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Cognitive effects of soy isoflavones in patients with Alzheimer's disease

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Abstract

BACKGROUND—In a previous trial, treatment with soy isoflavones was associated with improved nonverbal memory, construction, verbal fluency, and speeded dexterity compared to treatment with placebo in cognitively healthy older men and women.

Objective—The current trial aimed to examine the potential cognitive benefits of soy isoflavones in patients with Alzheimer's disease.

METHODS—Sixty-five men and women over the age of 60 were treated with 100mg/day soy isoflavone, or matching placebo capsules for six months. *APOE* genotype was determined for all participants. Cognitive outcomes and plasma isoflavone levels were measured at Baseline, and at two additional time points.

RESULTS—Fifty-nine subjects completed all study visits. Thirty-four were women (52.3%); average age was 76.3 (SD=7.2) years, and 31 (47.7%) were *APOE*4 positive. Plasma isoflavone levels increased in subjects treated with soy isoflavones compared to Baseline and to placebo, although intersubject variability in plasma levels was large. No significant differences in treatment effects emerged between treatment groups or genders. Analyses of associations between changes in cognition and plasma isoflavone levels revealed an association between equol levels and speed dexterity and verbal fluency.

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CONCLUSIONS—Six months of 100mg/day treatment with soy isoflavones did not benefit cognition in older men and women with Alzheimer's disease. However, our results suggest the need to examine the role of isoflavone metabolism, i.e., the ability to effectively metabolize soy isoflavones by converting daidzen to equol when attempting fully clarify the cognitive effects of isoflavones.

Keywords

Soy Isoflavones; Alzheimer's disease; cognition; genistein; daidzein; equol; phytoestrogens; clinical trial

1. INTRODUCTION

Studies reporting on the cognitive effects of soy isoflavones are mixed. Observational research studies suggest a range of effects, including benefit, [1] benefit for subgroups only, [2] no effect,[3–5] and possible harm.[6] For example, data from the Honolulu-Asia Aging Study [6] suggested that consumption of tofu, a whole soybean protein rich in isoflavones was associated with brain atrophy in older Japanese Americans. A number of intervention studies have been conducted, and provide similarly conflicting results. Several small clinical trials suggested a beneficial cognitive effect,[7–10] a mixed or partial benefit,[11–14] or no effect.[15–17] A large trial enrolling postmenopausal women age 45 to 92 found a benefit but only for a visual memory.[18] All but a few clinical trials [9, 11, 17] excluded men, and most enrolled younger, postmenopausal women. Altogether, there is little know about soy isoflavone's effect on cognition in older adults, especially older adults with Alzheimer's disease (AD).

Preliminary findings from a previous study by our group enrolling cognitive healthy older adults found that treatment with soy isoflavones was associated with improved performance across several cognitive domains, including nonverbal memory, construction, verbal fluency, and speeded dexterity compared to treatment with placebo.[11] Given the potential for soy isoflavones to improve thinking skills in cognitive healthy individuals, we explored whether soy isoflavones may influence cognition in individuals with cognitive impairment, such as Alzheimer's disease. Among animal models, evidence suggests a neuroprotective effect of soy isoflavones on spatial learning and memory in a rat model of Alzheimer's disease.[20] Similarly, Ma et al.[21] found a neuroprotective effect of soy isoflavones in combination with folic acid on learning and memory in an amyloid β rat model of Alzheimer's disease. In both studies, rats injected with amyloid β performed more poorly than healthy controls; however, exposure to soy isoflavones mitigated the negative effects of β-amyloid on learning and memory performance.

Human studies of soy isoflavones in Alzheimer's disease are sparse. Jefremov et al.[22] found that both estrogens and phytoestrogens exerted a protective effect on post-mortem human brain tissues in hippocampal and frontal cortices. To our knowledge, however, no studies have been published examining the effect of soy isoflavones on living individuals diagnosed with Alzheimer's disease. Accordingly, the present study was designed to evaluate whether dietary intake of soy isoflavones may benefit cognitive functioning among

individuals diagnosed with Alzheimer's disease. Based on our previous work [11] and that of others,[18] we hypothesized that older adults with AD, who were treated with soy isoflavones would demonstrate domain-specific cognitive changes, specifically benefits in visual spatial abilities, language fluency, and speeded dexterity, rather than global cognitive effects. Furthermore, we speculated that the soy isoflavone supplement would be well tolerated with minimal safety concerns.

2. MATERIALS AND METHODS

2.1 Subjects

Seventy-two men and women over the age of 60, recruited from the community provided informed consent for study procedures. All participants had study partners who also provided informed consent. The research was performed in accordance with the Helsinki Declaration of 1975. The University of Wisconsin-Madison Health Science Institutional Review Board reviewed and approved of all study procedures. Seven subjects were excluded prior to randomization: four subjects withdrew consent, another was excluded due to an abnormal lipase laboratory value, and two participants were excluded due to their inability to complete cognitive testing. Thus, sixty-five volunteers were randomized to treatment with 100mg/day soy isoflavone, or matching placebo capsules for 6 months. Fifty-nine participants completed 6 months of treatment; two in the isoflavone arm and four participants in the placebo arm discontinued study participation early. See Figure 1 for illustration of the study's participant flow.

Other than Alzheimer's disease, subjects were free of major medical, neurological, and psychiatric illnesses. Individuals with diabetes, colon disease, and cancer other than basal or squamous cell carcinomas were excluded. Chronic, well-controlled medical conditions such as hypertension were permitted for entry into the study. All women were postmenopausal, and had not used hormone therapy for at least six months prior to enrollment in the study. Medication doses, including those of approved treatments for AD were stable for two months prior to randomization. Extensive laboratory and cognitive screenings were performed along with a medical examination to screen for exclusionary conditions. Female subjects underwent additional mammographic examination to screen for any pre-existing pathology.

2.2 Study design and data collection

Study procedures included cognitive and mood assessment; blood collection for safety laboratory testing (amylase, lipase, and phosphate levels) and isoflavone assays; symptom interview; and brief medical evaluation. Subjects also completed a standardized and validated food frequency questionnaire (FFQ) [23] in order to estimate weekly dietary intake of soy isoflavones. Study procedures were performed at Baseline and repeated at three and 6 months after treatment initiation.

2.3 Study medications

At Baseline subjects were randomized to receive either capsules containing 50 mg/day of purified isoflavones (approximately 85% daidzin and genistin, mainly as glycosides;

Novasoy® brand isoflavones; Archer Daniels Midland Co., Decatur, IL) a matching placebo capsules containing maltodextrin and caramel food color, or in this double-blind, parallel-group design study. The specific content of the isoflavone and placebo capsules are provided as supplemental data in Supplementary Table A.

Subjects and study partners were provided written instructions for dosing and a list of foods to avoid while enrolled in the study. Specifically, subjects were instructed to take one capsule twice daily, with carbohydrate-rich, low fat meals. On study visit days, subjects were instructed to take the study medication 4h prior to their visit in order to standardize the time between dosing and blood draw. However, because there are individual differences in ability to derive aglycone forms of isoflavones from glycosides, exposure to bioactive forms of the soy isoflavones was estimated by measuring plasma levels of aglycones.

2.4 Safety Monitoring

The most common adverse effects reportedly associated with high doses of isoflavones included asymptomatic hypophosphatemia, and elevated levels of plasma lipase and amylase.[129, 130] Thusly, non-fasting, safety laboratory tests included measurement of serum phosphate, and plasma levels of amylase and lipase.

2.5 Plasma isoflavone measurement and *APOE* genotyping

Isoflavone assays were performed on non-fasting blood samples collected at baseline, and after months 3 and 6. Concentrations of total daidzein, genistein and equol in plasma (0.5 mL) were measured after enzymatic hydrolysis of the glucuronide and sulfate conjugates and by a stable-isotope dilution GC-MS method that has been described previously.[24] Concentrations of daidzein, and genistein, and the metabolite, equol were expressed as nmol/L and the assays were conducted under Good Laboratory Practice (GLP) procedures and with quality assurance by incorporating within-batch and between-batch plasma samples quality control specimens. The within day reproducibility for repeat analysis of the same plasma sample expressed as %CV was 0.5% for daidzein and 1.0% for genistein and the mean between-batch reproducibility over 19 separate analytical runs was 5% (range 1.0–11.9%) for daidzein and 7% (range 1–17%) for genistein at concentrations of 100–200 ng/mL.[25]

Determination of apolipoprotein E (*APOE*) genotype was performed on a non-fasting blood sample collected at Baseline, using standard PCR and DNA sequencing techniques in a CLIA certified laboratory. DNA extracted from whole blood was amplified by PCR using specific primers for the ApoE gene and the DNA then sequenced and analyzed for genotype using the FinchTV program (Version 1.3; Geospiza, Inc.).

2.6 Neuropsychological Battery

At every study visit, a trained psychometrician administered a battery of neuropsychological measures designed to thoroughly sample memory and executive function, following standardized testing procedures. Tests included measures of verbal and visuospatial memory (List Learning,[26] Paragraph Recall,[27] Benton Visual Retention test,[28] Complex Figure Recall [26]); language/executive function (phonemic fluency,[26] animal fluency [26]);

working memory/executive function (Digit Symbol,[27] Digit Span [27]); executive function (Stroop Color Word test [29], Mazes [30] and Trail Making Test A and B [26]); and visual-motor function (Complex Figure Copy,[26] Grooved Peg Board [31]).

2.7 Assessment of Mood

To assess psychiatric symptoms, the Profile of Mood States [32] and the Geriatric Depression Scale-Short Form [33] were administered at Baseline, and months three and six following randomization.

2.8 Statistical Analysis

The primary objective of this pilot study was to examine the safety and feasibility of soy isoflavone supplement administration in men and women over the age of 60 years. Data on plasma levels of isoflavones following 1 and 6 months administration of 100mg/day purified isoflavones are presented along with data on side-effects, safety laboratory tests, and study and medication adherence estimated by FFQ and pill count. A second major objective was to examine the potential efficacy of a soy isoflavone supplement to favorably alter cognition in older adults with dementia due to Alzheimer's disease. Neuropsychological data were compared for an effect of soy administration across visits. The effect of soy isoflavone withdrawal was also determined by comparing neuropsychological performance at month 6 to performance at month 8 (washout visit). Two additional exploratory analyses were conducted. First, data from baseline cognitive testing was compared to baseline FFQ data in order to estimate the effect of prior exposure to dietary soy isoflavones. Secondly, correlations between plasma levels of the soy isoflavones (genistein and daidzein, and the metabolite of daidzein, equol) while on study medication and change on neuropsychological variables were examined in order to estimate the relationship between soy metabolism and efficacy of intervention.

Given the small sample-size, nonparametric methods were employed for all comparisons. Chi-square tests were used to examine the association between categorical variables (e.g., sex and treatment group). Comparisons between continuous variables (e.g. age) were computed using the nonparametric Wilcoxon rank-sum test. Cognitive function test p-values were adjusted for age and years of education using nonparametric Wilcoxon score general linear models.[34] Correlations between continuous variables were computed using Spearman rank-order correlations. These analyses were considered exploratory; thus, corrections for multiple comparisons were not applied.

3. RESULTS

3.1 Subjects

Fifty-nine subjects finished all study visits; partial data were available from sixty-five participants. Among participants completing the trial, thirty were women (50.8%); average age was 76.3 (SD=7.2) years, and 31 (47.7%) were *APOE* e4 positive.

Table 1 lists demographic data by randomization group. There were no significant differences between isoflavone and placebo-treated groups in age, education, or *APOE* e4

status. Furthermore, dietary intake of isoflavones, global cognition, and self-report of mood symptoms were similar between treatment groups at Baseline. These data suggest that subjects were in the early stages of AD, non-depressed and generally naïve to dietary isoflavones. Finally, the groups did not differ in use of concomitant therapies or in anthropomorphic characteristics. Nearly all participants were prescribed a single standard cholinergic therapy, and about half were taking a combination of a cholinesterase inhibiter and memantine.

3.2 Adherence and Safety Outcomes; and Isoflavone assays

Table 2 provides data related to study adherence and safety outcomes. Overall, there was good adherence to study instructions as measured with pill counts and reports of dietary soy intake. For example, medications counts suggest adequate adherence with 97–98% of pill counts aligned with expectations. No subjects were discontinued due to abnormal safety laboratory values, mean values are provided in Table 2.

Plasma isoflavone concentrations increased in all subjects treated with soy isoflavones compared to Baseline levels and to placebo-treated subjects. Table 3 lists genistein, daidzein and equol concentrations in plasma in the isoflavone - and placebo-treated participants at 3 time points: at Baseline, and after 3 and 6 months of therapy.

Despite subjects' compliance with treatment, there was high intersubject variability in the plasma concentrations within the isoflavone-treated subjects. This is illustrated in Supplementary Figure 1, which portrays individual subjects' change from baseline for specific isoflavones for participants randomized to the active treatment condition. Only a quarter of subjects demonstrated a measurable increase in equol levels after 6 months of treatment (7/27 subjects randomized to receive soy isoflavones, from whom equol levels were obtained at their final visit).

3.3 Effect of treatment with soy isoflavones on cognition

As anticipated there was no significant change in global cognition with 6 months of treatment with soy isoflavones or placebo. Both treatment groups appeared to decline on the Mini Mental State Examination over the course of treatment (see Table 4).

Wilcoxon general linear models, examining treatment effects by time revealed no significant differences between treatment groups. Planned subgroup analyses explored the effect of gender and *APOE* e4 genotype and on treatment response. Neither men nor women with AD demonstrated enhanced cognitive performance with isoflavone treatment compared to placebo. Likewise, *APOE* e4 genotype (positive or negative for the risk factor) did not influence response to treatment with isoflavones.

A final planned analysis explored the relationship between plasma levels of isoflavones and cognitive changes. Specifically, the associations between changes in plasma isoflavone levels and changes in cognitive scores were tested for four domains identified in our previous study as favorably influenced by isoflavone treatment; specifically, the cognitive domains included visual spatial memory, visual-motor, verbal fluency, and speeded dexterity. Ten sub-test scores were derived from the tests measuring the four domains. Three

of the ten comparisons (e.g., two of four measures selected, speeded dexterity and verbal fluency) showed significant correlations.Table 5 lists all correlations. Specifically, total plasma equol was significantly correlated with performance on a measure of speeded dexterity (Grooved Pegboard, non-dominant hand: $r = -0.33$, $p = 0.02$; dominant hand $r = -0.29$, $p = 0.05$). A positive association between total plasma equol and performance on a verbal fluency test (Phonemic Fluency: $r = 0.29$, $p = 0.04$). Importantly, given the small sample size and multiple outcomes, these analyses must be considered merely exploratory.

4. DISCUSSION

This randomized, controlled, clinical trial investigated the cognitive effects of soy isoflavones in older adults with Alzheimer's disease (AD). The results show no cognitive benefits over placebo after 6 months of therapy with 100mg/day soy isoflavone tablets, and global cognition declined at similar rates in both treatment and control groups. Among individuals who were effectively able to metabolize the soy isoflavone daidzein to equol however, data suggested an association between plasma levels of equol and performance on two measures of neuropsychological function: verbal fluency and speeded manual dexterity. While preliminary, these results suggest a need to further clarify how soy isoflavone metabolism influences efficacy.. To our knowledge, these findings are the first to report on the function of soy isoflavones among older adults with cognitive impairment.

The present data suggest that soy isoflavones may exert a positive, albeit modest effect on cognition. However, differences were observed only for individuals able to metabolize isoflavones, as demonstrated by circulating plasma equol levels. These data confirm in part our previous results in cognitively healthy older adults,[11] and extend findings across a wider spectrum of cognitive ability, to individuals with AD. As noted previously, the few intervention studies conducted so far enrolled primarily younger, cognitively healthy, postmenopausal women. A large randomized, controlled trial enrolling postmenopausal women age 45 to 92 revealed a decline on general intelligence with soy treatment, but a beneficial effect for visual memory.[18] The conflicting results may explain why findings from small trials are highly mixed.[7–10][11–14][15–17]

While the findings of the present study were neutral overall, the findings related to equol production are encouraging given that research on AD therapies is most notable for a lack of therapeutic effect. Moreover, the potential for achieving positive effects with a relatively benign, natural agent is promising. Correlations between improved performance and plasma equol levels were found in two domains of cognitive function; specifically, on measures of verbal fluency and speeded dexterity. As both tasks are timed, successful performance in each relies on cognitive speed. The present results suggest the possibility that soy isoflavones may function to facilitate cognitive processing speed, either directly or indirectly. Conversely, our results did not show any group differences on measures of memory or other, more complex speeded tasks of visuomotor processing. Moreover, our sample sizes are small, particularly considering the limited number of participants who demonstrated a measurable change in equol levels following administration of soy isoflavone tablets. Additional research is necessary to replicate the findings, delineate the

cognitive domains potentially affected by isoflavones, and the mechanisms or conditions in which soy isoflavones may optimally influence cognition, e.g., equol production.

This study used an isoflavone supplement that consisted mainly of isoflavone glycosides, which are not the biologically active forms of isoflavones.[39, 40] Even though hydrolysis of the glycoside is efficient,[39, 41, 42] there are significant differences in the pharmacokinetics of glycosides when compared with aglycons, with the latter being absorbed much faster and reaching higher peak plasma concentrations.[43] While some controversy exists,[44, 45] numerous lines of evidence now suggest that biologically active aglycons are likely to have increased bioavailability relative to glycosides [46] and better efficacy.[36] These data can be contrasted to the findings from St. John et al (CITE) that revealed that change in urinary excretion of isoflavones, excluding equol, was inversely correlated with change in a general intelligence factor. In other words, genistein and diadzein production was associated with a potential harmful cognitive effect.

However, production the aglycons, genistein and diadzein is not necessarily aligned with equol production. Research on the pharmacokinetics of isoflavones has established a high level of individual variability in metabolism of isoflavones, such that not all adults are “equol producers,” that is, able to convert daidzen to equol.[36, 37] Possible contributing factors to successful conversion include content and availability of isoflavones from food, variability in lactase persistence status, presence of specific gastrointestinal microflora, gastric transit times, and systemic metabolic rates.[38] Data from subjects younger and healthier than the participants included in the present study indicate that approximately one one-third of non-vegetarians are equol producers. (Setchell 2006; Newton et al. 2015). In the present study, only for individuals able to make this conversion did a pattern emerge suggesting a beneficial effect of soy.

The mechanisms by which soy isoflavones appear to exert beneficial effects on cognition may be multifactorial given their wide range of biological actions. Isoflavones genistein and the dadizein metabolite, equol are selective estrogen receptor modulators, in having greater affinity for ERβ, a receptor that is widely distributed in brain tissue, than for ERα.[47, 48] Soy isoflavones may work to confer a neuroprotective effect on brain tissue via estrogenic receptor pathways [35] and thereby defend against cell apoptosis and neurotoxicity.[49] Additionally, isoflavones are very effective antioxidants [50, 51] having greater in vitro antioxidant activity than vitamin C or E. Thus soy isoflavones may improve cognition via protection against oxidative stress. Studies indicate that amyloid β is associated with oxidative stress and mitochondrial dysfunction in the Alzheimer’s brain.[52, 53] Soy isoflavones may counteract this process by up-regulating serum and brain tissue antioxidant levels.[53] In addition, soy isoflavones may reduce reactive oxygen species (oxidants) and ameliorate β amyloid induced cellular apoptosis.[54] A further mechanism of action for soy isoflavones may involve reduction of amyloid fibril accumulation.[55]

The strengths of this study include its prospective design, and measurement of plasma isoflavones concentrations. Additionally, our study examined the potential application of isoflavones in a unique cohort with high need for effective treatment. The weaknesses include its small sample size and regional, socioeconomic and racial homogeneity of the

sample. It is unknown to what extent cultural, ethnic, and dietary factors may influence the ability to metabolize soy isoflavones, and investigation of individuals from diverse cultural and ethnic backgrounds may yield different results. Identifying multiple outcomes increases the risk of spurious findings. Additionally, the study used a glycoside conjugate rather than aglycone forms of soy isoflavones, which may have yielded different results.

Conclusion

This small prospective study suggested no cognitive benefits over placebo from 6-months of therapy with 100mg/day a soy isoflavone supplement. However, there was evidence for mild to modest cognitive benefit with among older individuals with AD, who were able to metabolize the isoflavone supplement, specifically to equol. Given the lack of efficacy of current pharmacotherapy in AD, the possibility of any additional treatments is encouraging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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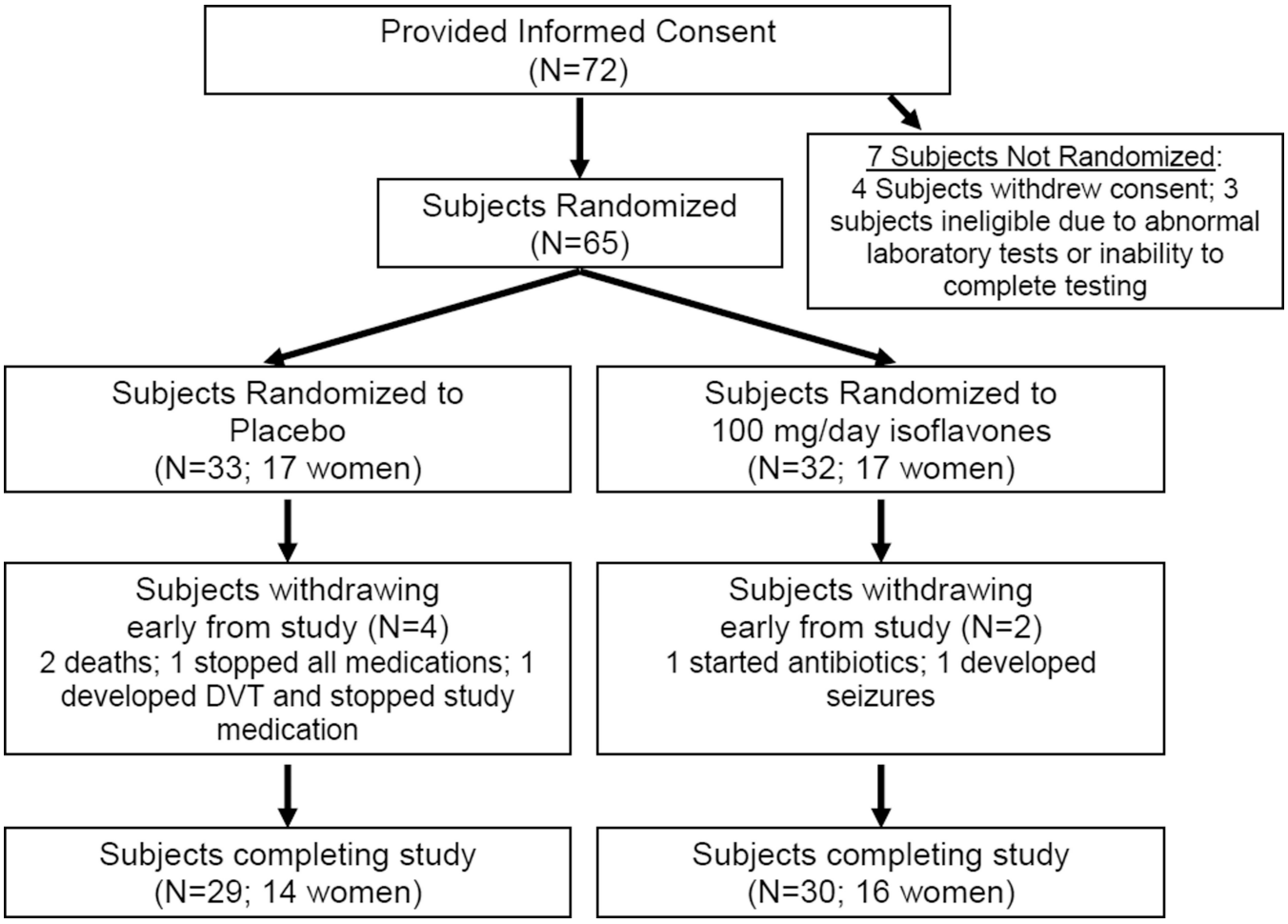

Figure 1.
Participant recruitment, randomization, and follow-up.

Table 1

Subject characteristics at entry into study.

SUBJECT BASELINE CHARACTERISTICS	Placebo Treated (N=33)	Isoflavone Treated (N=32)	P-value
Age in years: mean (SD)	76.8 (6.8)	75.7 (7.7)	0.54
Gender: Percent and number of Women	51.5% (n=17)	53.1% (n=17)	0.90
Education in years: mean (SD)	14.2 (2.5)	14.8 (2.7)	0.38
ApoE4 Status[1]: Percent and number of ApoE4 carriers	45.4% (n=15)	50.0% (n=16)	0.71
Total weekly Isoflavone intake at Baseline[2]: mean mg/week (SD)	6.5 (34.1)	16.5 (66.3)	0.45
Global Cognition: Mini-Mental State Examination score mean (SD)	22.4 (5.3)	23.5 (4.0)	0.35
Mood Symptoms: Geriatric Depression Scale-Short Form score mean (SD)	2.6 (2.4)	3.2 (3.3)	0.40
Concomitant treatment: Percent and number on AchE-I[3]	90.9% (n=30)	96.9% (n=31)	0.32
Concomitant treatment: Percent and number on NMDA-receptor blocker[4]	42.4% (n=14)	50.0% (n=16)	0.54
Body Mass Index	27.9 (5.4)	27.5 (5.0)	0.75

[1] One subject in each group declined to undergo ApoE genotyping

[2] Established with Food Frequency Questionnaire (FFQ)

[3] Acetylcholinesterase-Inhibitors included Donepezil, Galantamine Hydrobromide, and Rivastigmine

[4] NMDA-Receptor Blocker was Memantine

Table 2

Data on participant adherence to study procedures, and vitals and safety laboratory values after 6 months of treatment with Soy Isoflavones or Placebo.

	Placebo Treated MEAN VALUE (SE)	**Isoflavone Treated MEAN VALUE (SE)**	***P*-value**
Adherence to study protocol			
Average weekly dietary soy intake[1] during study participation; mg/week (SD) (N=55)	19.9 (34.7)	4.9 (10.9)	0.76
Pill Count – Average percent of expected number of pill used between visits (SD) (N=55)	97.5% (2.2%)	98.1% (5.7%)	0.68
Vital signs after 6 months of treatment (N=58)			
Average systolic blood pressure (SD) mmHg	126.9 (15.7)	128.8 (18.5)	0.67
Average diastolic blood pressure (SD) mmHg	66.8 (7.2)	69.3 (9.3)	0.26
Safety Laboratory Tests after 6 months of treatment (N=59)			
Amylase – Average blood level (SD) U/L	69.9 (36.6)	58.5 (14.9)	0.12
Lipase – Average blood level (SD) U/L	287.8 (222.3)	267.5 (103.0)	0.65
Phosphate – Average blood level (SD) mmol/L	3.5 (1.2)	3.3 (0.4)	0.39

Table 3

Plasma Isoflavones at Baseline, Month 3 and Month 6.

	Placebo Treated (N=33)	Isoflavone Treated (N=32)	P-value
Genistein ng/mL (SD)			
Baseline[1]	4.8 (11.4)	1.5 (2.6)	0.12
Month 3[2]	2.1 (3.7)	277.7 (188.4)	0.00
Month 6[3]	15.6 (35.4)	338.6 (308.8)	0.00
Daidzein ng/mL (SD)			
Baseline[1]	2.8 (6.1)	1.3 (2.5)	0.21
Month 3[2]	1.1 (2.3)	197.3 (150.5)	0.00
Month 6[3]	10.6 (37.8)	206.6 (156.8)	0.00
Equol ng/mL (SD)			
Baseline[1]	0.3 (1.5)	0.1 (0.5)	0.55
Month 3[2]	0.2 (0.5)	20.3 (50.7)	0.03
Month 6[3]	0.1 (0.6)	19.6 (40.5)	0.01

[1]Baseline assay data unavailable for three subjects: two placebo- and one isoflavone-treated subject

[2]Month 3 assay data unavailable for two subjects: one from each group

[3]Month 6 assay data unavailable for four subjects: one placebo- and three isoflavone-treated Subjects

Table 4

Estimated Marginal Means for Cognitive Tests after 6 months of treatment with Soy Isoflavones or Placebo.

	Domain	Test	Placebo Treated MEAN VALUE (SE)	Isoflavone Treated MEAN VALUE (SE)	*P*-value
Cognitive Outcomes	Global Cognition	Mini-Mental State Examination (MMSE) (N=59)	21.3 (1.0)	23.4 (1.0)	0.15
	Verbal Memory	List Learning Delayed Free Recall; Number of words recalled (N=57)	1.8 (0.50)	2.2 (0.50)	0.56
		Logical Memory Delayed Recall; Number of story elements recalled (n=57)	4.6 (0.95)	5.7 (0.9)	0.40
	Executive Function	Mazes; Time to complete (N=53)	32.2 (5.3)	22.6 (5.4)	0.22
		Trail Making Test B; Time to complete (N=52)	95.5 (16.7)	95.7 (16.0)	0.99
		Stroop Color Word Test; Time to complete (N=54)	105.0 (12.0)	91.8 (12.0)	0.44
	Language Executive Function	Category Fluency; Number of words generated (N=55)	21.5 (1.9)	25.7 (2.0)	0.13
		Phonemic Fluency; Number of words generated (N=56)	25.9 (2.4)	35.2 (2.4)	0.04*
	Visual Memory	Complex Figure Delayed Recall; Number of points (N=57)	10.6 (1.8)	13.4 (1.8)	0.27
		Benton Visual Retention Test; Number of correct figures (N=56)	2.7 (0.4)	2.8 (0.4)	0.78
		Benton Visual Retention; Number of errors (N=56)	14.5 (1.2)	13.1 (1.2)	0.42
	Visual Motor	Complex Figure Copy; Number of points (N=57)	27.6 (1.1)	29.0 (1.1)	0.35
		Grooved Pegboard Dominant Hand; Time to complete (N= (N=51)	134.3 (17.6)	149.8 (16.6)	0.53
		Grooved Pegboard Non-Dominant Hand; Time to complete (N=48)	161.8 (24.4)	156.0 (22.4)	0.87
Mood Outcomes	Depression	Geriatric Depression Scale – Subject Report (N=57)	2.1 (0.6)	3.3 (0.6)	0.41
		Geriatric Depression Scale – Study Partner Report (N=57)	5.2 (0.7)	6.3 (0.7)	0.25
	Multiple-Mood States	Profile of Mood States (POMS) – Tension Scale (N=56)	8.2 (1.3)	9.3 (1.3)	0.55
		POMS – Depression Scale (N=56)	9.0 (1.8)	11.4 (1.7)	0.34
		POMS – Anger Scale (N=56)	6.8 (1.4)	8.3 (1.4)	0.45
		POMS – Fatigue Scale (N=56)	10.3 (1.2)	10.3 (1.2)	0.97
		POMS – Vigor Scale (N=56)	12.4 (1.1)	12.0 (1.1)	0.79
		POMS – Confusion Scale (N=56)	15.5 (1.1)	14.0 (1.1)	0.35

All means were adjusted for covariates including age at baseline (76.4 years) and education (14.5 years). Subjects performed similarly on tests at baseline with the exception of Phonemic Fluency. Individuals randomized to receive Soy Isoflavones out-performed those on Placebo in Baseline Phonemic Fluency.

*When model is corrected for baseline differences, difference between groups is no longer significant.

Table 5

Estimated Marginal Means for Cognitive Tests after 6 months of treatment with Soy Isoflavones or Placebo.

Domain	Test	Spearman's rho Correlation of test score with 6 month Change in Plasma Isoflavones (ng/mL)					
		ΔGenistein		ΔDiadzein		ΔEquol	
		r	*p*	*r*	*p*	*r*	*p*
Verbal Fluency	Animal Fluency; Number of words generated	0.11	0.45	0.19	0.20	0.23	0.12
	Vegetable Fluency; Number of words generated	0.17	0.26	0.20	0.17	0.09	0.55
	Fruit Fluency; Number of words generated	0.19	0.19	0.25	0.08	0.22	0.13
	Phonemic Fluency; Number of words generated	0.19	0.18	0.28	0.11	**0.29**	**0.04**
Visual Spatial Memory	Complex Figure Delayed Recall; Number of points	0.04	0.80	0.03	0.85	0.14	0.34
	Benton Visual Retention Test; Number of correct figures	0.24	0.10	0.20	0.17	0.20	0.17
	Benton Visual Retention Test; Number of errors	−0.27	0.06	−0.24	0.10	−0.21	0.15
Visual-Motor Construction	Complex Figure Copy; Number of points	0.13	0.37	0.10	0.51	0.22	0.13
Speeded Dexterity	Grooved Pegboard Dominant Hand; Time to complete	−0.07	0.64	−0.04	0.77	**−0.29**	**0.05**
	Grooved Pegboard Non-Dominant Hand; Time to complete	−0.11	0.45	−0.13	0.40	**−0.33**	**0.02**