Effect of Soy Protein Containing Isoflavones on Cognitive Function, Bone Mineral Density, and Plasma Lipids in Postmenopausal Women

A Randomized Controlled Trial

Sanne Kreijkamp-Kaspers, MD, PhD
Linda Kok, MD, PhD
Diederick E. Grobbee, MD, PhD
Edward H. F. de Haan, PhD
André Aleman, PhD
Johanna W. Lampe, PhD, RD
Yvonne T. van der Schouw, PhD

Context Postmenopausal estrogen therapy has been posited to have some beneficial effects on aging processes, but its use has risks. Isoflavones, estrogenlike compounds naturally occurring in plant foods, might confer positive effects with fewer adverse effects.

Objective To investigate whether soy protein with isoflavones improves cognitive function, bone mineral density, and plasma lipids in postmenopausal women.

Design, Setting, and Participants Double-blind, randomized, placebo-controlled trial of 202 healthy postmenopausal women aged 60 to 75 years, recruited from a population-based sample in the Netherlands, conducted between April 2000 and September 2001.

Intervention Participants were randomly assigned to receive 25.6 g of soy protein containing 99 mg of isoflavones (52 mg genistein, 41 mg daidzein, and 6 mg glycetein or total milk protein as a powder on a daily basis for 12 months.

Main Outcome Measures Cognitive function was assessed using the following instruments: dementia, Mini-Mental State Examination; memory, Rey Auditory Verbal Learning Test, immediate recall, delayed recall, and recognition, the Digit Span forward and reversed, and the Doors test; complex attention tasks, Digit Symbol Substitution and Trailmaking, A1, A2, and B; and verbal skills, Verbal Fluency A and N, animals and occupations, and the Boston Naming Task. Bone mineral density of the hip and lumbar spine was assessed using dual-energy x-ray absorptiometry scanning. Lipid assessment included lipoprotein(a), total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides.

Results A total of 175 women completed the baseline and at least 1 postintervention analysis and were included in the modified intent-to-treat analysis. Adherence was good (median plasma genistein levels, 17.2 and 615.1 nmol/L for placebo and soy group, respectively). Cognitive function, bone mineral density, or plasma lipids did not differ significantly between the groups after a year.

Conclusion This double-blind randomized trial does not support the hypothesis that the use of soy protein supplement containing isoflavones improves cognitive function, bone mineral density, or plasma lipids in healthy postmenopausal women when started at the age of 60 years or later.

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THE SUDDEN DECLINE IN ESTROgen levels after menopause coincides with acceleration of several aging processes.[1] On average, bone mineral density (BMD) decreases and cognitive function declines, whereas total cholesterol and low-density lipoprotein cholesterol (LDL-C) increase. It has been suggested that postmenopausal estrogen therapy might counteract some of these changes. However, short-term estrogen use is associated with the recurrence of vaginal bleeding,[2] and long-term use has been associated with an increased risk of breast cancer,[3,4] stroke,[5] and cardiovascular disease.[4]

Phytoestrogens, including isoflavones and lignans, are estrogenlike compounds naturally occurring in plant foods such as soy, beans and peas, fruits, vegetables, and nuts and grains.[6] These compounds can activate the estrogen receptor and cause messenger RNA transcription.[7] Depending on the situation, binding to the receptor in the presence of endogenous estrogen in premenopausal women could result in an antagonist action by competitive binding, whereas in the postmenopausal state, phytoestrogens have been hypothesized to act as an agonist. If that is the case, they could provide an alternative for traditional estrogen therapy. The normal consumption of

Author Affiliations are listed at the end of this article.
Corresponding Author: Yvonne T. van der Schouw, PhD, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, PO Box 85500, Room D 01.335, 3508 GA Utrecht, the Netherlands (y.t.vanderschouw@umcutrecht.nl).

phytoestrogens in western populations is very low, less than 5 mg/d,[6,8] but the consumption in Asian populations is 10- to 40-fold higher; the estimated intake for women in Shanghai is 40 mg/d.[9] A meta-analysis on the effects of soy protein supplementation on plasma lipids comprising 38 studies reported a decrease in total cholesterol levels by 9.3% and LDL-C levels by 12.9%.[10] Both animal and human research has suggested a preventive effect of isoflavones on bone loss[11] and recent trials with isoflavones reported an improvement in cognitive function in both college students and postmenopausal women.[12-14] Their main limitations were that the studies were small and had methodological issues, in particular the absence of blinding.

The aim of our study was to determine the effects of supplementation with soy protein, which naturally contains large amounts of the isoflavones genistein and daidzein,[15] on cognitive function, BMD, and plasma lipids in older postmenopausal women.

METHODS

The Institutional Review Board of the University Medical Center Utrecht approved the study protocol and all participants gave written informed consent.

Participants

Participants were identified via the database of a breast cancer screening program in Utrecht. We invited by mail women aged 60 to 75 years to participate. Details of the study have been published previously.[16] We excluded women with conditions for which estrogens are contraindicated (active liver disease, impaired renal function, history of breast cancer or other malignancy, history of thromboembolism or deep venous thrombosis); women with an endometrial thickness of more than 4 mm, current and recent (within past 6 months) estrogen users; and women with a known allergy or hypersensitivity to soy or cow's milk. The study was conducted between April 2000 and September 2001.

Randomization and Blinding

After completing the baseline tests, participants were randomly assigned to the intervention or the placebo group in blocks of 10. A list of randomization numbers was computer-generated. Each randomization number corresponded to 1 of the 2 possible interventions, and personnel not involved in the trial attached a label with the number to the identical boxes containing soy or total milk protein. To assess the efficacy of blinding, at the end of intervention the participants were asked whether they thought they had been assigned to the placebo or the soy group.

Intervention

The intervention consisted of 25.6 g of isoflavone-rich soy protein containing 52 mg genistein, 41 mg daidzein, and 6 mg glycitein (aglycone weights) in 36.5 g of powder (Solae, Solae Co, St Louis, Mo) that could be mixed with food or beverages. The placebo (25.6 g of total milk protein) looked and tasted identical to the soy and contained the same nutrients other than isoflavones. Extra vitamins and minerals were added to the supplement for both groups (riboflavin, pyridoxine hydrochloride, cyanocobalamin, folic acid, cholecalciferol, and calcium). One supplement was taken per day for a total of 12 months.

A certified dietitian assessed the usual dietary pattern using a food frequency questionnaire at baseline, at 3 and 6 months, and at the final visit.[17] This questionnaire has been validated and was modified slightly to capture dietary phytoestrogen intake. The dietitian counseled the participants on incorporating the supplement into their diets by providing recipes and making other suggestions. The participants were individually advised from which sources to decrease their protein intake to compensate for the extra protein intake from the supplement.

Measurements

For all participants, we measured vital signs (pulse rate, blood pressure, and weight) and recorded adverse events every 3 months during the visits. A fasting blood sample was taken at baseline and at 12 months, the final visit. Because the primary goal of this study was to investigate the effects of phytoestrogens on clinical end points, we did not plan to assess the effects of the intervention on sex hormone levels beforehand, but we collected and stored additional blood samples during the study to explore biological mechanisms for potential soy effects. Adherence was checked by assessing plasma genistein levels in the final-visit blood sample. Blood levels reflect intake from the preceding 24 to 36 hours. Participants did not know that adherence was determined by blood analysis. During the trial, women kept diaries to record the time and amount they took in. Nonused supplements were also counted.

Cognitive Testing

The participants were tested during a morning visit in a quiet room by neuropsychologically trained personnel. Cognitive testing was performed at baseline and at the final visit. Since the performance on cognitive tests can be influenced by concomitant depression,[18] we assessed the presence of depression using the self-rated Geriatric Depression Scale (GDS).[19] Depression was defined as a score of at least 11.

Cognitive tests were selected that have been documented to be sensitive to the effects of aging and that have been included in previous trials of estrogen treatment. More specifically, due to its receptor affinity in brain structures subserving memory (ie, hippocampal formation), estrogen has been associated with memory performance.[20,21] We therefore included measures of short-term and long-term verbal and visual memory. In addition, estrogen has been related to verbal processing.[22] Hence, we included measures of naming and verbal fluency. Finally, estrogen could have a more general beneficial effect on brain metabolism, which potentially could benefit complex attention functions that are universally compromised with increasing age. Therefore, we included tests of complex attention that have been

widely documented to be sensitive to cognitive aging.

The Mini-Mental State Examination[23] was used as a global test for Alzheimer disease or dementia from other causes (maximum score, 30). The Rey Auditory Verbal Learning Test was used as a measure of verbal episodic memory.[24] In this test, the participants are asked to recall a 15-word list immediately (immediate recall) for 5 times consecutively (maximum score, 75), and, after 25 to 30 minutes (delayed recall; maximum score, 15). Furthermore, the participants were asked to recognize the words out of a list of 30 (recognition, maximum score, 30). The Doors test was used to assess visual memory.[25] Participants are shown 2 series of 12 photographs of doors, which they subsequently have to recognize from arrays of 4 pictures of doors (maximum score, 24). In the Digit Span test, a subtest of the Wechsler Adult Intelligence Scale,[26] participants are asked to repeat a string of digits in the original order (digit span forward, maximum score, 8) and in the reverse order (digit span reversed, maximum score, 7) to give an impression of short-term memory and working memory. To test verbal fluency, the participant is asked to list as many nouns as possible beginning with the letters *N* and *A* and to name as many animals and occupations, each in 1 minute (score, number of nouns named). In the Boston naming task for verbal competence and semantic retrieval, the participant is shown line drawings, which have to be properly named[27] (maximum score, 200). The digit symbol substitution test, also from the Wechsler Adult Intelligence Scale,[26] measures cognitive and perceptual speed. The participant is given a code that pairs symbols with digits. The test consists of pairing as many digits to their corresponding symbols as possible in 90 seconds (score, number of paired digits). The Trailmaking test A1, A2, and B is a complex attention and mental flexibility task. In the Trailmaking test, pseudorandomly placed circles with numbers (Trailmaking A1), with letters (Trailmaking A2), and with both letters and numbers (Trailmaking B) have to be connected with a line as fast as possible in a fixed order[28] (score, seconds needed to complete the task). At baseline, we also assessed the verbal intelligence quotient using the Dutch Adult Reading Test (a Dutch version of the National Adult Reading Test[29,30]) in which the participants have to read out loud a list of words with irregular pronunciation. Completion of the entire test battery took 1 hour on average.

Bone

Bone Mineral Density. At baseline and at the end of the 12-month intervention, BMD of the left proximal femur and the lumbar spine (L1-L4) was measured by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR 1000 densitometer (Hologic Inc, Waltham, Mass). All scans were analyzed according to written manufacturer procedures.

At baseline, 201 participants underwent a measurement of BMD of the hip. In one participant scanning was not possible because of hip prostheses on both sides. In 198 participants we were able to scan the left hip, but in 3 we had to scan the right hip because of a prosthesis on the left side. Because of this small number, we analyzed the results of the right-sided scan together with those on the left side. At the end of participation the hip was scanned in 174 participants.

Dual-energy x-ray absorptiometry of the lumbar spine (L1-L4) was obtained in 202 participants at baseline and in 175 at the end of participation. Vertebral measurements had to be excluded in 12 scans for several reasons, eg, insufficient scanning of L1 or L4 in 7, projection of a corpus alienum over 1 of the 4 vertebrae with subsequent overestimation of BMD in 3, and exclusion by the machine of vertebrae during the analysis procedure in 2 patients. When we excluded a vertebra we also excluded the result of the total spine, because this is calculated from the results of the 4 vertebrae and consequently also not correct.

Bone Parameters. Ostase immunoradiometric assay (Beckman Coulter, Inc, Fullerton, Calif) is a quantitative measurement of the bone formation marker bone-specific alkaline phosphatase. We used a timed-rate method to determine inorganic phosphorus concentration and indirect potentiometry to determine calcium concentration (Synchron LX System and Access2, Beckman Coulter). All bone parameters were measured in blood plasma.

Plasma Lipids

Plasma lipid levels were assessed at baseline and the final visit. Cholesterol was assessed by an enzymatic method with the Cholesterol Reagent (Synchron LX Systems, Beckman Coulter). High-density lipoprotein-cholesterol was assessed using a direct HDL-C assay with a timed–end point method. Triglycerides were also determined with a timed–end point method (Synchron LX Systems; Beckman Coulter).

Adherence

Plasma genistein levels were measured with blood obtained at the final visit using Labmaster TR-FIA kits (Turku, Finland). Fluorescence was measured on the Wallac Victor 2 model 1420 spectrofluorimeter (Labmaster, Turku, Finland). Data were analyzed using GraphPad Prism software (GraphPad Software Inc, San Diego, Calif). Intraassay and interassay coefficients of variation were 2.2% and 14.8%, respectively. Equol is a highly active metabolite produced by the intestinal flora from the isoflavone daidzein. This metabolite is only produced in a proportion of the population. It has been suggested that possibly only this subgroup benefits from the intervention. Equol producer status was defined as equol of higher than 83 nmol/L in plasma.[31] The proportion of equol producers was 29.9%.

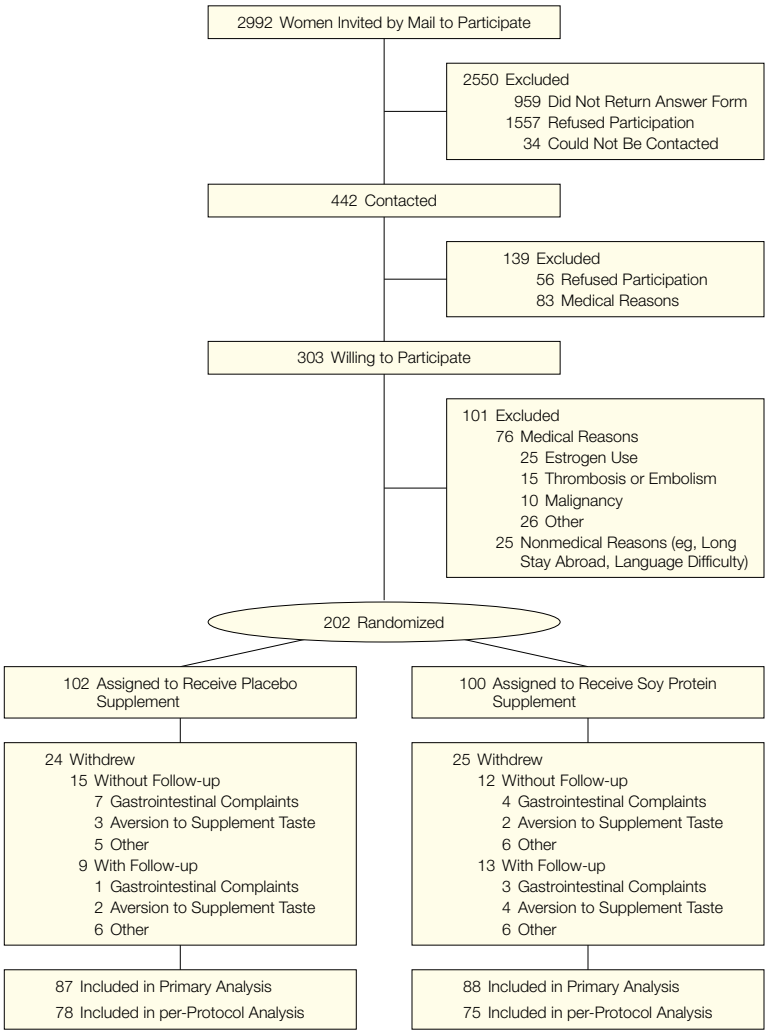
Statistical Analysis

We performed power calculations for 3 primary end points: the Rey Auditory Verbal Learning Test for cogni-

tive function, density of the lumbar spine for BMD, and total cholesterol for lipids. We planned to recruit a total of 200 participants, 100 for each group. This number was based on conventional assumptions of $\alpha = .05$ and $\beta = .20$ and a 25% rate of withdrawal from the intervention group. Assuming that soy isoflavones are as effective as conventional hormone therapy, we would be able to demonstrate an improvement of 13% on the Rey Auditory Verbal Learning Test,[20] detect a total cholesterol decrease of 7.4%, and a BMD increase of 6.7%. Similar changes in total cholesterol and BMD have been reported for soy isoflavone supplementation.[32,10]

Data were analyzed according to a modified intention-to-treat principle, including all those who had 2 measurements, including baseline, in the groups to which they were randomized. Linear regression analysis was used with baseline-to-final visit changes as the dependent variable and group allocation as the independent variable. This procedure could result in slight rounding effects in the tables. We performed a closeout visit when a participant had remained for at least 1 month. Fourteen percent of participants did not complete 1 month of treatment or were unable or unwilling to participate in a final visit. The 175 participants (86%) who completed a closeout or final visit were included in the primary, modified intention-to-treat analysis. Secondary analyses comprised a per-protocol analysis, including only the 153 participants (76%) who had completed the whole treatment protocol, and an analysis of the cognitive function results excluding 17 participants who were depressed. Furthermore, we studied whether the effect of soy differed across subgroups of postmenopausal years (<14, 14-22, >22 years), equol-producer status, body mass index (<24, 24-28, >28), smoking history (ever/never), and history of estrogen use was studied by looking at effects of intervention in the specific subgroups. We tested this effect modification by adding interaction terms between intervention and group variables to the basic model containing the 2 individual variables. To assess whether baseline differences in smoking and BMD influenced our results, we repeated the analysis adjusting for the baseline values. At the outset of the study, we decided not to adjust for multiple comparisons.[33,34] SPSS 11.0 statistical software was used to perform all the analyses (SPSS Inc, Chicago, Ill). $P<.05$ was considered statistically significant.

Figure. Participant Flow Diagram



RESULTS

Participant recruitment and enrollment are shown in the FIGURE. Between March 2000 and September 2000, we randomly assigned 202 women to the 2 treatments.

Forty-nine participants (24%) did not complete the trial for various reasons, the most important being gastrointestinal tract complaints and aversion to the taste of the supplement. Median duration of participation for the dropouts was 79 days (range, 4-285 days). There was no difference in dropout rate between the 2 groups (24 placebo, 25 soy).

TABLE 1 shows the baseline characteristics of the participants by intervention group included in the modified intent-to-treat analysis. There were no major differences between the 2 groups. Characteristics of these 2 groups also are

very similar to all those who enrolled, except that those who had at least 2 assessments were somewhat less likely to smoke, were better educated overall, and were more likely to be taking statins than those who did not. (A table showing all randomized participants is available from the author on request.) TABLE 2 shows the adverse events reported during the trial. The mean number of events per participant was 2.54 in the soy group and 2.56 events in the placebo group. There were also no differences in types of adverse events. The nutrient intake, as calculated from the food frequency questionnaire, showed similar dietary patterns for the 2 groups. Both groups reduced their intake of protein and total calories as a result of the nutrients provided by the supplement (TABLE 3). TABLE 4 shows the efficacy of the blinding, confirming that blinding had been effective. Genistein levels during the trial were markedly different between the intervention group and the placebo group (median [interquartile range]) was 17.2 nmol/L (10.29-54.0 nmol/L) for the placebo group and 615.1 nmol/L (234.3-1634.6 nmol/L) for the soy group; *P* value for difference <.001), demonstrating that adherence was good. In addition, 90% of participants used at least 80% of their supplements. Analyses evaluating whether genistein concentrations were associated with treatment effects showed no significant differences from the main effects.

Cognitive Function

Baseline performance on the cognitive function tests was similar for the 2 groups (TABLE 5). On most of the tests focusing on memory the soy group scored slightly higher, but the differences were clinically small and not statistically significant. The tests for verbal skills and the more complex tasks requiring concentration and visual attention showed no significant between-group differences. When participants with depression[11] were excluded from the analysis, the results were virtually identical. Years since menopause, equol production, body mass index, smoking status, and history of estrogen therapy did not affect the results, and adjustment for smoking to control for baseline differences also did not alter the results. (Results are available from the author on request.)

Bone

Baseline BMD and plasma bone-specific alkaline phosphatase, calcium, and phosphorus levels are shown in TABLE 6. Both groups showed a decrease in BMD after a year. At 1 year, the placebo group had a mean BMD decrease of 0.009 g/cm^2 in the intertrochanter region of the hip, whereas the soy group had a mean BMD increase of 0.004 g/cm^2, resulting in a difference in BMD change of 1.31% (*P*=.02). In the other regions, both in hip and lumbar spine, there were no major differences

Table 1. Baseline Characteristics of the Modified Intent-to-Treat Population*

Characteristics	Placebo (n = 87)	Soy (n = 88)	*P* Value
Physical characteristics, mean (SD)			
Age, y	66.7 (4.8)	66.5 (4.7)	.72
Body mass index†	25.9 (3.5)	26.4 (4.1)	.34
Blood pressure, mm Hg			
Systolic	143.9 (19.7)	138.4 (18.5)	.06
Diastolic	75.7 (14.6)	73.4 (12.2)	.25
Glucose, mg/dL	97.3 (25.2)	99.1 (18.0)	.48
Years postmenopausal	17.6 (6.2)	18.3 (7.7)	.45
Physical activity (Voorrips score)	13.6 (7.6)	14.4 (9.1)	.65
Smoking status			
Current	7 (8.0)	14 (15.9)	.11
Former	31 (35.6)	30 (34.1)	.13
Educational achievement			
Primary school	6 (6.9)	12 (13.6)	.14
Lower vocational education	17 (19.5)	11 (12.5)	.21
Lower general secondary education	22 (25.3)	21 (23.9)	.82
Higher general secondary education	24 (27.6)	19 (21.6)	.36
Higher vocational education	13 (14.9)	18 (20.5)	.34
University	5 (5.7)	7 (8.0)	.57
Medical history			
≥1 Fractures after age 40 y	24 (27.6)	28 (31.8)	.54
Hysterectomy	28 (32.2)	25 (28.4)	.59
Bilateral oopherectomy	6 (6.9)	6 (6.8)	.98
Medication use			
Current use of cholesterol-lowering medication	21 (24.1)	19 (21.6)	.80
Current use of antihypertensive medication	24 (27.6)	13 (14.8)	.04
Ever use of estrogens	21 (24.1)	19 (21.6)	.87

SI conversion: To convert glucose to mmol/L, multiply by 0.0555.
*Data are presented as number (percentage) unless otherwise indicated. Percentages may not sum to 100 due to rounding.
†Body mass index is calculated as weight in kilograms divided by the square of height in meters.

Table 2. Reported Adverse Events During the Study

Adverse Events	Placebo (n = 102)	Soy (n = 100)	*P* for Difference
Gastro intestinal complaints, eg, obstipation and gastric complaints	33	48	.14
Musculoskeletal complaints	70	68	.86
Lower and upper airway complaints, including ear, nose, and throat	70	62	.45
Urogenital complaints, eg, urinary tract infections or vaginal infections	16	16	.98
Dermatological complaints, eg, dermatitis or eczema	28	29	.23
Miscellaneous	41	30	.94
Total No. of Adverse Events	**258**	**253**	.77

Table 3. Nutrient Intake at Baseline and Final Visit for the Modified Intent-to-Treat Population

	Nutrient Intake, Mean (SD)					
	Placebo (n = 87)		Soy (n = 88)		*P* for Difference	
Variables	Baseline	Final Visit*	Baseline	Final Visit*	Baseline	Final Visit
Isoflavones, mg	7.60 (11.1)	5.27 (8.6)	7.30 (10.0)	4.91 (8.0)	.85	.78
Coumestans, mg	2.67 (4.2)	1.35 (3.2)	2.66 (3.2)	1.81 (3.6)	.99	.38
Lignans precursors, mg	1.85 (0.86)	1.68 (0.67)	1.97 (0.85)	1.94 (0.78)	.36	.02
Total phytoestrogens, mg	13.32 (15.3)	9.37 (11.3)	13.15 (13.1)	9.68 (11.5)	.94	.86
Energy, kcal	2153 (439.7)	1862 (429.9)	2129 (466.8)	1887 (447.3)	.73	.72
Protein, g						
Total	103.75 (22.8)	81.33 (18.4)	99.64 (22.4)	82.51 (19.5)	.23	.69
Plant	39.46 (11.5)	34.37 (9.4)	39.50 (11.9)	35.75 (11.2)	.98	.38
Animal	64.67 (17.8)	47.11 (14.7)	60.48 (18.6)	46.85 (14.7)	.13	.91
Fat, g						
Total	76.96 (22.4)	71.91 (24.2)	74.26 (24.8)	69.49 (21.0)	.45	.49
Saturated	32.41 (11.6)	31.80 (12.6)	30.11 (11.1)	28.79 (9.8)	.18	.09
Monounsaturated fat	28.20 (8.8)	26.20 (9.3)	27.25 (9.6)	25.09 (8.6)	.50	.42
Polyunsaturated fat	15.77 (5.6)	13.46 (5.8)	16.37 (7.8)	15.15 (6.7)	.56	.08
Carbohydrates, g	243.46 (59.9)	206.15 (56.4)	246.88 (65.1)	213.77 (59.6)	.72	.40
Fiber, g	37.52 (10.2)	31.66 (8.2)	39.01 (10.6)	33.51 (8.8)	.35	.16
Alcohol, g	10.24 (11.4)	9.29 (10.5)	10.68 (10.9)	10.90 (11.0)	.80	.34
Calcium, mg	1762 (583.2)	1319 (483.5)	1623 (533.8)	1212 (393.0)	.10	.12
Vitamin D, µg	4.28 (2.4)	3.23 (1.4)	4.27 (2.1)	3.54 (1.6)	.97	.18
Vitamin C, mg	147.20 (57.5)	135.69 (53.8)	152.69 (72.4)	131.83 (53.0)	.58	.64

*Nutrients in the supplement are not included.

Table 4. Efficacy of Blinding

Question Options	Placebo (n = 102)	Soy (n = 100)
Participant thought she was in the placebo group	18	15
Participant thought she was in the soy group	12	10
Participant did not know	52	56
Not asked	20	19

between the 2 groups. No significant differences were seen for bone-specific alkaline phosphatase, calcium, and phosphorus measurements. Adjustment for smoking history and baseline BMD did not change the results. (Results are available on request from the author.)

Subgroup analysis by the number of postmenopausal years showed that women in the lowest tertile, ie, who had the most recent start of menopause, had better results after a year of soy intervention, while women in the highest tertile did slightly worse compared with the placebo group (TABLE 7). This was the case for both hip and lumbar spine BMD measurements, but the interaction term did not reach statistical significance. Only the intertrochanter region of the hip showed a statistically significant interaction ($P=.04$) with years since menopause. Again, there was no significant interaction with equol production, body mass index, smoking history, or history of estrogen therapy.

Plasma Lipids

At baseline, the levels of plasma lipids were similar between the 2 groups (TABLE 8). In the soy group, the LDL-C and total cholesterol levels remained constant while the placebo group showed a small decrease, but the differences were not statistically significant. There was no significant interaction with age, equol production, baseline cholesterol level, smoking history, body mass index, and history of estrogen use. Adjustment for smoking to control for baseline differences did not change the results.

COMMENT

In this longer-term, relatively large double-blind, placebo-controlled, randomized trial, we did not find any effect of soy protein supplementation, which naturally contains large amounts of isoflavones, on cognitive function, BMD, or plasma lipids in the relevant population of aging women. It should be acknowledged that although BMD of the intertrochanter region of the hip was significantly higher in the soy group, it was only 1 comparison among 13 BMD measurements and may well be a chance finding.

To fully appreciate these results, some issues need to be addressed. First, a null finding in a trial could be caused by insufficient statistical power. However, the number of participants needed according to the power calculation was reached and the withdrawal from treatment did not exceed the predefined 25%. Furthermore, previous positive trials for isoflavones and cognitive function enrolled a maximum of 56 participants and the first trial to find an effect on BMD comprised only 66 participants. Since our trial had 202 participants, it seems unlikely that lack of power is a major concern.

Second, adherence is always a concern, especially with a relatively unat-

tractive intervention like a dietary supplement. However, serum genistein levels were markedly different between the intervention group and the placebo group, demonstrating good adherence.

The null finding for the Mini-Mental State Examination, the digit span, and the Rey Recognition could be caused by a

Table 5. Results for Cognitive Function for the Modified Intent-to-Treat Population

Tests*	Cognitive Function Score, Mean (SD)				Difference in Change (95% CI for Difference)	*P* Value
	Baseline		Final Visit			
	Placebo (n = 87)	Soy (n = 88)	Placebo (n = 87)	Soy (n = 88)		
Memory						
Rey Auditory Verbal Learning Test, maximum score						
Immediate recall, 75	39.8 (8.2)	41.5 (8.7)	44.5 (10.6)	47.1 (9.3)	1.03 (–1.20 to 3.27)	.36
Delayed recall, 15	7.9 (2.4)	8.2 (2.5)	9.1 (3.1)	9.7 (3.0)	0.14 (–0.54 to 0.82)	.68
Recognition, 30	28.4 (1.6)	28.4 (1.5)	28.8 (1.3)	28.9 (1.3)	0.14 (–0.29 to 0.57)	.53
Digit span, maximum score						
Forward, 8	6.1 (1.0)	6.1 (1.2)	5.8 (1.1)	5.9 (1.2)	0.19 (–0.14 to 0.51)	.27
Reversed, 7	4.5 (1.2)	4.6 (1.2)	4.4 (1.1)	4.7 (1.2)	0.18 (–0.18 to 0.54)	.32
Doors test, 24	19.2 (2.8)	19.0 (2.9)	19.9 (2.7)	19.6 (2.6)	–0.08 (–0.84 to 0.68)	.84
Complex attention						
Trailmaking test, s						
A1	43.7 (14.3)	41.5 (15.5)	40.2 (20.4)	39.0 (17.7)	0.94 (–5.26 to 7.14)	.76
A2	44.1 (18.5)	43.1 (17.9)	42.0 (32.9)	38.5 (18.7)	–0.98 (–9.70 to 7.73)	.82
B	89.4 (30.2)	85.1 (40.4)	80.3 (23.6)	78.1 (37.0)	6.54 (–2.16 to 15.23)	.14
Digit symbol substitution	50.1 (10.3)	50.4 (9.8)	51.5 (11.2)	52.4 (9.9)	0.73 (–0.89 to 2.36)	.38
Verbal tasks, score number of nouns named						
Fluency N	6.5 (3.6)	6.5 (3.6)	6.7 (3.0)	6.8 (3.2)	0.10 (–0.93 to 1.13)	.85
Fluency A	8.2 (3.1)	8.8 (3.7)	7.9 (2.6)	8.3 (2.9)	–0.23 (–1.11 to 0.64)	.60
Fluency animals	21.9 (5.5)	21.6 (5.0)	20.8 (4.1)	19.7 (4.0)	–0.78 (–2.18 to 0.61)	.27
Fluency occupations	16.2 (4.3)	16.1 (4.3)	15.4 (3.8)	14.9 (3.9)	–0.27 (–1.29 to 0.74)	.59
Boston Naming Task, maximum score, 200	157.5 (14.8)	156.7 (13.4)	160.4 (10.6)	159.3 (13.3)	–0.11 (–2.63 to 2.41)	.93
Mini-Mental State Examination, maximum score, 30	27.5 (1.7)	27.8 (1.6)	27.4 (2.2)	27.4 (1.8)	–0.24 (–0.87 to 0.40)	.47

Abbreviation: CI, confidence interval.
*The tests are described in the "Methods" section.

Table 6. Results for Bone Mineral Density and Bone-Related Laboratory Values for the Modified Intent-to-Treat Population

Variables	Bone Density Measurement, Mean (SD)				Difference in Change (95% CI for Difference)	*P* Value
	Baseline		Final Visit			
	Placebo	Soy	Placebo	Soy		
Bone mineral density, g/cm^2						
Total hip	0.831 (0.119)	0.861 (0.112)	0.826 (0.121)	0.860 (0.113)	0.005 (–0.004 to 0.013)	.27
Neck	0.695 (0.108)	0.722 (0.101)	0.691 (0.108)	0.718 (0.100)	0.001 (–0.007 to 0.008)	.89
Trochanter	0.644 (0.097)	0.667 (0.100)	0.638 (0.097)	0.666 (0.101)	0.004 (–0.003 to 0.011)	.28
Intertrochanter region	0.978 (0.149)	1.006 (0.138)	0.969 (0.149)	1.010 (0.139)	0.013 (0.002 to 0.025)	.02
Ward triangle	0.526 (0.125)	0.555 (0.126)	0.527 (0.126)	0.549 (0.130)	–0.007 (–0.021 to 0.007)	.33
Lumbar spine						
L1–L4	0.895 (0.166)	0.917 (0.150)	0.893 (0.165)	0.919 (0.160)	–0.001 (–0.010 to 0.008)	.79
L1	0.783 (0.163)	0.807 (0.154)	0.78 (0.163)	0.794 (0.158)	–0.009 (–0.024 to 0.007)	.29
L2	0.869 (0.169)	0.886 (0.147)	0.867 (0.173)	0.870 (0.183)	–0.001 (–0.018 to 0.015)	.87
L3	0.920 (0.175)	0.940 (0.168)	0.916 (0.182)	0.931 (0.205)	0.008 (–0.006 to 0.022)	.27
L4	0.988 (0.182)	0.999 (0.183)	0.984 (0.179)	1.009 (0.184)	0.003 (–0.011 to 0.016)	.73
Calcium, mg/dL	9.4 (0.3)	9.4 (0.4)	9.6 (0.3)	9.7 (0.6)	0.064 (–0.068 to 0.196)	.35
Phosphorus, mg/dL	3.8 (0.4)	3.7 (0.5)	3.7 (0.5)	3.7 (0.5)	0.043 (–0.074 to 0.158)	.47
Bone-specific alkaline phosphatase, µg/L	12.9 (4.0)	12.7 (3.9)	12.4 (3.7)	12.1 (4.4)	–0.229 (–0.983 to 0.525)	.55

Abbreviation: CI, confidence interval.
SI conversion factors: to convert calcium to mmol/L, multiply by 0.25; phosphorus, multiply by 0.323.

Table 7. Outcomes for Bone End Points Stratified by Number of Postmenopausal Years for the Modified Intent-to-Treat Population

Outcome by Years Postmenopause	Difference (95% Confidence Interval)	*P* Value for Interaction
Total hip, g/cm^2		
<14	0.013 (–0.001 to 0.027)	.07
14-22	0.008 (–0.006 to 0.022)	
>22	–0.005 (–0.022 to 0.011)	
Neck, g/cm^2		
<14	–0.004 (–0.017 to 0.008)	.89
14-22	0.018 (0.002 to 0.034)	
>22	–0.007 (–0.021 to 0.008)	
Trochanter, g/cm^2		
<14	0.006 (–0.005 to 0.017)	.49
14-22	0.007 (–0.006 to 0.020)	
>22	0.000 (–0.013 to 0.013)	
Intertrochanter region, g/cm^2		
<14	0.028 (0.005 to 0.050)	.04
14-22	0.015 (–0.004 to 0.034)	
>22	–0.002 (–0.021 to 0.018)	
Ward triangle, g/cm^2		
<14	0.004 (–0.021 to 0.028)	.49
14-22	–0.013 (–0.043 to 0.016)	
>22	–0.009 (–0.030 to 0.013)	
Lumbar spine, g/cm^2		
L1-L4		
<14	0.009 (–0.005 to 0.022)	.27
14-22	0.009 (–0.010 to 0.029)	
>22	–0.005 (–0.024 to 0.014)	
L1		
<14	0.005 (–0.013 to 0.023)	.50
14-22	–0.045 (–0.123 to 0.032)	
>22	–0.015 (–0.050 to 0.020)	
L2		
<14	0.003 (–0.022 to 0.028)	.86
14-22	–0.022 (–0.107 to 0.063)	
>22	–0.003 (–0.03 to 0.025)	
L3		
<14	0.016 (–0.007 to 0.040)	.64
14-22	–0.037 (–0.127 to 0.052)	
>22	0.001 (–0.025 to 0.026)	
L4		
<14	0.008 (–0.013 to 0.029)	.58
14-22	0.005 (–0.021 to 0.031)	
>22	–0.002 (–0.029 to 0.025)	
Calcium, mg/dL		
<14	–0.108 (–0.224 to –0.012)	.01
14-22	–0.024 (–0.184 to 0.136)	
>22	0.176 (–0.028 to 0.376)	
Phosphorus, mg/dL		
<14	–0.144 (–0.340 to 0.024)	.01
14-22	0.022 (–0.217 to 0.260)	
>22	0.183 (–0.015 to 0.380)	
Bone-specific alkaline phosphatase, µg/L		
<14	–0.121 (–1.347 to 1.105)	.79
14-22	–0.509 (–1.852 to 0.834)	
>22	–0.351 (–1.786 to 1.085)	

SI conversions: to convert calcium to mmol/L, multiply by 0.25; phosphorus, multiply by 0.323.

ceiling effect because the average score in this relatively healthy population was close to the maximum score. However, our participants had a wide range of scores for the other cognitive function tests, making a ceiling effect unlikely.

The lack of an effect on cognitive function contrasts with findings in animal experiments and a few recent trials. Animal studies using a rat model showed a clear effect of an isoflavone-rich diet on cognitive function.[35,36] In humans, 3 intervention studies have been published. The first[37] reported improvements in both long-term and short-term memory and better performance on a mental flexibility task after a 10-week soy diet. However, in addition to the small number of participants, participants were not blinded for the intervention, and comprised male and female college students, which limits the comparability with our study. In postmenopausal women, one study showed improvement on picture recall, learning rule reversals, and a planning task while another reported improvements on verbal fluency only. These studies were small and used isolated isoflavones in pills.

For BMD, several studies have indicated encouraging effects of isoflavones on osteoporosis. A recent review[11] lists the available data. Both in vitro cell cultures and in vivo animal studies almost invariably show positive effects of isoflavones on parameters related to BMD. Clinical studies are less consistent, with results varying from an improvement of 2.2% in lumbar BMD after 6 months of treatment[32] to no effect.[38] In another study, soy milk containing 85 mg isoflavones was effective in preserving bone.[39] Interestingly, the interim analyses of the second study after 1 year did not disclose a significant effect on BMD, suggesting that the duration of the intervention in our 1-year trial may still have been too short. In several clinical studies the bone formation marker bone-specific alkaline phosphatase was not affected by the intervention,[40-42] which agrees with our findings.

For plasma lipids, a large body of evidence points to a favorable effect of soy

Table 8. Results for Plasma Lipids for the Modified Intent-to-Treat Population

	Measurement, Mean (SD)					
	Baseline		Final Visit			
Variables	Placebo	Soy	Placebo	Soy	Difference in Change (95% CI for Difference)	*P* Value
Cholesterol, mg/dL						
Total	236.2 (36.6)	240.15 (45.1)	229.3 (31.2)	238.9 (42.8)	0.15 (–0.06 to 0.36)	.15
LDL	159.4 (33.9)	161.0 (38.2)	152.8 (28.1)	159.8 (39.3)	0.14 (–0.04 to 0.33)	.12
HDL	59.0 (13.14)	59.8 (15.8)	56.7 (13.1)	1.54 (59.4)	0.05 (–0.01 to 0.10)	.09
Triglycerides, mg/dL	110.6 (52.2)	120.3 (63.7)	121.2 (50.57)	122.12 (52.2)	–0.09 (–0.22 to 0.03)	.14
Lipoprotein(a), g/L	0.24 (0.27)	0.27 (0.37)	0.24 (0.31)	0.31 (0.43)	0.03 (–0.01 to 0.06)	.14

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Conversions: To convert total cholesterol, HDL-C, and LDL-C from mg/dL to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

protein. A meta-analysis of 38 studies reported a decrease in total cholesterol of 9.3% and LDL-C of 12.9%.[10] However, in contrast to the meta-analysis, several subsequent studies aimed at postmenopausal women did not demonstrate any effect on cholesterol, LDL-C, or HDL-C levels[43-48] except for 2[49,50] that showed effects on LDL-C and HDL-C but not on total cholesterol. Possibly, the findings in the meta-analyses, based mainly on trials performed in men, do not hold for postmenopausal women.

A possible explanation for the striking discrepancy between the promising findings in animal research and subsequent lack of confirmation in human trials, especially for BMD and cognitive function, may be found in species differences in the metabolism of isoflavones. One of the main metabolites of the isoflavone daidzein in rodents is equol. In humans the production is dependent on the individual's intestinal flora, and research shows that only about one third of people will produce equol when exposed to high amounts of daidzein.[51,52] Equol may be an important modifier of the effects of isoflavones.[53] However, equol production was assessed in our trial and the effects of soy on the different end points were not different from equol producers when compared with the nonproducers although statistical power was limited.

Another important factor may be the timing of the supplementation. In our trial women were on average 18 years menopausal. In a rat model, isoflavones were very effective in preventing bone loss shortly after ovariectomy, but late supplementation may not restore bone or prevent further losses.[54] Similarly, the most pronounced effects of estrogen on cognitive function have been reported in perimenopausal women, and not in late postmenopausal women.[1,55] With respect to bone, it has been suggested that it is easier to prevent changes or losses after menopause than reverse them when they have already taken place.[11] Our findings in subgroups according to years since menopause appear to support this hypothesis; in the women who were recently menopausal, our intervention seemed to improve BMD while in the late menopausal women such effect was absent. However, only the intertrochanter region of the hip showed a statistically significant interaction ($P = .04$). The influence of the timing of supplementation needs to be elucidated in further research.

In conclusion, the results of this large, double-blind, 1-year randomized trial do not support the hypothesis that isoflavones from soy protein have beneficial effects on cognitive function, BMD, or plasma lipids in older postmenopausal women.

Author Affiliations: The Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht (Drs Kreijkamp-Kaspers, Kok, Grobbee, and van der Schouw), Division of Psychonomics, Helmholz Research Institute, Utrecht University (Dr de Haan), and Department of Neuroscience, University Medical Center Utrecht (Dr Aleman), Utrecht, the Netherlands; and Fred Hutchinson Cancer Research Center, Seattle, Wash (Dr Lampe).

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Study concept and design: Grobbee, de Haan, Aleman, Lampe, van der Schouw.
Acquisition of data: Kreijkamp-Kaspers, Kok, Grobbee, Aleman, van der Schouw.
Analysis and interpretation of data: Kreijkamp-Kaspers, Kok, Grobbee, de Haan, Aleman, Lampe, van der Schouw.
Drafting of the manuscript: Kreijkamp-Kaspers, Grobbee.
Critical revision of the manuscript for important intellectual content: Kok, Grobbee, de Haan, Aleman, Lampe, van der Schouw.
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