Long-term soy isoflavone supplementation and cognition in women

A randomized, controlled trial

V.W. Henderson,
MD, MS
J.A. St. John, MPH
H.N. Hodis, MD
N. Kono, MPH
C.A. McCleary, PhD
A.A. Franke, PhD
W.J. Mack, PhD
For the WISH Research
Group

Correspondence & reprint
requests to Dr. Mack:
wmack@usc.edu

ABSTRACT

Objective: To determine the cognitive effects of long-term dietary soy isoflavones in a daily dose comparable to that of traditional Asian diets.

Methods: In the double-blind Women's Isoflavone Soy Health trial, healthy postmenopausal women were randomly allocated to receive daily 25 g of isoflavone-rich soy protein (91 mg of aglycone weight of isoflavones: 52 mg of genistein, 36 mg of daidzein, and 3 mg glycitein) or milk protein–matched placebo. The primary cognitive endpoint compared between groups at 2.5 years was change from baseline on global cognition, a composite of the weighted sum of 14 neuropsychological test score changes. Secondary outcomes compared changes in cognitive factors and individual tests.

Results: A total of 350 healthy postmenopausal women aged 45–92 years enrolled in this trial; 313 women with baseline and endpoint cognitive test data were included in intention-to-treat analyses. Adherence in both groups was nearly 90%. There was no significant between-group difference on change from baseline in global cognition (mean standardized improvement of 0.42 in the isoflavone group and 0.31 in the placebo group; mean standardized difference 0.11, 95% confidence interval [CI] –0.13 to 0.35). Secondary analyses indicated greater improvement on a visual memory factor in the isoflavone group (mean standardized difference 0.33, 95% CI 0.06–0.60) but no significant between-group differences on 3 other cognitive factors or individual test scores, and no significant difference within a subgroup of younger postmenopausal women.

Conclusion: For healthy postmenopausal women, long-term dietary soy isoflavone supplementation in a dose comparable to that of traditional Asian diets has no effect on global cognition but may improve visual memory.

Classification of evidence: This study provides Class I evidence that long-term dietary supplementation with isoflavone-rich soy protein does not improve global cognition of healthy postmenopausal women. *Neurology*® 2012;78:1841–1848

GLOSSARY

CI = confidence interval; ISP = isoflavone-rich soy protein; WISH = Women's Isoflavone Soy Health.

Isoflavones are plant-derived diphenolic micronutrients with structural similarities to steroidal estrogens. Soybeans (*Glycine max*) and soy-based food products are the major dietary source of isoflavones. The primary soy isoflavones, genistin, daidzin, and glycitin, are hydrolyzed within the intestines to their respective aglycones (genistein, daidzein, and glycitein) before systemic absorption.^{1,2}

Soy is a staple of many traditional Asian diets.^{3,4} High soy intake may reduce the risk of some chronic diseases among Asian women compared with their Western counterparts, and soy

Supplemental data at
www.neurology.org

Supplemental Data

From the Departments of Health Research and Policy (Epidemiology) and Neurology and Neurological Sciences (V.W.H.), Stanford University, Stanford, CA; Atherosclerosis Research Unit (J.A.S., H.N.H., N.K., W.J.M.) and Departments of Preventive Medicine (J.A.S., H.N.H., N.K., W.J.M.), Medicine (H.N.H.), Pharmacy (H.N.H.), and Neurology (C.A.M.), University of Southern California Keck School of Medicine, Los Angeles; and University of Hawaii Cancer Center (A.A.F.), Honolulu, HI.

WISH Research Group Coinvestigators are listed on the *Neurology*® Web site at www.neurology.org.

Study funding: Supported by the National Center of Complementary and Alternative Medicine, the Office of Dietary Supplements, and the Office of Research on Women's Health (NIH grant U01-AT001653). Solae LLC (St. Louis, MO) provided study products without charge.

Go to Neurology.org for full disclosures. Disclosures deemed relevant by the authors, if any, are provided at the end of this article.

products are suggested as a treatment option for menopausal vasomotor symptoms.^{5,6} Soy isoflavones may also benefit cognitive health in postmenopausal women, but evidence from adequately powered clinical trials is lacking. Improved cognition is reported in some clinical trials^{7–10} but not others.^{11–15} Cognitive benefit is possibly restricted to younger postmenopausal women⁶ or to specific cognitive domains.⁹ Adverse outcomes are occasionally described,¹³ and observational findings raise concern of cognitive harm.^{16,17}

Given widespread interests in soy products among postmenopausal women^{5,6} and controversial findings on cognitive function,^{7–15} we sought to examine the long-term cognitive efficacy of soy isoflavones in the Women's Isoflavone Soy Health (WISH) trial. This study of healthy midlife and older women was designed to assess the effects of dietary supplementation with isoflavone-rich soy protein (ISP) containing naturally occurring isoflavones in quantities within the upper range of traditional Asian diets.^{3,4} The primary WISH trial endpoint was the effect of ISP compared with placebo on progression of subclinical atherosclerosis.¹⁸ A secondary endpoint, reported here, was the ISP effect on cognitive change.

METHODS Design. Between June 2004 and July 2008, we conducted a randomized, double-blind, parallel-group, controlled trial of daily soy isoflavone supplements or matched placebo. Interventions occurred on the Health Sciences Campus of the University of Southern California, Los Angeles. Our primary research question assessed by Class I evidence was whether long-term ISP supplements improve global cognition among healthy postmenopausal women. Secondary research questions assessed by Class II evidence concerned long-term effects on specific cognitive domains and on global cognition in specific subgroups of women. We hypothesized that ISP improves episodic memory (because of high estrogen receptor- β concentrations in the hippocampus^{19,20}) and executive functions^{7–9} and improves global cognition among younger women closer in time to menopause⁶ and among consistent producers of equol, an isoflavone metabolite.²¹ We had no specific predictions regarding other cognitive domains or subgroups defined by menopausal status²² or vasomotor symptoms.²³

Standard protocol approvals, registrations, and participant consents. The WISH study was approved by institutional review boards of the University of Southern California and Stanford University. Participants provided written informed consent before study-related procedures were performed. The protocol is registered at ClinicalTrials.gov, identifier number NCT00118846.

Participants. Eligible participants were healthy postmenopausal women. Postmenopausal status was based on a serum

level of total estradiol <20 pg/mL and absence of vaginal bleeding for at least 1 year (natural menopause) or bilateral oophorectomy at least 1 year before enrollment (surgical menopause). Exclusion criteria included use of menopausal hormone therapy or isoflavone dietary supplements during the preceding month, history or signs of cardiovascular disease, history of diabetes mellitus or fasting serum glucose >126 mg/dL, triglyceridemia, uncontrolled hypertension, untreated thyroid disease, renal insufficiency, alcohol or substance abuse, life-threatening disease, or inability to participate in outcome assessments.¹⁸ Demographic information, reproductive history, and medication and nutraceutical use were obtained by structured questionnaires. Women recorded in a diary the number of daily hot flashes and rated their intensity as mild, moderate, or severe²⁴ (median duration of recording = 31 days, SD 3.0). Body mass index (kg/m²) was calculated from measured weight and height.

Randomization, masking, and interventions. Assignment to treatment group in a 1:1 ratio used blocked randomization (masked block size of 4) within 2 strata of carotid artery intima-media thickness, assessed by high-resolution B-mode ultrasonography as described previously.¹⁸ The computer-generated randomization sequence (SAS statistical software) was prepared before trial initiation by a statistician. For each stratum defined by carotid artery intima-media thickness, the randomization list included a unique randomly assigned product identification number and the allocation code (ISP or placebo). The database link between product identification number and allocated treatment was unavailable to other study staff. Packets of blinded study product were prepared based on the randomization list. Upon determination of trial eligibility for a given participant, clinic staff pulled the next packet in sequence from the appropriate stratum and recorded the product identification number. Apart from the single unblinded statistician, all participants, investigators, staff, and data monitors were masked to group assignment.

Participants were assigned to a total daily dose of 25 g ISP or to milk protein—matched placebo (0 mg isoflavones) offered in powder or bar form (Solae LLC, St. Louis, MO). ISP and placebo product were identical in appearance and taste. ISP contained 91 mg aglycone weight isoflavones (154 mg total isoflavone equivalents) composed of genistein (52 mg aglycone equivalents), daidzein (36 mg aglycone equivalents), and glycitein (3 mg aglycone equivalents). Protein, isoflavone, and select vitamin and mineral contents were determined by analytical testing before release to ensure target levels for macronutrients and micronutrients. Clinic visits occurred every month for the first 6 months and every 2 months thereafter, for a planned treatment period of 2.5 years. The study dietician instructed women not to consume soy supplements, but participants were not otherwise asked to alter usual dietary habits.

Outcomes and follow-up. A comprehensive neuropsychological battery (table e-1 on the *Neurology*[®] Web site at www.neurology.org) assessed a broad spectrum of cognitive skills, emphasizing standardized tests sensitive to age-associated change in middle-aged and older adults. Cognitive ability was estimated at baseline with the Wechsler Test of Adult Reading.²⁵ Mood was assessed with the Center for Epidemiologic Studies-Depression scale.²⁶ The battery was administered in a fixed order; the median baseline completion time was 70 minutes (interquartile range 63–76 minutes).

The primary outcome was change in global cognition compared between treatment groups, evaluated at baseline and after 2.5 years. Planned secondary analyses included cognitive factors and individual test scores. Subgroup analyses examined treatment group differences on global cognition based on age (<60

years or ≥ 60 years), time since menopause (< 5 years, 5–10 years, or > 10 years), menopause type (natural or surgical), baseline vasomotor symptoms (any or none), and equol producer status (nonproducer, intermittent producer, or consistent producer). At each trial visit, unused portions of the study product and the amount consumed were determined; adherence was calculated as amount consumed divided by amount that should have been consumed.

As ancillary measures of adherence, plasma and urinary genistein, daidzein, glycitein, and equol were quantified at baseline and at 6-month intervals, using high-pressure liquid chromatography with isotope dilution electrospray ionization (negative mode) tandem mass spectroscopy.²⁷ For ISP recipients, equol producer status was defined by plasma equol levels at postrandomization visits: nonproducer, equol level never > 20 nmol/L; intermittent producer, equol level > 20 nmol/L at some visits; or consistent producer, equol level > 20 nmol/L at all visits.

Statistical analysis. Sample size estimates based on the primary outcome of progression of carotid intima-media thickness required randomization of 300 women to detect a treatment effect size of 0.4 at 90% power and 2-sided α of 0.05, assuming 10% annual dropout. From the 350 women who were randomly assigned, 313 women in this analysis of cognitive outcomes allowed detection of treatment effect sizes of 0.32 at 80% power and 0.37 at 90% power (2-sided α of 0.05). For individual cognitive tests, we adopted an α level of 0.004 (0.05/14), with min-

imum detectable treatment effect sizes of 0.44 (80% power) and 0.49 (90% power).

An intention-to-treat analysis was performed for all participants who completed cognitive assessments at baseline and at the end of the trial. Baseline characteristics were compared between treatment groups with independent-sample t tests for continuous variables and χ^2 tests for categorical variables. Planned data reduction was undertaken to reduce type 1 error. The specified primary cognitive endpoint compared between treatment groups was change from baseline on a global cognitive composite score calculated as an average of standardized scores for 14 neuropsychological tests, weighted by the inverse intertest correlation matrix. Test means and SDs for standardization used baseline test data for the entire sample. Because cognitive effects of neuroactive compounds might vary by cognitive domain, secondary analyses compared change scores for cognitive factors and individual neuropsychological tests. Cognitive factor scores were derived from raw test scores using principal component analysis with an orthogonal varimax rotation.

Analysis of covariance was used to test for treatment group differences on the weighted cognitive composite change score, adjusting for baseline composite score and carotid intima-media stratum. Secondary analyses of cognitive factors and individual tests were performed in the same manner. Subgroup secondary analyses used analysis of covariance to evaluate whether treatment effects on the cognitive composite change score differed by subgroup membership, with treatment as the class variable and adjustment for baseline values of the composite score and intima-media stratum. Effect modification was tested by adding subgroup $\times$ treatment interaction terms. To evaluate whether ISP-associated equol production contributed to cognitive change, equol nonproducers, intermittent producers, and consistent producers were compared with the placebo group with analysis of covariance on the composite change score, controlling for baseline score and intima-media stratum. Data were analyzed using SAS 8.02 statistical software (SAS Institute Inc., Cary, NC).

RESULTS Of 1,063 community volunteers screened for eligibility, 350 women were randomly assigned to a treatment group (175 ISP and 175 placebo); 313 women with both baseline and endpoint cognitive test data were included in intention-to-treat analyses (figure 1). Treatment groups did not differ at baseline on demographic or clinical characteristics (table 1) or neuropsychological test performances (table e-1). Participants not contributing to this analysis (20 ISP and 16 placebo) did not significantly differ from other women on demographic or clinical variables or cognitive composite score. They performed less well at baseline on 4 individual neuropsychological tests (table e-1).

Adherence. Median (interquartile range) packet and bar adherence was 90% (80%–97%) for women receiving ISP and 89% (76%–96%) for women receiving placebo ($p = 0.41$, Wilcoxon rank sum test). ISP-treated women had significantly higher plasma and urinary isoflavone levels during the trial compared with placebo-treated women (table e-2). No participant reported on-trial use of soy or isoflavone supplements.

Table 1 Participant characteristics at baseline

Characteristic ^a	ISP group (n = 154)	Placebo group (n = 159)
Age, y, mean (SD)	61 (7)	61 (7)
Race or ethnicity, n (%)		
White, non-Hispanic	91 (59)	106 (67)
Black, non-Hispanic	11 (7)	8 (5)
Hispanic	28 (18)	21 (13)
Asian	17 (11)	19 (12)
Other	7 (5)	5 (3)
Education, n (%)		
Less than high school	2 (1)	2 (1)
High school or some college	66 (43)	56 (35)
College graduate	86 (56)	101 (64)
Age at menopause, y, mean (SD) ^b	49.4 (4.6)	49.6 (4.7)
Time since menopause, y, mean (SD)	11 (8)	11 (8)
< 5 y, n (%)	31 (20)	32 (20)
5–10 y, n (%)	45 (29)	43 (27)
> 10 y, n (%)	65 (42)	63 (40)
Unknown, n (%) ^b	13 (9)	21 (13)
Type of menopause, n (%)		
Natural	141 (92)	139 (88)
Surgical	13 (8)	19 (12)
Unknown	0	1 (<1)
Past use of hormone therapy, n (%)		
Yes	114 (74)	110 (69)
No	40 (26)	49 (31)
Hot flashes, n (%) ^b		
None	73 (50)	69 (46)
Any, within previous month	72 (50)	81 (54)
Hot flashes among those with any, mean no./d (SD)		
Mild	1.2 (1.6)	0.8 (1.0)
Moderate	0.6 (1.2)	0.8 (1.7)
Severe	0.3 (0.9)	0.3 (1.2)
Any	2.0 (2.4)	1.9 (2.7)
Body mass index, kg/m ² , mean (SD)	26.5 (5.0)	26.7 (5.3)
Systolic blood pressure, mm Hg, mean (SD)	117 (13)	118 (15)
Diastolic blood pressure, mm Hg, mean (SD)	75 (9)	75 (9)
Verbal intelligence quotient estimate, mean (SD) ^b	107 (12)	109 (11)
Mood (CES-D score), mean (SD)	6.9 (6.4)	7.5 (7.1)

Abbreviations: CES-D = Center for Epidemiological Studies-Depression scale; ISP = isoflavone-rich soy protein.

^a Between-group differences were not significant for any baseline comparison (all $p \geq 0.10$).

^b Age at menopause and time since menopause were unknown for 34 women (hysterectomy without oophorectomy or hysterectomy where it was not known whether oophorectomy was performed), hot flash information was missing for 18 women (9 in the ISP group and 9 in the placebo group), and IQ estimates (Wechsler Test of Adult Reading scores) were missing for 4 women (2 in the ISP group and 2 in the placebo group).

Efficacy. On average, women in the ISP and placebo groups improved over time on the global cognitive composite change score, but the mean between-

group difference of 0.11 standardized units (95% confidence interval [CI] -0.13 to 0.35) was not significant (table 2, figure e-1). In the principal component analysis of individual neuropsychological test change scores, a 4-factor solution (table 2) accounted for 66% of the variance. We computed factor scores by selecting tests with factor loading >0.54 , standardizing test scores using the overall sample mean and SD at baseline, and summing these standardized scores. The first factor (eigenvalue = 3.53, 25% of variance) represented aspects of executive function (including working memory), expression, and visuospatial skills (executive/expression/visuospatial [general intelligence] factor). The other factor scores represented aspects of episodic memory: verbal learning factor (eigenvalue = 1.99, 14% of variance), verbal logical memory factor (eigenvalue = 1.95, 14% of variance), and visual memory factor (eigenvalue = 1.71, 12% of variance). Participants with missing component tests (60 of 8,764, $<1\%$ of all test scores) did not have corresponding factor scores. Cognitive factor change scores were better for the ISP group compared with the placebo group for the visual memory factor (mean standardized difference 0.33, 95% CI 0.06 – 0.60); differences for other factors were not significant (table 2). Change scores did not differ significantly between treatment groups for any individual neuropsychological test, with nonsignificant trends noted for Letter-Number Sequencing (favoring placebo) and Faces I (favoring ISP) (table 2, figure e-1).

Subgroup analyses. Effects of treatment group on the cognitive composite change score were not significantly modified by age, time since menopause, menopause type, or presence of vasomotor symptoms (table 3). There was a nonsignificant trend based on time since menopause ($p = 0.07$). Post hoc analyses suggested that women between 5 and 10 years postmenopause receiving ISP were more likely to show cognitive improvement than women within 5 years of menopause or more than 10 years after menopause. There was also a nonsignificant trend based on type of menopause ($p = 0.06$), with surgically menopausal women receiving ISP performing significantly worse on the cognitive composite than surgically menopausal women receiving placebo (mean standardized difference of -0.78 ; 95% CI -1.53 to -0.02); naturally menopausal women showed small changes in the opposite direction (mean standardized difference of 0.18, 95% CI -0.07 to 0.43). More than one-fifth of participants randomly assigned to ISP were consistent equal producers. Analyses comparing consistent equal producers with women receiving placebo suggested a

Table 2 Standardized changes in cognitive scores over 2.5 years^a

Outcome variable (sample sizes for ISP and placebo groups)	ISP group (n = 154) ^b	Placebo group (n = 159) ^b	p Value
Primary endpoint: cognitive composite score (154, 159)	0.42 (0.09)	0.31 (0.08)	0.36
Secondary endpoints: individual cognitive test scores, grouped by factor			
Executive/expressive/visuospatial factor (143, 143)	0.08 (0.20)	0.45 (0.20)	0.20
Symbol Digit Modalities Test (154, 158)	−0.01 (0.04)	0.01 (0.04)	0.68
Trail Making Test, Part B (151, 155)	0.01 (0.07)	0.09 (0.06)	0.41
Shipley Abstraction (153, 152)	−0.03 (0.04)	−0.04 (0.04)	0.84
Letter-Number Sequencing (152, 158)	−0.09 (0.05)	0.08 (0.05)	0.033
Block Design (152, 151)	0.21 (0.05)	0.23 (0.05)	0.79
Judgment of Line Orientation (154, 157)	−0.04 (0.06)	−0.05 (0.06)	0.92
Category Fluency (154, 157)	−0.11 (0.05)	−0.05 (0.05)	0.41
Boston Naming Test (151, 154)	−0.01 (0.04)	0.02 (0.04)	0.66
Verbal episodic memory (list learning) factor (151, 154)	0.22 (0.12)	0.30 (0.11)	0.61
California Verbal Learning Test, immediate recall (152, 157)	0.10 (0.07)	0.17 (0.07)	0.49
California Verbal Learning Test, delayed recall (151, 154)	0.11 (0.07)	0.14 (0.06)	0.77
Verbal episodic memory (logical memory) factor (154, 157)	0.15 (0.14)	−0.12 (0.14)	0.19
East Boston Memory Test, immediate recall (154, 158)	0.08 (0.07)	−0.06 (0.07)	0.17
East Boston Memory Test, delayed recall (154, 157)	0.06 (0.08)	−0.05 (0.08)	0.32
Visual episodic memory factor (154, 157)	0.74 (0.10)	0.41 (0.10)	0.018 ^c
Faces I, immediate recall (154, 158)	0.50 (0.06)	0.30 (0.06)	0.024
Faces II, delayed recall (154, 158)	0.24 (0.06)	0.11 (0.06)	0.15

Abbreviation: ISP = isoflavone-rich soy protein.

^a For all scores, positive change represents improved performance (signs were reversed for the timed Trail Making Test). Significance was defined as $p < 0.05$ for the cognitive composite score and for cognitive factors and as $p < 0.004$ for individual test scores.

^b Values are least squares mean change (SEM).

^c Significant difference.

nonsignificant trend ($p = 0.08$) favoring consistent equol producers (mean standardized difference of 0.34, 95% CI −0.04 to 0.72) (table 3). In exploratory analyses of postmenopausal women younger than age 60 years, cognitive factor change scores were somewhat better among women receiving ISP but did not differ significantly between treatment groups (table e-3).

Safety. There were no deaths and one cardiovascular event (stroke in an ISP participant) during the trial. Five participants in the placebo group and no participant in the ISP group reported cancer; other adverse events were generally mild.¹⁸

Sensitivity analyses. Exclusion of one woman diagnosed with AD after study entry, a woman with a 2 SD drop in her East Boston Memory Test delayed recall score, and 2 women whose test scores appeared

affected by limited English did not affect study results. Primary endpoint analyses were repeated for 277 women after exclusion of 36 women who withdrew from study treatment but returned for endpoint cognitive testing. Results were unaltered, except that allocation to ISP was no longer significantly negatively associated with global cognition among surgically menopausal women ($p = 0.059$). Exclusion of 76 participants who consumed less than 80% of assigned ISP or placebo product did not further change the significance of results for global cognition or subgroup analyses.

DISCUSSION This 2.5-year trial showed that healthy postmenopausal women randomly allocated to receive daily 25-g ISP dietary supplements did not differ from women allocated to placebo on a composite change score of global cognition. Findings were similar for changes in 3 of 4 cognitive factors and individual test scores. The validity of these results is supported by the low rate of participant withdrawal and excellent on-trial adherence, assessed by product count and by isoflavone levels in plasma and urine.

Putative cognitive effects of soy isoflavones are usually ascribed to the ability of these compounds to bind estrogen receptors²⁸ but could involve other properties.^{29,30} Genistein and daidzein bind with greater affinity to estrogen receptor- β than to estrogen receptor- α .²⁸ Within the brain, estrogen receptor- β expression is relatively high in the hippocampus,^{19,20} suggesting that soy isoflavones might particularly benefit episodic memory. Poor performance on tests of episodic memory are associated with increased risk of AD.^{31,32} We found no ISP benefit for verbal episodic memory (list learning or logical memory) but noted better visual episodic memory (memory for faces). In other isoflavone trials, better picture recall has been reported for postmenopausal women⁸ and better complex figure recall for older men and women,³³ but absence of effect on similar visual memory tests is also described.^{9,14}

In a search that included PubMed and the Cochrane Central Register of Controlled Trials electronic databases (with search terms for isoflavone, soya, or tofu combined with memory or cognition, without restriction on publication date or language), we identified 9 randomized double-blind placebo-controlled trials in midlife or later-life women with objective cognitive endpoints.^{7–15} The trials identified were of shorter duration than WISH with lower power to detect potential effects of study interventions. The ISP supplement in the WISH trial, which provides principal isoflavones in a soy protein-based

Table 3 Standardized changes in global cognition over 2.5 years, subgroup analyses

Characteristic (sample sizes for ISP and placebo groups)	ISP group ^a	Placebo group ^a	p Value	p Value, subgroup × treatment interaction
Age group				0.24 ^b
<60 y (69, 72)	0.61 (0.11)	0.47 (0.11)	0.36	
≥60 y (85, 87)	0.26 (0.12)	0.18 (0.12)	0.62	
Time since menopause				0.070 ^b
<5 y (31, 32)	0.41 (0.17)	0.49 (0.16)	0.73	
5–10 y (45, 43)	0.74 (0.15)	0.41 (0.16)	0.13	
>10 y (65, 63)	0.17 (0.14)	0.25 (0.14)	0.69	
Type menopause				0.058
Natural (141, 139)	0.46 (0.09)	0.28 (0.09)	0.15	
Surgical (13, 19)	−0.10 (0.28)	0.68 (0.23)	0.045 ^c	
Vasomotor symptoms, within previous month				0.80
Any (72, 81)	0.53 (0.11)	0.45 (0.10)	0.58	
None (73, 69)	0.24 (0.14)	0.10 (0.14)	0.49	
Equol producer status				0.37 ^d
Placebo (159)		0.31 (0.08)		
ISP, nonproducer (74)	0.33 (0.12)		0.89 ^e	
ISP, intermittent producer (34)	0.40 (0.18)		0.66 ^e	
ISP, consistent producer (37)	0.65 (0.18)		0.083 ^e	

Abbreviation: ISP = isoflavone-rich soy protein.

^a Values are least squares mean change (SEM).

^b Modeled as a continuous variable; interactions remained nonsignificant when modeled categorically.

^c Significant difference.

^d Comparing means of the 4 equol status groups, $F = 1.05$.

^e Compared with placebo group.

Asian diet, was similar in composition and dose to supplements in most trials.^{7,8,10,12,14,15} By far the longest prior trial was a 12-month study of Dutch women aged 60–75 years; when compared with placebo, daily soy protein showed no significant effect on cognitive endpoints.¹² In WISH, the absence of significant ISP impact on global cognition confirms and extends this finding within a larger group of women receiving supplements for 2.5 years. In addition, the WISH trial included younger women closer to the time of menopause. Our findings fail to support the suggestion that soy isoflavones might provide particular cognitive benefit to younger postmenopausal women⁶; ISP allocation did not significantly benefit global cognition among women younger than age 60 years or within 5 years of menopause. Several smaller trials reported better performance on tasks implicating aspects of executive functions.^{7–9} We found no substantial effects of ISP on similar tests. However, different executive functions are not tightly correlated,^{34,35} and our neuro-

psychological battery did not, for example, include a sequential planning task.^{8,9}

Trial results generalize to healthy postmenopausal women. However, cognitive effects of soy isoflavones might differ for women of reproductive age and for men. Effects might also differ for producers of equol, a daidzein metabolite produced by intestinal flora in about one-third of the population.³⁶ Equol has selective affinity for estrogen receptor- β and has other biologic properties that might modify the effects of soy product consumption.²¹ Among consistent equol producers who received ISP compared with women given placebo, we noted nonsignificant improvement in global cognition.

Effects of ISP on global cognition may differ after surgical menopause compared with natural menopause. Surgically menopausal women receiving ISP showed a significant decline in global cognition; women in the placebo group improved. The between-group difference was about three-fourths of a SD, but the inference of cognitive decline is tempered by the relatively few surgically menopausal women and the relatively large mean improvement (practice effect) shown by surgically menopausal women allocated to placebo.

The WISH trial was larger and longer than prior trials of soy isoflavones, but small cognitive effects could have been missed. Soybeans have biologically active constituents other than genistin, daidzin, and glycitin,^{37,38} which individually or in aggregate could modify the cognitive effects of isoflavones. Our trial did not assess phytoestrogens more abundant in non-soy food products (e.g., flavones, coumestans, and lignans), and results cannot be generalized to these compounds. However, to the extent that other phytoestrogens involve similar estrogenic and nonestrogenic mechanisms, cognitive outcomes might be predicted to be similar.

Overall clinical trial results indicate that soy isoflavone consumption should not be pursued by postmenopausal women of any age with the goal of global cognitive benefit but that a high soy diet can be consumed without undue concern for adverse effects on memory skills associated with late-life dementia. Secondary analyses suggest that soy isoflavone consumption after surgical menopause is associated with worse global cognition, and this possibility merits further study, as does the possibility of improved global cognition in women who are consistent equol producers.

For healthy postmenopausal women, this clinical trial provides Class I evidence that ISP supplements

in a daily dose of 25 g over a 2.5-year period do not improve global cognition (mean standardized difference 0.11, 95% CI -0.13 to 0.35 , $p = 0.36$). This trial provides Class II evidence for secondary outcomes where we had specific hypotheses. Long-term ISP supplementation improves one aspect of episodic memory (visual memory) but does not improve performance on tests assessing aspects of executive functions or global cognition among postmenopausal women younger than age 60 years or within 5 years of menopause. Because of small sample sizes, evidence is insufficient to support or refute cognitive benefit among consistent equol producers.

AUTHOR CONTRIBUTIONS

Dr. Henderson: drafting/revising the manuscript for content, study concept or design, analysis or interpretation of data, obtaining funding, and technical support for cognitive assessment. J.A. St. John: drafting/revising the manuscript for content, analysis or interpretation of data, acquisition of data, and statistical analysis. Dr. Hodis: drafting/revising the manuscript for content, study concept or design, analysis or interpretation of data, and obtaining funding. N. Kono: analysis or interpretation of data and statistical analysis. Dr. McCleary: analysis or interpretation of data and technical support for cognitive assessment. Dr. Franke: analysis or interpretation of data and acquisition of data. Dr. Mack: drafting/revising the manuscript for content, study concept or design, analysis or interpretation of data, statistical analysis, study supervision or coordination, and obtaining funding.

ACKNOWLEDGMENT

The authors thank the following WISH trial contributors: Meir Stampfer, MD, J. Christopher Gallagher, MD, and Ronald M. Krauss, MD (members of Data and Safety Monitoring Board); Josh Berman, MD, PhD, Catherine Stoney, PhD, and Shan S. Wong, PhD (National Center for Complementary and Alternative Medicine, ex officio members of Data and Safety Monitoring Board); Lisa Begg, DrPH, RN, and Rebecca B. Costello, PhD (Office of Dietary Supplements, ex officio members of Data and Safety Monitoring Board); Liny Zurbrugg, RN (study coordinator), Martha Charlson, RD, Irma Flores, MA, Martha Huerta, Thelma LaBree, MA, Sonia Lavender, MA, Violetta McElreath, RN, Janie Teran, and Philip Zurbrugg (members of Clinical Center); Mei Feng, MD, Yanjie Li, MD, Lora Whitfield-Maxwell, RN, and Mingzhu Yan, MD, PhD (members of Ultrasound Image Acquisition and Processing Laboratory); Carlos Carballo, Farzana Choudhury, MS, Laurie Dustin, MS, Adrian Herbert, Michael Hutchinson, Chun Ju-Chien, George Martinez, Nitya Mathew, Olga Morales, Connie Wu, MS, and Mingzhu Xiang, MS (members of Data Coordinating Center); Gail Izumi, CLS, Arletta Ramirez, CLS, and Luci Rodriguez, CLS (members of Core Lipid/Lipoprotein Laboratory); Livia Y. Wei (member of Bone Density and Metabolic Laboratory); Chui-chen Tseng, MS, and Giske Ursin, PhD (members of Mammography Breast Density Laboratory); and Sandra M. Hebshi and Ian Pagano (members of Isoflavone Laboratory).

DISCLOSURE

The authors report no disclosures relevant to the manuscript. **Go to Neurology.org for full disclosures.**

Received November 8, 2011. Accepted in final form January 31, 2012.

REFERENCES

- Nielsen IL, Williamson G. Review of the factors affecting bioavailability of soy isoflavones in humans. *Nutr Cancer* 2007;57:1–10.
- Cederroth CR, Nef S. Soy, phytoestrogens and metabolism: a review. *Mol Cell Endocrinol* 2009;304:30–42.
- Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. *Nutr Cancer* 2006;55:1–12.
- Mortensen A, Kulling SE, Schwartz H, et al. Analytical and compositional aspects of isoflavones in food and their biological effects. *Mol Nutr Food Res* 2009;53(suppl 2):S266–S309.
- Nelson HD, Vesco KK, Haney E, et al. Nonhormonal therapies for menopausal hot flashes: systematic review and meta-analysis. *JAMA* 2006;295:2057–2071.
- The role of soy isoflavones in menopausal health: report of The North American Menopause Society/Wulf H. Utian Translational Science Symposium in Chicago, IL (October 2010). *Menopause* 2011;18:732–753.
- Kritz-Silverstein D, Von Muehlen D, Barrett-Connor E, Bressel MAB. Isoflavones and cognitive function in older women: the SOY and Postmenopausal Health in Aging (SOPHIA) Study. *Menopause* 2003;10:196–202.
- Duffy R, Wiseman H, File SE. Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacol Biochem Behav* 2003;75:721–729.
- File SE, Hartley DE, Elsabagh S, Duffy R, Wiseman H. Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause* 2005;12:193–201.
- Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F, Unfer V. Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebo-controlled study. *Fertil Steril* 2006;85:972–978.
- Howes JB, Bray K, Lorenz L, Smerdely P, Howes LG. The effects of dietary supplementation with isoflavones from red clover on cognitive function in postmenopausal women. *Climacteric* 2004;7:70–77.
- Kreijkamp-Kaspers S, Kok L, Grobbee DE, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *JAMA* 2004;292:65–74.
- Fournier LR, Ryan Borchers TA, Robison LM, et al. The effects of soy milk and isoflavone supplements on cognitive performance in healthy, postmenopausal women. *J Nutr Health Aging* 2007;11:155–164.
- Ho SC, Chan AS, Ho YP, et al. Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. *Menopause* 2007;14:489–499.
- Basaria S, Wisniewski A, Dupree K, et al. Effect of high-dose isoflavones on cognition, quality of life, androgens, and lipoprotein in post-menopausal women. *J Endocrinol Invest* 2009;32:150–155.
- White LR, Petrovitch H, Ross GW, et al. Brain aging and midlife tofu consumption. *J Am Coll Nutr* 2000;19:242–255.
- Hogervorst E, Sadjimim T, Yesufu A, Kreager P, Rahardjo TB. High tofu intake is associated with worse memory in elderly Indonesian men and women. *Dement Geriatr Cogn Disord* 2008;26:50–57.
- Hodis HN, Mack WJ, Kono N, et al. Isoflavone soy protein supplementation and progression of subclinical ath-

- erosclerosis in healthy postmenopausal women: a randomized controlled trial. *Stroke* 2011;42:3168–3175.
19. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol* 1997;388:507–525.
 20. González M, Cabrera-Socorro A, Pérez-García CG, et al. Distribution patterns of estrogen receptor α and β in the human cortex and hippocampus during development and adulthood. *J Comp Neurol* 2007;503:790–802.
 21. Setchell KD, Clerici C. Equol: pharmacokinetics and biological actions. *J Nutr* 2010;140:1363S–1368S.
 22. Henderson VW, Sherwin BB. Surgical versus natural menopause: cognitive issues. *Menopause* 2007;14:572–579.
 23. Maki PM, Drogos LL, Rubin LH, Banuvar S, Shulman LP, Geller SE. Objective hot flashes are negatively related to verbal memory performance in midlife women. *Menopause* 2008;15:848–856.
 24. Guidance for Industry: Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulva and Vaginal Atrophy Symptoms: Recommendations for Clinical Evaluation. Rockville, MD: Food and Drug Administration, Center for Drug Evaluation and Research; 2003.
 25. WTAR: Wechsler Test of Adult Reading Manual. San Antonio, TX: Harcourt Assessment; 2001.
 26. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1977;1:385–401.
 27. Franke AA, Hebshi SM, Pagano I, Kono N, Mack WJ, Hodis HN. Urine accurately reflects circulating isoflavonoids and ascertains compliance during soy intervention. *Cancer Epidemiol Biomarkers Prev* 2010;19:1775–1783.
 28. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology* 1998;139:4252–4263.
 29. Djuric Z, Chen G, Doerge DR, Heilbrun LK, Kucuk O. Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. *Cancer Lett* 2001;172:1–6.
 30. Lee YB, Lee HJ, Sohn HS. Soy isoflavones and cognitive function. *J Nutr Biochem* 2005;16:641–649.
 31. Kawas CH, Corrada MM, Brookmeyer R, et al. Visual memory predicts Alzheimer's disease more than a decade before diagnosis. *Neurology* 2003;60:1089–1093.
 32. Small BJ, Fratiglioni L, Viitanen M, Winblad B, Bäckman L. The course of cognitive impairment in preclinical Alzheimer disease. *Arch Neurol* 2000;57:839–844.
 33. Gleason CE, Carlsson CM, Barnett JH, et al. A preliminary study of the safety, feasibility and cognitive efficacy of soy isoflavone supplements in older men and women. *Age Ageing* 2009;38:86–93.
 34. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howarter A. The unity and diversity of executive functions and their contributions to complex “frontal lobe” tasks: a latent variable analysis. *Cogn Psychol* 2000;41:49–100.
 35. Henderson VW, Popat RA. Effects of endogenous and exogenous estrogen exposures in midlife and late-life women on episodic memory and executive functions. *Neuroscience* 2011;191:129–138.
 36. Atkinson C, Frankenfeld CL, Lampe JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Exp Biol Med* 2005;230:155–170.
 37. Kang J, Badger TM, Ronis MJ, Wu X. Non-isoflavone phytochemicals in soy and their health effects. *J Agric Food Chem* 2010;58:8119–8133.
 38. Klein MA, Nahin RL, Messina MJ, et al. Guidance from an NIH workshop on designing, implementing, and reporting clinical studies of soy interventions. *J Nutr* 2010;140:1192S–1204S.

Editor's Note to Authors and Readers: Levels of Evidence in *Neurology*®

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*® that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the AAN classification scheme requirements. While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care. For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.^{1–3}

1. French J, Gronseth G. Lost in a jungle of evidence: we need a compass. *Neurology* 2008;71:1634–1638.
2. Gronseth G, French J. Practice parameters and technology assessments: what they are, what they are not, and why you should care. *Neurology* 2008;71:1639–1643.
3. Gross RA, Johnston KC. Levels of evidence: taking *Neurology*® to the next level. *Neurology* 2009;72:8–10.