Soy Protein Containing Isoflavones and Mammographic Density in a Randomized Controlled Trial in Postmenopausal Women

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Abstract

Background: **The relatively high dietary intake of soy in Asian countries has been hypothesized to, at least partly, explain the lower breast cancer incidence patterns in these countries compared with the Western world. The aim of the present study was to determine the effect of daily soy supplementation on mammographic density, one of the strongest known risk factors for breast cancer.**
Methods: **A double-blind, randomized, controlled trial was conducted to compare the effects of soy protein intake containing 99 mg isoflavones daily with intake of milk protein (placebo) for the duration of 1 year. Two hundred and two Dutch postmenopausal women ages 60 to 75 years were randomized. Mammographic density was assessed using a quantitative computer-assisted method on digitized mammograms. Equol producer status was assessed in plasma provided at the final visit (soy group) or after a 3-day challenge with soy after the trial was finished (placebo group).**
Results: **A total of 175 women completed the baseline visits and at least one follow-up visit and were included in the intention-to-treat analyses. For 126 women, both pre- and post-trial mammograms were available. Mammographic density decreased in both study arms, but the decrease did not differ significantly between intervention and placebo groups. Equol producer status did not modify the results.**
Conclusion: **The results of this trial do not support the hypothesis that a diet high in soy protein among postmenopausal women decreases mammographic density.** (Cancer Epidemiol Biomarkers Prev 2008;17(10):2632–8)

Introduction

Isoflavones in soy (genistein, daidzein, and glycitein) are a type of phytoestrogens that are characterized by their structural similarity to mammalian estrogens and their ability to bind to the estrogen receptor. Because of these characteristics, phytoestrogens have weak estrogenic potential, possibly increasing breast cancer risk. Phytoestrogens can, however, also compete with endogenous estrogens for estrogen receptors, which may lead to a decrease in the carcinogenic potential of endogenous estrogens. Other possible mechanisms of action that may decrease breast cancer risk include inhibition of enzymes involved in endogenous estrogen production (1).

In approximately 25% to 40% of the population, daidzein can be metabolized in the intestinal tract to form the estrogenically more potent equol. Hence, equol producers have been hypothesized to experience the largest effects from soy consumption (2).

Compared with Western countries, breast cancer incidence rates in Asian countries are much lower. As the dietary intake of soy and soy-derived foods is much higher in Asia, it has been hypothesized that a high dietary intake of soy could protect against breast cancer (3). Meta-analyses including observational studies on habitual soy consumption and breast cancer risk indeed reported that soy intake may be associated with a small reduction in breast cancer risk (4), although the effect may be restricted to soy food consumption at levels commonly consumed in Asian populations (5). Observational studies with circulating levels of phytoestrogens show inconclusive results (6-9).

Mammographic density is often used as a biomarker for breast cancer risk. It represents the amount of stromal and glandular tissue in the female breast. High mammographic density has been associated with a 3- to 6-fold increase in breast cancer risk (10). Only few intervention studies with isoflavones from soy or other sources and mammographic density have been published (11-14). Two studies in premenopausal women living in Hawaii showed no effect of an intervention with soy isoflavones on mammographic density (11, 12). In postmenopausal women, the relationship between phytoestrogens and mammographic density may be different because circulating levels of endogenous hormones decline after menopause. Two intervention

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studies with phytoestrogens from red clover (13) or black cohosh (14) did not find effects on mammographic density in postmenopausal women; however, the effects of isoflavone supplements may differ from the effects of isoflavones consumed as soy (15).

In this study, we aim to investigate the effect of a 1-year soy supplementation on mammographic density as a biomarker for breast cancer risk using a double-blind, randomized, placebo-controlled trial among postmenopausal women.

Materials and Methods

Subjects. For the present study, we made use of data from a double-blind, randomized, placebo-controlled trial that was initiated to study the effect of soy consumption on bone mineral density, cardiovascular disease, cognitive function, performances in daily life, and well being (16-19). Detailed information about the study is given elsewhere (17). For each woman, the total duration of the trial (12 mo) was planned in between two consecutive breast cancer screening dates, which are every 2 y in the Netherlands. A total of 202 women were included in the trial between March and September 2000 (Fig. 1). Of these, 175 women completed the baseline visit and at least one follow-up visit and comprised the original study population. The Dutch population-based breast cancer screening invites women ages 50 to 75 y. Fourteen women (8 and 6 in placebo and soy groups, respectively) were aged over 75 y at the end of the intervention period and therefore no post-trial mammogram was available for these women. For another 31 women (20 in the placebo group and 11 the soy group), either the pre-trial or the post-trial breast cancer screening mammogram could not be collected from the archives. Three women (two in the placebo group and one in the soy group) had breasts too large to fit on a

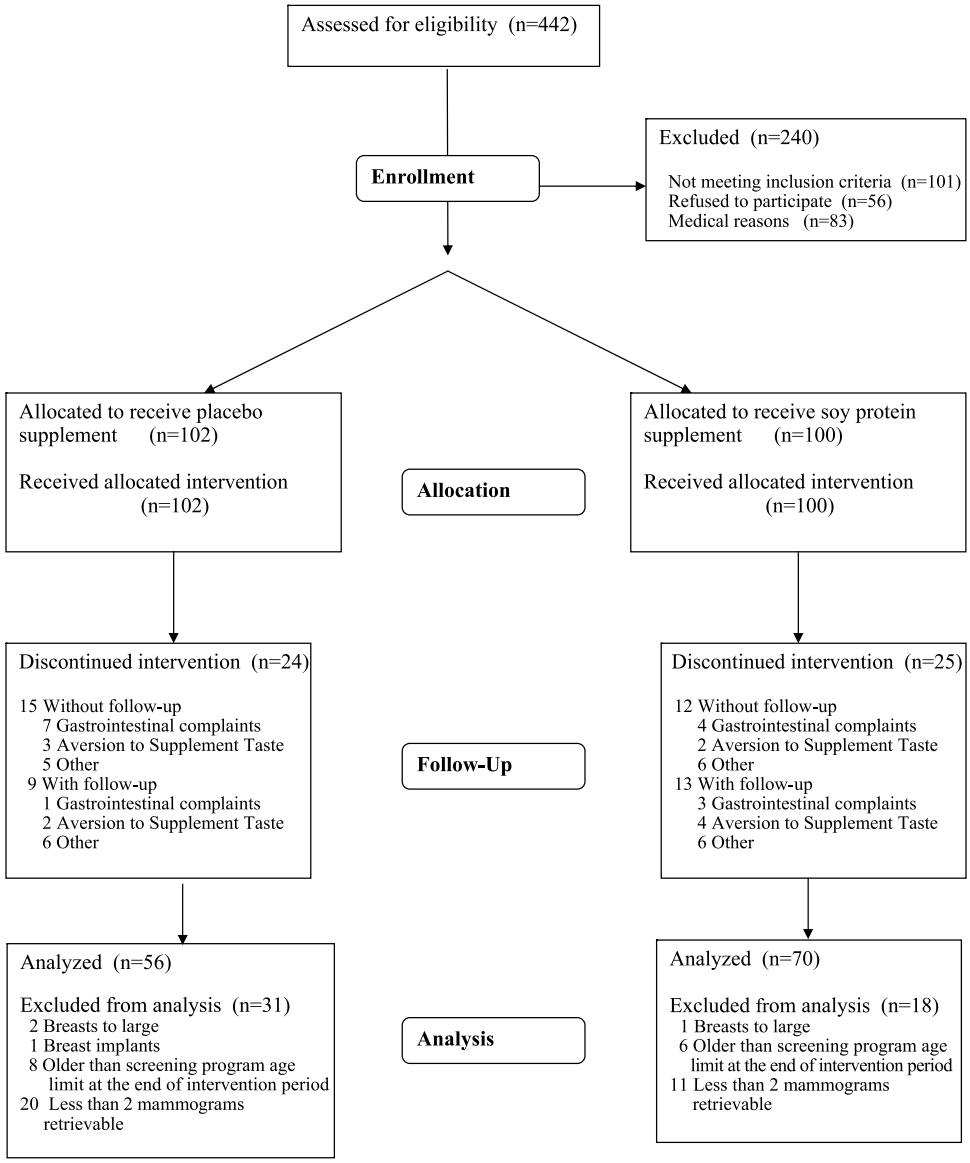

Figure 1. Flow diagram of recruitment.

single mammogram and were therefore excluded. One woman was excluded from the placebo group because she had silicone implants. The study population for analysis comprised 126 women (56 and 70 in placebo and soy groups, respectively).

All participating women signed informed consent and the study was approved by the Institutional Review Board of the University Medical Center Utrecht.

Intervention. Participants in the intervention group received 36.5 g of soy powder (Solae brand soy protein; The Solae Company) daily, containing 52 mg genistein, 41 mg daidzein, and 6 mg glycitein (aglycone weights). The placebo consisted of 36.5 g of identical-looking and identical-tasting milk protein powder (The Solae Company). Extra vitamins and minerals (riboflavin, pyridoxine hydrochloride, cyanocobalamin, folic acid, cholecalciferol, and calcium) were added to both the soy and the placebo supplement. Participants were provided with the supplements by dieticians who also provided recipes and advised on incorporation of the supplements in the individual diets of the participants.

Equol-Metabolizing Status. In addition to genistein concentrations, which were measured to assess compliance, equol levels were measured to determine which women were able to metabolize this compound. Genistein and equol concentrations in plasma were measured with TR-FIA kits (Labmaster) as previously described (20). Exposure to a sufficient amount of daidzein is required to assess equol producer status. After finishing the study, women in the placebo group with at least one follow-up visit ($n = 87$) were therefore asked to use the soy supplement for 3 consecutive days and provide a blood sample. Fifty-seven women were willing to participate. Intra-assay and inter-assay coefficients of variation were 2.2% and 14.8%, respectively. The cutoff value of 83 nmol/L, proposed by Setchell et al. (2), was used to classify participants as equol producers or equol nonproducers.

Mammographic Density Analysis. After digitizing the films using a laser film scanner (Lumiscan 50, Lumisys Eastman Kodak Co.), the total breast area on the mammogram as well as the area of dense tissue within the breast were quantified using a computer-assisted method based on gray levels of pixels in the digitized mammogram. This computer-assisted method to determine mammographic density has proved to be very reliable and the method is described elsewhere in detail (21). To compute percent breast density, the dense area is divided by the total breast area and multiplied by 100. Percent breast density is used in most publications on mammographic density. However, in women with equal percent density, the absolute amount of glandular and stromal tissue, which is regarded as the target tissue for breast cancer (22, 23), is higher in larger breasts (24). Hence, we will present results on both relative and absolute measures of mammographic density.

Mammographic density on craniocaudal views and mediolateral oblique views as well as on left and right views shows very strong correlation (25). As representative information on mammographic density can be provided in a single view and because the mediolateral view is the standard view used during breast cancer screening in the Netherlands, we used the left mediolateral mammogram for all women. All 260 mammograms were assessed by one observer in sets composed of randomly ordered films. Eight mammograms of women with breasts too large to fit on a single mammogram ($n = 3$) and women with silicone implants ($n = 100$) were not used in the data analyses. Both images of the same woman (i.e., before and after intervention/placebo) were always read in the same set, which contained 36 images. The order in which the mammograms within a set were presented to the reader was also randomized. To assess the reliability of the reader, a library set was created, which consisted of 35 randomly chosen films from our study subjects. This library set was read before the first set and after the last set. The images in the library set were randomly ordered both times. In this study, intraclass correlation coefficients of 1.00, 0.90, and 0.91 were reached between the repeated readings for total breast area, dense area, and percent density, respectively.

Data Analyses. Both measures of mammographic density (dense area and percent density) were log10 transformed to normalize their distributions. These transformed values were used in linear regression analyses. For ease of interpretation, geometric means and 95% confidence intervals (95% CI) are presented. The nondense area was normally distributed.

The difference in baseline-to-final visit change in mammographic measures between the soy and the placebo groups was determined using linear regression. The models included change as dependent variable and group allocation and baseline measurement of the breast measure under study as independent variables. The intention-to-treat analyses of the original study consisted of 175 women. Because mammographic data were unavailable for 49 women, the intention-to-treat analyses included 126 women and will therefore be referred to as modified intention-to-treat analyses. Original per-protocol analyses included 153 women. Modified per-protocol analyses included 112 women who finished the trial and of whom mammograms were available.

To determine the potential effect of the time interval between finishing the trial and the day of second mammography, subgroup analyses were done using the median time period as cutoff point. The same procedure was used for analyses with subgroups of high and low values of mammographic measures at baseline, as women with high baseline values may experience larger decrease. Finally, subjects were stratified according to equol metabolizer status. As not all women in the placebo group participated in a post-trial soy challenge, metabolizer status could be assessed in only 38 of the 56 women in the placebo group of the modified intention-to-treat analyses. Differences in effect between subgroups were tested by adding interaction terms between subgroup and intervention group to the regression models also containing the individual terms.

Two-sided P values of <0.05 were considered statistically significant. All analyses were conducted using the Statistical Analysis System software package, release 9.1 (SAS Institute).

Results

On average, women in the placebo group were 1 year younger than women in the soy group (Table 1). They were 1 year older at menopause, had slightly lower body mass index, and were less likely to currently smoke. At baseline, the average size of the dense area as well as percent density were higher in the placebo group, whereas the nondense area was larger in the soy group (Table 2). The statistically significant difference in genistein plasma levels between the soy group and the placebo groups (1,259 ± 1,610 and 55 ± 101 nmol/L, respectively; $P < 0.001$) showed good compliance.

Absolute density and percent density decreased in both study groups. This decrease was similar between study groups in both the modified intention-to-treat and the modified per-protocol analyses. The increase in nondense area was also similar between soy and placebo groups (Table 2). Including age and body mass index at time of randomization, age at menopause and smoking status to the models did not change the results materially (data not shown).

A total of 28.7% of the study participants was classified to be equol producers (21.1% and 32.9% in the placebo and soy groups, respectively). Equol producer status did not affect the outcome in either the modified intention-to-treat analyses (Table 3) or modified per-protocol analyses (data not shown). Women in the intervention group that had a relative short time period between ending the study and the date of the second mammography (below median) on average had slightly increased dense area after the intervention (Table 4). This increase was, however, not statistically significantly different from the decrease in mammographic density in the placebo group. In the subgroup of women with a time period between study ending and second mammography larger than median, the dense area decreased in both the placebo and intervention groups.

In women with baseline dense area above median, the decrease in dense area was stronger in the intervention group compared with the placebo group (Table 5). In the subgroup of women with baseline dense area below median, there was a small increase in dense area, which was not seen in the placebo group. There were, however, no statistically significant differences in baseline-to-final visit changes between any of the groups and tests for interaction were not significant (Table 5).

Discussion

In the present study, we did not observe changes in mammographic density after 1 year of daily supplementation of soy protein containing 99 mg isoflavones in a randomized placebo-controlled trial among elderly women. The observed decrease in mammographic density was expected, as natural involution of the breast occurs with age. The decrease in both the dense area and percent density was similar in both study groups and the increase of the nondense area was also similar between the soy and the placebo groups.

Major strengths of our study include the duration of the study and the large amount of soy protein consumed, which was in the upper range of dietary intake in Asian countries (26, 27). Compliance was good as genistein levels measured at the final visit differed markedly between the soy and the placebo group (19). Another strong point of the study was the assessment of participant's capability to produce equol in the intestinal tract, as this trait may define who will benefit most from soy consumption. Equol-producing capacity did, however, not modify the results.

The present trial was initially conducted to assess the effect of soy isoflavone supplementation on a range of health measures (17). The trial was designed to include women who were in between two consecutive breast cancer screening dates to assess possible changes in mammographic density. As there was a time delay between the finish of the trial and the date of post-trial mammography, a possible effect of treatment may have diminished after cessation and may have become

Table 1. Baseline characteristics of the modified intention-to-treat population

	Placebo, $n = 56$ [mean (SD) or n (%)]	Intervention, $n = 70$ [mean (SD) or n (%)]
Age (y)	65.3 (4.0)	66.3 (4.3)
BMI (kg/m^2)	25.7 (3.4)	26.3 (4.0)
Age at menarche (y)	13.5 (1.7)	13.5 (1.8)
Age at first child birth (y)	26.7 (4.7)	26.1 (3.2)
Nulliparous (n)	10 (18%)	9 (13%)
Age at menopause (y)	49.3 (3.6)	48.3 (5.5)
Time since menopause (y)	16.1 (5.8)	18.1 (7.0)
HT ever use (n)	15 (27%)	17 (25%)
Time since stop HT use (y)*	5.5 (1.5-13.5)	15 (5-18)
Never smoking (n)	32 (57%)	34 (49%)
Past smoking (n)	19 (34%)	24 (34%)
Current smoking (n)	5 (9%)	12 (17%)
Alcohol intake (g/d)*	8.1 (1.4-18.6)	10.0 (0.5-19.9)
Time periods		
First mammography to second mammography (d)*	748 (741-753)	747 (740-755)
First mammography to trial start (d)*	299 (276-324)	289 (265-312)
Trial start to trial finish (d)*	362 (357-364)	361 (357-364)
Trial finish to second mammography (d)*	98 (70-120)	101 (74-159)

Abbreviations: BMI, body mass index; HT, postmenopausal hormone therapy.
*Median and interquartile range.

Table 2. Differences in baseline-to-final visit change in mammographic density between placebo and intervention groups

	Placebo group		Intervention group		Difference in change (95% CI)	*P*
	Baseline	Final visit	Baseline	Final visit		
Modified intention-to-treat (n = 126)						
Dense area (cm^2)	11.5	10.1	9.9	8.8	−1.09 (−3.76 to 1.58)	0.42
Nondense area (cm^2)	78.5	95.3	94.4	107.7	−0.83 (−9.12 to 7.45)	0.84
% Density	15.4	10.8	10.6	8.1	−0.44 (−3.61 to 2.72)	0.78
Modified per-protocol (n = 112)						
Dense area (cm^2)	11.0	9.8	10.0	8.7	−1.11 (−4.01 to 1.78)	0.45
Nondense area (cm^2)	78.9	95.2	96.4	108.6	−0.92 (−9.94 to 8.10)	0.84
% Density	15.1	10.7	10.3	7.9	−0.91 (−4.36 to 2.54)	0.60

invisible at the time the second mammogram was taken. We do not know how fast a possible increase or decrease may go back to normal levels after cessation. Small fluctuations in mammographic density have been noted between the luteal and the follicular phase of the menstrual cycle and short (2 weeks) cessation of hormone therapy use has been shown to decrease mammographic density. This suggests that breast tissue, as reflected by mammographic density, may respond very quickly to hormonal changes (28-30). Restricting the analyses to women with a relative short time period between the ending of the study and the date of second mammography (below median, that is, <99.5 days) showed a small increase in the dense area in the soy group that was not seen in the placebo group or in the subgroup of women with a relative large time period between ending of the study and second mammography. The differences were, however, small and statistically nonsignificant, making it hard to draw conclusions about the effect of the time delay on the results.

A disadvantage of the study was the large proportion of women for whom mammograms were not available, resulting in reduced statistical power. From the 202 women in the original trial, 175 (87%) had at least one follow-up visit. Of these, 49 women did not have a complete set of mammograms. The original trial was designed to study other health-related outcomes as well, but there was no funding for making mammograms. Given all the trial logistics, we did not succeed in planning the intervention exactly between consecutive breast cancer screening dates for all women. As women over 75 years of age are no longer included in the national breast cancer screening program in the Netherlands, the women that reached this age during the intervention did not have a post-trial mammogram (n = 14). For another number of women, one or both pre- and post-trial mammograms were missing from the archives or were no longer stored (n = 31). Furthermore, a few women were excluded as their breasts were too large to fit on a single mammogram (n = 3) or because they had silicone implants (n = 1). In the intervention group, 70 (80%) had a complete set of mammograms versus 56 (64%) in the placebo group. We do not know what may have caused this difference other than chance. The time period between the date of first mammography and the start of the trial was very similar between groups, as were the total duration of the study, the time period between the finish of the trial and the second mammogram, and the total time between the first and second date of mammography. Mean mammographic density at baseline (measured both as absolute measure and as the proportion of the total breast) was higher in the placebo group compared with the soy group. The nondense area was larger in the soy group. These baseline differences may be explained, at least partly, by the small baseline differences in characteristics associated with higher mammographic density [i.e., age, body mass index (lower in the placebo group), and age at menopause (higher in the placebo group)]. As the study was randomized and as we have no reason to believe that the dropout in intervention and placebo groups was differential, we assume that the difference in baseline mammographic densities is a coincidence. Baseline differences between study groups were taken into

Table 3. Differences in baseline-to-final visit change in mammographic density between placebo and intervention groups, stratified for equol-producing capacity

		Placebo group		Intervention group		Difference in change (95% CI)	*P* difference	*P* interaction
		Baseline	Final visit	Baseline	Final visit			
Modified intention-to-treat (n = 108)								
Dense area (cm^2)	Prod*	11.9	9.9	9.2	8.3	0.56 (−6.69 to 7.80)	0.88	0.66
	Non-prod†	11.2	9.0	10.4	9.0	−0.50 (−3.50 to 2.49)	0.74	
Nondense area (cm^2)	Prod	86.3	101.8	82.0	97.7	−0.81 (−22.47 to 20.86)	0.94	0.99
	Non-prod	79.8	95.8	101.7	113.6	−1.91 (−11.92 to 8.09)	0.70	
% Density	Prod	12.2	9.0	11.8	8.3	−0.00 (−0.09 to 0.09)	0.94	0.95
	Non-prod	15.5	10.2	10.0	7.8	−0.01 (−0.04 to 0.03)	0.79	

*Equol producers, n = 31.
† Equol nonproducers, n = 77.

Table 4. Differences in baseline-to-final visit change in mammographic density between placebo and intervention groups, stratified on median time period between finishing the study and the second mammogram

		Placebo group		Intervention group		Difference in change (95% CI)	*P* difference	*P* interaction
		Baseline	Final visit	Baseline	Final visit			
Modified intention-to-treat (n = 126)								
Dense area (cm^2)	<Median*	10.1	9.1	9.0	9.3	0.46 (−3.70 to 4.63)	0.82	0.24
	≥Median†	13.2	11.2	10.9	8.4	−2.61 (−6.09 to 0.87)	0.14	
Nondense area (cm^2)	<Median	70.7	92.3	92.6	113.1	4.55 (−7.88 to 16.97)	0.47	0.45
	≥Median	86.7	98.6	96.1	102.7	−4.91 (−15.17 to 5.36)	0.34	
% Density	<Median	17.3	11.2	10.3	7.7	−0.02 (−0.06 to 0.02)	0.37	0.94
	≥Median	13.6	10.4	11.0	8.4	0.00 (−0.05 to 0.05)	0.96	

NOTE: Median time period was 99.5 and 96.5 d in the intention-to-treat and the per-protocol analyses, respectively.
*Below median time period between finishing the study and the second mammogram.
†Median or larger time period between finishing the study and the second mammogram.

account by including the baseline breast measures as covariate in the regression models.

Our results are in line with the null results of two previous studies on soy isoflavones among premenopausal women in Hawaii (11, 12), of which one was small (n = 30) and used an isoflavone tablet. The second study was more comparable with our study not only in size (220 women were randomized) but also in the intervention being isoflavones consumed as soy or soy-based foods (equivalent of 50 mg of isoflavones daily; ref. 12). Two studies with isoflavones from nonsoy sources [i.e., red clover in a 1-year trial with mostly postmenopausal women (13) and black cohosh in a 6-month trial with postmenopausal women (14)] also did not find effects on mammographic density.

In contrast to the findings of randomized trials, most (12, 31-33) but not all (34) cross-sectional studies show that women with high habitual consumption of soy have lower mammographic density. This might suggest that the duration of the intervention in the trials was too short. Trials with hormone therapy and trials with tamoxifen, however, have shown that a change in mammographic density is visible in a 1-year time interval (35-37), making it unlikely that isoflavones do affect mammographic patterns, at least not to the same extent as hormone therapy or tamoxifen. The amount of isoflavones used in our study was probably sufficient as it was at the upper range of dietary intake in Asian countries (26, 27). In these ranges of intake, high phytoestrogen intake has been shown to relate inversely to breast cancer risk in observational studies.

As the SD of the change in mammographic density during the trial was quite large [mean change, −1.84 cm^2 dense area (SD, 8.95) and −4.34 percent density (SD, 9.58)] and because we lost some women without available mammograms, our study was underpowered to find small differences in change between soy and placebo groups. We had 70% power to detect a difference in effect of 3.7 cm^2 dense area or 3.9 percent density, with a significance level of 0.05. Another possibility for not finding a treatment-induced effect is the relatively old age of our study population. Decreased mammographic density as observed in intervention studies with tamoxifen is generally much stronger among premenopausal women or young postmenopausal women compared with postmenopausal women in the age range of the present study (35, 38, 39). This difference in effect between younger and older women may be caused by the natural age-related involution of the breast. A potential effect of soy may be less conspicuous in the involuted breast compared with a breast that is still dense. Our results in the subgroup of women who had larger than median breast density at baseline support this. Numbers in this analysis were, however, small and the stronger decrease in the intervention group was not statistically significantly different from the decrease in the placebo group. It has furthermore been suggested that soy consumption at pubertal age has the largest effect on breast cancer risk. Studies with adolescent soy intake and breast cancer risk support this (40, 41); however, there is, to our knowledge, no experimental support for this hypothesis. The effects of isoflavones

Table 5. Differences in baseline-to-final visit change in mammographic density between placebo and intervention groups, stratified on median baseline breast measures

		Placebo group		Intervention group		Difference in change (95% CI)	*P* difference	*P* interaction
		Baseline	Final visit	Baseline	Final visit			
Modified intention-to-treat (n = 126)								
Dense area (cm^2)	<Median*	5.7	5.5	5.8	6.4	1.07 (−1.91 to 4.05)	0.47	0.16
	≥Median†	19.5	15.8	19.6	13.2	−2.47 (−6.45 to 1.51)	0.22	
Nondense area (cm^2)	<Median	49.0	69.2	57.2	74.8	0.61 (−13.76 to 14.98)	0.93	0.95
	≥Median	117.7	130.2	124.0	133.9	−1.69 (−9.91 to 6.52)	0.68	
% Density	<Median	6.5	4.7	5.9	4.9	0.61 (−0.71 to 1.94)	0.36	0.75
	≥Median	25.8	17.8	26.0	16.9	−0.72 (−6.63 to 5.18)	0.81	

NOTE: Median baseline dense area (cm^2), nondense area (cm^2) and % density were 11.1, 88.4 and 14.6 in the intention-to-treat analyses and 11.3, 89.6 and 14.6 in the per-protocol analyses, respectively.
*Below median baseline breast measure.
†Median or larger baseline breast measure.

may therefore differ according to a woman's age of consumption.

In conclusion, the findings in this 1-year double-blind, randomized, placebo-controlled trial do not support any effect (beneficial or adverse) of a large quantity of soy protein containing isoflavones on mammographic density in postmenopausal women. Effects of soy intervention at other ages may be possible but have to be investigated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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