total isoflavone intake (Table 6). The OR between S(-)equol producing status and overall breast pathology (OR 0.78; 95% CI 0.31 – 1.99), hyperplasia (OR 0.99; 95% CI 0.28 – 3.44), or breast cancer (OR 0.65; 95% CI 0.22 – 1.90) were not statistically significant. The final models were adjusted for all these above-mentioned covariates as well as for total energy intake (data were available for 125 subjects). There were no statistically significant associations between S(-)equol status and overall breast pathology (OR 0.68; 95% CI 0.23 – 1.89), hyperplasia (OR 0.84; 95% CI 0.20 – 3.41), or breast cancer (OR 0.56; 95% CI 0.16 – 1.87).

4. Discussion

We report for the first time, the prevalence of S(-)equol producers in US postmenopausal women undergoing a physician recommended breast biopsy (25 out of 143, 17.5%). The observed prevalence is lower in our study population as compared to the previous reports of about 30% in general Western populations. It is likely that the observed lower prevalence in our population is because of the underlying population differences such as biopsy

recommendation, abnormal mammogram, and postmenopausal status, as compared to previous reports among general Western population. However, future studies with direct comparison between healthy and breast biopsy recommended population are needed for further clarification.

The threshold value of -1.60 used for defining S(-)equol status in our population is comparable to a previously documented ratio of -1.75 by Setchell *et al* [58]. It is possible that our cut-off was higher because of the underlying population differences, or also due to the different soy challenge (a soy bar) as compared to the soy milk challenge by Setchell *et al* [58]. Our study is the first to test the S(-)equol producing status of African-American women with a relatively large sample size ($n = 51$). 13.72% (7 out of 51) of the African-American participants were S(-)equol producers as compared to 19.56% (18 out of 92) Caucasian-Americans, but the difference was not statistically significant. To date, the majority of the S(-)equol phenotyping studies have been conducted mainly among Caucasian Americans and Asians [59,60], with the exception of the Frankenfeld *et al* [61] study that included three African American women, and all three were not S(-)equol producers. The lower prevalence observed among AA women in our study are similar to observed by Hedlund *et al* [62] among Caucasian men who were long-term low soy consumers. Future studies are needed to confirm the observed lower prevalence of S(-)equol phenotype among AA postmenopausal women in our study, and to further understand the dietary or lifestyle factors responsible for the observed lower prevalence among this group.

To confirm if the participants who have a breast biopsy positive for cancer might be less likely to return urine samples, a chi-square test was performed for urine sample availability from 143 subjects by biopsy status for the entire 200 subjects. The p-value of the chi-square test was not significant, suggesting there were no differences in compliance across biopsy results.

We observed no associations between S(-)equol producing status and breast pathology, hyperplasia, or breast cancer. Based on our results of null associations, we rejected our hypothesis of lower prevalence of ductal hyperplasia, breast cancer, or overall breast pathology among S(-)equol producers as compared to non-producers.

Previously, Verhues *et al* [63] and Ward *et al* [64] have measured the absolute values of S(-)equol levels among study participants and its relation with risk of breast cancer within the European Prospective Investigation into Cancer cohort, and reported no association between plasma levels of S(-)equol and breast cancer risk. However, there was no soy challenge performed prior to the sample collection. The presence of S(-)equol will depend upon whether or not the gut microflora was exposed to any daidzein through usual food intakes. The latter was confirmed by Liu *et al* [35]; misclassification in the absence of such challenge is observed even in a much higher soy consuming population of Asians. To avoid such misclassification, S(-)equol phenotyping in the current study was performed after a three consecutive days of soy challenge.

The ability to produce S-(–)equol has not been measured longitudinally in humans; only limited data measured at 1 to 3 years apart suggested that the phenotype was stable for majority of the subjects over this time period [65]. More recently, Franke *et al* [66,67] reported different intra-individual variations during high- vs. low-soy diet [67], and using different cut-off methods and thresholds [66] for S-(–)equol producers, non-producers and those who crossed-over to either from being a producer to non-producer or vice-versa. Following antibiotic therapy, the majority of the subjects (n = 40 out of 43, or 91%) maintained their relative S-(–)equol producer or non-producer status, while one subject lost the S-(–)equol producing ability following the therapy. However, the urine sample was collected shortly after (1.7 months) the completion of antibiotic therapy and their microflora may have been incompletely restored.

Higher mammographic density is an independent risk factor for breast cancer, and is hormone sensitive. Frankenfeld *et al* [61] reported a 39% lower breast density among the S-(–)equol producing postmenopausal women as compared to non-producers. However, Atkinson *et al* [68] observed no significant association between S-(–)equol producing status and breast density among premenopausal women (mean percent breast density S-(–)equol producers: 39.34%; non-producers: 37.68%). The authors reported that the study might not have been adequately powered to detect the breast density differences between S-(–)equol producers and non-producers, and future studies with similar breast density distribution would require a sample size of 680.

Although not statistically significant, an interesting finding was that none of the S-(–)equol producers in our study had ER- breast cancer (all 8 were ER+). If this were confirmed in a larger study, it would be a clinically important finding because ER+ tumors respond much better to the treatment and have improved prognosis as compared to ER- tumors.

The inclusion of relatively high number of AA women (51 out of 127) is a major strength of our study. Given the nature of the cross-sectional study design, these are one time point measurements. Although evidence suggests that the S-(–)equol producing status is stable, additional follow-up data in-terms of cancer prognosis, and disease free survival will be very important to understand the associations between S-(–)equol producing status and disease status. Additionally, the limited sample size of the study, and lack of similar studies in the literature to serving as a basis of power calculations are among the limitations. Post-hoc sample size based on biopsy outcome and prevalence of S-(–)equol producing status revealed that a total sample size of 400 would have been needed to detect the differences between S-(–)equol producers and non-producer with alpha at 0.05 and 80% power.

We report for the first time that the prevalence of S-(–)equol producing status among postmenopausal women with abnormal mammogram undergoing a physician recommended breast biopsy is lower (17%) as compared to previous reports among general Western populations. We report a lack of any statistically significant association between S-(–)equol producing status and ductal hyperplasia, breast cancer, or overall breast pathology. If production of S-(–)equol is beneficial, the exposure will depend on daidzein intake through the diet. However, with our data it was not possible to test the hypothesis that under higher soy intake this phenotype might have protective effects. Therefore, future studies of S-

(–)equal status and breast cancer risk are needed in population with higher soy intake than ours and also where variation in soy intake exists.

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List of Abbreviations

AA	African American
AS	Asian American
BMI	Body Mass Index
CA	Caucasian American
CI	Confidence Interval
ER –ve	Estrogen receptor negative
ER +ve	estrogen receptor positive
FFQ	Food Frequency Questionnaire
LC-MRM-MS	Liquid Chromatography-Multiple Reaction ion Monitoring Mass Spectrometry
O-DMA	O-desmethylangolensin
SSQ	Soy Screen Questionnaire
UAB	University of Alabama at Birmingham

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Fig. 1.

Study recruitment and sample size overview. The breast biopsy patients at the Kirklin Clinic of the University of Alabama at Birmingham, AL, were screened on the day of biopsy for study eligibility. The study schematic shows the number of patient screened, the number eligible, the number recruited, the number non-compliant, and the final sample size for various analyses.

^aSSQ, soy screen questionnaire

^bFFQ, food frequency questionnaire

Urinary S-(-)equol analyses of study participants (n = 144)

Fig. 2.

Urinary levels of isoflavones and S-(-)equol following a three-day soy challenge were detected and quantified using liquid chromatography-multiple reaction ion monitoring mass spectrometry analysis. The values are Log₁₀S-(-)equol/daidzein, and a cut-off of -1.60 was used to define S-(-)equol producing status.

Prevalence of S-(–)equol producers in the study population (n = 144)

Fig. 3.

Urinary levels of isoflavones and S-(–)equol following a three-day soy challenge were detected and quantified using liquid chromatography-multiple reaction ion monitoring mass spectrometry analysis. Prevalence of S-(–)equol producers in the study population was calculated using $\log_{10}$ S-(–)equol/daidzein cut-off of -1.60 (represented with the solid line). Subjects with $\log_{10}$ S-(–)equol/daidzein values above the cut-off were identified as S-(–)equol producers (◆), those with values below the cut-off were identified as non-producers (○). Using this cut-off value, there were 26 (18.06%) S-(–)equol producers and 118 (81.94%) non-producers.

Table 1Baseline characteristics by S-(–)equol producing status^a

	Group S-(–)equol producers (n=25)	Group Non-producers (n=118)	Total (n=143)	p ^b
Age (years)	60.40 ± 9.89	60.99 ± 8.71	60.89 ± 8.81	0.76
Body Mass Index (kg/m ²)	28.72 ± 6.09	30.38 ± 7.80	30.09 ± 7.56	0.32
Age at menarche	12.54 ± 1.14	12.48 ± 1.75	12.49 ± 1.66	0.87
Age at menopause	46.22 ± 6.80	48.62 ± 5.94	45.27 ± 8.28	0.14
Total isoflavone intake (mg/day)	4.78 ± 11.89	4.89 ± 17.17	4.51 ± 15.78	0.54
Race: n (%)				
African American	7 (28.00%)	44 (37.28%)	51 (35.66%)	0.38
Caucasian	18 (72.00%)	74 (62.71%)	92 (64.34%)	
Hormone therapy: n (%)				
Yes	8 (32.00%)	24 (20.34%)	32 (22.38%)	0.20
No	17 (68.00%)	94 (79.66%)	111 (77.62%)	
Parous: n (%)				
Yes	19 (76.00%)	88 (74.58%)	107 (74.83%)	0.88
No	6 (24.00%)	30 (25.42%)	36 (25.17%)	
Family history of breast cancer: n (%)				
Yes	7 (28.00%)	28 (23.73%)	35 (24.48%)	0.65
No	18 (72.00%)	90 (76.27%)	108 (75.52%)	

^aData are presented as means ± SD, except where otherwise noted.^bFor continuous variables, significant differences were assessed using a 2-sample t test. For categorical variables, significant differences were tested using χ^2 analyses.

Table 2

Breast biopsy outcome of the study population by urine sample^a

	Total enrolled (n = 200)	Subjects that didn't provide their urine samples n = 56, (28%)	Subjects that provided their urine samples n = 144 (72%)	p ^b
Normal tissue (0)	97 (48.5%)	31 (55%)	66 (46%)	0.429
Hyperplasia (1)	45 (22.5%)	12 (21%)	33 (23%)	
Cancer (2)	58 (29.0%)	13 (23%)	45 (31%)	

^aData are presented as frequency (percent).

^bsignificant differences between breast biopsy outcome and study compliance in terms of subjects providing urine samples was tested using χ^2 analyses. The χ^2 value of 1.692 at p f 0.429 was not significant.

Table 3

Estrogen and progesterone receptor status of breast cancer patients by S-(-)-equol producing status^a

Receptors	Total (n = 40)	S-(-)-equol producers (n = 8)	Non-producers (n = 32)	p ^b
Estrogen receptor:				
Positive	35 (87.5%)	8 (100.0%)	27 (84.0%)	0.31
Negative	5 (12.5%)	0 (0.0%)	5 (16.0%)	
Progesterone receptor:				
Positive	28 (70.0%)	6 (75.0%)	22 (69.0%)	0.54
Negative	12 (30.0%)	2 (25.0%)	10 (31.0%)	

^a Data are presented as frequency (percent).

^b Statistical significance was tested using the Fisher's exact test and its associated 2-sided P-values.

Usual soy isoflavone intake assessments using the Block Soy Screen Questionnaire by S-(-)equol producing status^a

Table 4

Assessments from soy based food items only	Subjects with SSQ measurements (n = 143)		S-(-)equol producers (n = 25)		Non-producers (n = 118)	
	Median (P25 – P75)		Median (P25 – P75)		Median (P25 – P75)	
Kcal/d	3.17 (0.72 – 10.28)		4.30 (2.61 – 10.67)		2.47 (0.37 – 9.89)	
Soy protein (g/d)	0.23 (0.07 – 0.83)		0.33 (.20 – 0.87)		0.18 (0.035 – 0.81)	
Genistein (µg/d)	105.86 (2.97 – 892.22)		180.46 (4.95 – 598.76)		105.86 (2.97 – 1010.92)	
Daidzein (µg/d)	74.21 (4.75 – 615.17)		76.01 (7.46 – 379.82)		73.93 (4.75 – 654.97)	
Total isoflavones (µg/d)	220 (0.00 – 1235)		380 (0.0 – 1360)		145 (0.0 – 1307)	

^a Data are presented as median (interquartile range)

Table 5

Crude relative odds of biopsy outcome by S-(-)equol producing status^a

Model 1: Unadjusted model for association between S-(-)equol producing status and biopsy outcome (N = 143)						
	S-(-)equol producer	Non-producer	Odds ratio	95% Confidence Intervals		p ^b
	n	n		Lower limit	Upper limit	
Normal	10	55	--	--	--	--
Hyperplasia	6	27	0.82	0.27	2.49	0.72
Breast cancer	9	36	0.73	0.27	1.97	0.53
Breast pathology (Hyperplasia + breast cancer)	15	63	0.76	0.32	1.83	0.55

^aCrude relative odds of biopsy outcome by S-(-)equol status among 143 postmenopausal women undergoing a physician recommended breast biopsy.

^bStatistical significance was tested using logistic regression models.

Table 6

Adjusted relative odds of breast biopsy outcome by S(-)equol producing status

Model 2*: Adjusted model for association between S(-)equol producing status and breast pathology (N = 125)						
	S(-)equol producers		Non-producers		Odds ratio	
	n		n		95% Confidence intervals	p ^b
					Lower limit	Upper limit
Normal biopsy	8		51	--	--	--
Hyperplasia	5		24	0.84	0.20	3.41
Breast cancer	7		30	0.56	0.16	1.87
Breast pathology (hyperplasia + breast cancer)	12		54	0.68	0.23	1.89

* Model 2 represents the relative odds of breast biopsy outcome by S(-)equol producing status. This model was adjusted for: age, race, BMI, menarche, parity, smoking, hormone therapy, family history of breast cancer in first degree relative, total isoflavone intake from SSQ, and kcal/d from the FFQ.

^b Statistical significance of these associations was tested using logistic regression model, and was adjusted for above mentioned co-variables.