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The effects of dietary supplementation with isoflavones from red clover on cognitive function in postmenopausal women

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ABSTRACT

Objective To examine the effects of dietary isoflavone supplementation with an extract from red clover on cognitive function in postmenopausal women.

Design Thirty postmenopausal women aged greater than 60 years received either two tablets of an extract of aglycone isoflavones from red clover (each containing formononetin 25 mg, biochanin 2.5 mg and less than 1 mg of daidzein and genistein) for 6 months in a randomized, controlled clinical trial. Cognitive function tests were performed at baseline and at the end of isoflavone or placebo therapy.

Results Isoflavone supplementation was associated with an apparent improvement in block design (a test of visual-spatial intelligence) compared to placebo (isoflavone +12%, placebo −3%; $p = 0.03$), no improvement in verbal memory compared to an improvement on placebo (isoflavone +1%, placebo +29%; $p = 0.023$) and a deterioration in digit recall compared to placebo (isoflavone −6%, placebo +12%; $p = 0.029$). However, these findings were not statistically significant when corrections were made for potential chance findings due to multiple comparisons.

Conclusion Isoflavone supplementation does not appear to have major short-term effects on cognitive function in postmenopausal women. However, further clinical trials are required to determine whether small effects or long-term effects on cognitive function occur during isoflavone supplementation.

INTRODUCTION

There is conflicting evidence relating to the effects of estrogen replacement therapy on cognitive function in postmenopausal women. While a number of epidemiological and short-term interventional studies have suggested beneficial effects[1–4], a recent long-term, randomized, placebo-controlled study (The Women's Health Initiative trial) reported an adverse effect of combined treatment with estrogens and a progestogen on cognitive function[5]. Isoflavone phytoestrogens bind to estrogen receptors and may have either partial agonist or antagonist effects on estrogen receptors under certain circumstances[6,7]. Isoflavone phytoestrogens are widely used because of the

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belief that they may alleviate symptoms of menopause or help to prevent postmenopausal diseases associated with estrogen deficiency. The effects of isoflavone phytoestrogens on cognitive function in postmenopausal women have not been previously investigated. The effects of dietary supplementation with an extract of isoflavones from red clover in postmenopausal women, who had never received estrogen replacement therapy and who were consuming a diet low in isoflavones, were therefore investigated in a randomized, placebo-controlled, double-blind trial.

METHODS

Participants

Thirty women aged greater than 60 years who had been postmenopausal for at least 5 years, had never received hormone replacement therapy and who complained of memory difficulties, participated in a randomized, double-blind, crossover study. The subjects were recruited over a 6-month period from the general population following an advertisement in a local newspaper. A Minnesota Mini Mental State Examination (a widely used screening test for cognitive dysfunction) was performed when screening potential volunteers, and a result of 27 or greater was required for participation in the study.

The exclusion criteria were regular soy or isoflavone use, adherence to a vegetarian diet, more than two standard alcoholic drinks per day and any serious medical illness that may have influenced the outcome of the study (known malignancy, diabetes, ischemic heart disease, cerebrovascular or peripheral vascular disease, inflammatory arthritis, autoimmune disease or inflammatory bowel disease). Patients were also excluded if they had symptoms of major depression, current treatment of depression or therapy in the last 3 months with any of the following drugs: anticonvulsants, antidepressants, phenothiazines, benzodiazepines, ergot derivatives, β-blockers, centrally acting antihypertensives, cholinesterase inhibitors or anticholinergic drugs. Any other medications were kept constant for the duration of the study.

Study design

The study consisted of four phases. The first phase was a 1-month run-in period during which the subjects received single-blind placebo therapy and consumed a low isoflavone diet. The subjects were given dietary instructions and a list of foods to avoid. The low isoflavone diet was continued for the duration of the study. A 24-h urine collection was performed in the last week of this phase to confirm that the subjects were consuming a low isoflavone diet. A record of the patient's concomitant medication was taken and the subjects' medical history, demographic data, blood pressure and height and weight were recorded. Fasting plasma cholesterol, triglyceride and high density lipoprotein (HDL) cholesterol levels were measured. In addition, an intelligence quotient test (FSIQ, National Adult Reading Test) was performed by one of three experienced psychologists. The test assessed the ability of the subjects to read 50 irregular words. This provides an estimate of the subjects' premorbid cognitive abilities.

At the end of the run-in period, the subjects were randomized to receive either active therapy or placebo therapy for 6 months. A randomization code was generated by the Clinical Trials pharmacist of the St. George Hospital Pharmacy Department. The randomization sequence was generated in blocks of six and the numbers were placed in envelopes that were sequentially numbered. Patients were allocated an envelope sequentially by the clinical trial coordinator in the order that they signed the consent form. Medication containers that had been prepared by the Clinical Trials pharmacist were then dispensed by the study coordinator to the subjects. The medication containers were identical in appearance except for the randomization number on the label, and each container had an excess of tablets to allow the assessment of compliance from tablet counts of the returned medication. The Clinical Trials pharmacist had no contact with the subjects and no involvement in the data collection. The randomization code was not broken until the last patient had completed the study and all of the data had been checked and entered into the case report forms. The conduct of the trial was monitored by an independent, external clinical trial auditing company, and was completed over a 2-year period.

At the end of this first 6-month treatment period, the subjects entered into a further 1-month single-blind placebo wash-out period, after which they crossed over to the alternate therapy for a further 6 months. At the beginning of the run-in period and at the end of each 6-month treatment period, a full medical history and examination were performed along with cognitive function testing. Blood pressure, body weight, heart rate and plasma cholesterol, triglyceride and HDL

levels were also measured. A 24-h urine collection was performed during the last week of each 6-month treatment period for the measurement of urinary isoflavone excretion.

Study medication

The active therapy consisted of a standardized extract of isoflavones from red clover (Rimostil®, Novogen, Sydney, Australia) formulated as tablets containing approximately 25 mg of formononetin, 2.5 mg of biochanin and less than 1 mg of genistein and daidzein. Subjects took two tablets each morning with the morning meal. Formononetin and biochanin have been demonstrated to be extensively converted to daidzein and genistein in humans[8]. During the placebo phases of the study, the subjects took two placebo tablets which were identical in appearance and taste to the active medication but which contained no isoflavones. The subjects returned the bottles containing their study medication, including any remaining tablets, at the end of each study period for a tablet count to assess compliance.

Cognitive function testing

Cognitive function tests were performed by three experienced psychologists from the Community Rehabilitation and Geriatric Services (CRAGS). Wherever possible, the same psychologists performed repeated assessments of each volunteer. A battery of standard psychological tests was administered that had previously been developed and validated for the assessment of cognitive function in the elderly by the St. George Hospital Aged Care Department.

The tests administered were:

(1) *Digit recall* (Wechsler Memory Scale Revised, a test of immediate auditory attention) – digit sets of increasing length are read to the subject who is required to immediately recite them back[9];

(2) *Arithmetic test* (Wechsler Memory Scale Revised, a test of working memory) – a series of increasingly complex arithmetic tasks are given to the patient[9];

(3) *Trail A test* (a test of speed of information processing) – the subject is required to join a sequentially numbered pattern of dots as quickly as possible with a minimum of errors[10];

(4) *Trail B test* (a test of prefrontal lobe function) – the subject is required to join a pattern of dots labeled with alternating, sequential numbers and letters as quickly as possible with a minimum of errors[10];

(5) *The memory 1 and memory 2 tests* (Wechsler Memory Scale Revised, a test of word association memory) – the subject is asked to remember two sets of four word pairs, one set easy and the other set harder. They are then required to recall the word pairs immediately and after 30 min[10];

(6) *The verbal memory 1 and verbal memory 2 tests* (Wechsler Memory Scale Revised, tests of verbal memory) – the subject is read two narrative stories, each with 25 elements. The subject is asked to recall as many elements of the story as they can immediately and after a 30-min delay[11];

(7) *The visual 1 and visual 2 memory tests* (Wechsler Adult Intelligence Scale Revised, a test of visual memory) – the subject is shown four geometric designs for 10 s and asked to draw the designs immediately and after a 30-min delay[11];

(8) *The block design test* (Wechsler Adult Intelligence Scale Revised, a test of visual-spatial intelligence) – the subject is required to match patterns of increasing complexity made from four to nine blocks of different colors[11];

(9) *Digit symbol* (Wechsler Adult Intelligence Scale Revised, a test of information processing speed) – the subject is required to record digits from displayed numbers using a code[11];

(10) *Boston naming test* (a test of word naming) – subjects are shown pictures of objects of increasing difficulty and asked to name them[10];

(11) *FAS test* (Controlled Oral Word Association Test, a test of verbal fluency) – subjects are asked to name as many words as they can beginning with F, A and S for 1 min each[12];

(12) *Animals test* (a test of verbal fluency) – the subject is required to name as many animals as they can in 60 s[10];

(13) *Similarities test* (Wechsler Adult Intelligence Scale Revised, a test of verbal reasoning) – the subject is asked to explain the logic underlying the abstract association of two objects[11];

Expected results for the tests drawn from the Wechsler Memory Scale Revised were adjusted for age using data from the Mayo Older Americans Normative Studies[13].

The results of tests of similar areas of cognition were grouped together for analysis. The results for each of the individual tests in each group were added to obtain a composite result.

The groups were:

(1) Tests of speed of information processing (Trail A and digit symbol);

(2) Tests of memory (memory 1 and 2, verbal memory 1 and 2, visual memory 1 and 2);

(3) Tests of verbal ability (Boston naming test, FAS test, animals naming test, and similarities naming test);

(4) Tests of frontal cortex function (Arithmetic test, trail B test, and block design test).

The remaining test (digit recall) did not readily fit into any of these groups.

Urinary isoflavone estimation

The total volume of 24-h urine collections was measured and a 10–20 ml aliquot of each was stored at less than –20°C until assay. Daidzein, genistein, formononetin, biochanin, equol and ODMA (principal metabolites of isoflavones) were assayed in 1 ml of urine by gas chromatography–mass spectrometry with a limit of detection of 5 μg/ml and a within-day coefficient of variation of less than 20%. Peak separation was adequate, ensuring a high specificity of the assay. The assay was performed by the Scientific Laboratory of Novogen Pty Ltd, Sydney, Australia. This laboratory observes Good Laboratory Practice Principles. Total urinary isoflavone excretion was calculated as the sum of these isoflavones and metabolites. Samples from individual subjects were assayed in the same batch and all study samples were assayed using the same column within a period of 4 consecutive days.

The protocol allowed for the exclusion of any subject with a urinary excretion of greater than 3000 μg/day of total isoflavones while receiving placebo in either the baseline or active treatment periods.

Statistical analysis

The number of patients chosen for study was determined from a power calculation based on differences between previously published studies on estrogen therapy and placebo[3,4] and on the variability and reproducibility of cognitive function tests from the results of similar patients assessed previously in the St. George Hospital Department of Aged Care. The analysis of data was performed on an intention-to-treat basis. The power calculation was based on demonstrating a difference of greater than 30% between treatments during the first treatment phase of the study if significant carry-over effects, treatment order effect or time effect precluded the use of the cross-over data. The sample size was determined from a power calculation made using a variance of approximately 30%, which was obtained from previous data collected within the Department of Aged Care for similar patients. The power calculation was based on the individual ability of each test to detect the above treatment effect and was calculated using standard statistical techniques. As multiple pairwise comparisons were made in the overall analysis of the study, the p value required to show a significant difference was adjusted using the Bonferroni correction. The adjusted p value (α) was 0.003. All results are presented as the mean ± standard deviation (SD).

Age-adjusted normalized results (mean = 10, SD = 3) were calculated for each patient from population data for subjects of similar age, sex and education level. The data were analyzed using Student's t test or analysis of variance (Statistica 6.0, Statsoft, Tulsa, OK, USA) using normalized results. Analysis of the raw data was also performed to determine whether similar results were obtained to normalized data.

General conduct of the study

The study was approved by the South Eastern Sydney Area Health Service Ethics Committee (Southern Section) and all of the patients gave written informed consent. The first patient was randomized in May 1999 and the last patient was randomized in October 1999. The last patient completed the study in October 2000.

RESULTS

Thirty women were screened and all fulfilled the inclusion and exclusion criteria and were enrolled in the study; 28 patients completed the study. One patient died from a previously undiagnosed pancreatic cancer and one withdrew consent. The intention-to-treat analysis of the data was therefore performed on evaluable data from 28 patients, 14 in each treatment group. The

disposition of patients in the study is illustrated in Figure 1. Two patients were receiving cisapride, two were receiving omeprazole, two were receiving frusemide, one was receiving calcitriol and one was receiving diltiazem. These medications were kept constant for the duration of the study. No new medications were commenced by any of the patients during the study. Compliance with study medications, assessed by tablet counts, was greater than 80% for all patients who completed the study.

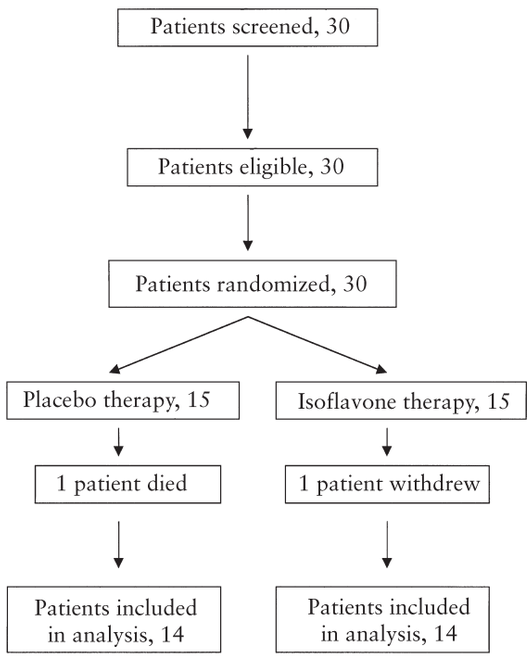

Figure 1 Disposition of patients

The groups were well matched with respect to age (isoflavones group, 68.5 ± 6.6 years; placebo group, 67.7 ± 5.5 years), FSIQ intelligence quotient (isoflavones, 109 ± 6; placebo, 106 ± 11), plasma cholesterol (isoflavone, 5.43 ± 1.16 mmol/l; placebo, 5.53 ± 1.43 mmol/l), triglyceride (isoflavone, 1.26 ± 0.78 mmol/l; placebo, 1.06 ± 0.41 mmol/l), HDL cholesterol levels (isoflavone, 1.65 ± 0.60 mmol/l; placebo, 1.62 ± 0.32 mmol/l), blood pressure (isoflavone, 139 ± 18/81 ± 10 mmHg; placebo, 145 ± 16/82 ± 8 mmHg), and heart rate (isoflavone, 69 ± 10 beats/min; placebo, 74 ± 10 beats/min). The women in the isoflavone group had 8.6 ± 1.96 years of education compared to 9.5 ± 1.38 years in the placebo group. None of these differences between the isoflavone and placebo groups was statistically significant.

Significant treatment order interactions were found between the two study phases for total digit span ($p = 0.002$), memory testing (test 1, $p = 0.009$) and the similarities test ($p = 0.009$). Results were therefore analyzed as the change in normalized values from baseline at the end of the first study period during which 14 patients received placebo and 14 received isoflavone therapy.

Urinary isoflavone excretion results are presented in Table 1. A number of urine samples were lost, resulting in complete sets of data for eight subjects who received isoflavones and 12 who received placebo. The missing urine samples were inadvertently discarded when the assaying laboratory moved premises. Isoflavone excretion was very low in both groups during the run-in phase and in the placebo group during the treatment

Table 1 24-h urinary isoflavone excretion (μg/day) at baseline and at the end of 6 months of treatment with isoflavone supplementation ($n = 12$) or placebo ($n = 8$). Equol and ODMA are principal metabolites of daidzein, genistein, formononetin and biochanin. Values are the mean ± standard deviation. All isoflavones and their metabolites were significantly higher at 6 months in the isoflavone group ($p < 0.05$). Isoflavones and metabolites did not differ significantly between the isoflavone or placebo groups at baseline

	24-h urinary isoflavone excretion (μg/day)						
	Isoflavone			*Placebo*			
	Baseline	*6 months*	*Change*	*Baseline*	*6 months*	*Change*	*p Value*
Daidzein	419 ± 165	4918 ± 917	4498 ± 1389	439 ± 135	429 ± 745	–9 ± 187	0.001
Genistein	274 ± 147	1143 ± 373	869 ± 393	275 ± 120	357 ± 305	82 ± 250	0.092
Equol	87 ± 52	808 ± 500	799 ± 801	61 ± 43	9 ± 408	–51 ± 55	0.204
ODMA	57 ± 34	853 ± 212	769 ± 335	91 ± 28	109 ± 173	18 ± 53	0.012
Formononetin	1 ± 1	776 ± 178	775 ± 286	2 ± 1	4 ± 145	2 ± 3	0.003
Biochanin	2 ± 1	13 ± 6	11 ± 8	2 ± 1	3 ± 5	1 ± 2	0.145
Total isoflavones	762 ± 334	8512 ± 1740	7750 ± 2613	870 ± 272	912 ± 1420	42 ± 454	0.002

Table 2 Baseline average (standardized) results for cognitive function tests in women who received isoflavone supplementation or placebo ($n = 14$ in each group). Values are the mean and standard deviation. Statistical comparisons between the groups were made using the Student's independent t test. The level for statistical significance was $p < 0.003$ (Bonferroni correction for multiple comparisons)

	Baseline test results		
	Isoflavone	*Placebo*	*p Values*
Digit recall test	10.7 ± 3.6	9.9 ± 3.7	0.570
Arithmetic test	8.1 ± 2.2	9.1 ± 3.2	0.309
Trail A test	8.3 ± 2.2	10.0 ± 3.1	0.116
Trail B test	9.0 ± 1.8	8.8 ± 2.9	0.816
Memory 1 test	9.7 ± 3.4	8.9 ± 4.2	0.591
Memory 2 test	9.3 ± 4.3	9.1 ± 4.1	0.929
Verbal memory 1 test	8.9 ± 1.9	8.1 ± 3.0	0.380
Verbal memory 2 test	10.8 ± 2.3	8.4 ± 3.6	0.037
Visual memory 1 test	9.5 ± 3.3	10.0 ± 3.9	0.718
Visual memory 2 test	9.1 ± 4.1	8.9 ± 3.4	0.882
Block design test	9.1 ± 2.8	9.8 ± 2.7	0.492
Digit symbol test	9.4 ± 2.9	9.5 ± 2.9	0.949
Boston naming test	0.0 ± 1.45	0.1 ± 1.0	0.694
FAS test	0.3 ± 0.9	–0.1 ± 1.0	0.373
Animals naming test	0.4 ± 1.1	–0.1 ± 1.0	0.314
Similarities test	10.3 ± 2.7	9.3 ± 3.4	0.369

phase. In contrast, isoflavone excretion was high in the active treatment group during the treatment phase. One subject had a 24-h urinary excretion in excess of 3000 μg/day while receiving placebo during the treatment phase of the study (4360 μg/day). Exclusion of this patient from the analysis did not significantly alter the results.

The results for baseline normalized cognitive function tests in each group are presented in Table 2. The groups were well matched at baseline, except for the possible exception of the second verbal memory test, in which the subjects who were to receive isoflavones appeared to perform better than the subjects who were to receive placebo. However, the differences between the two groups at baseline ($p = 0.039$) was not statistically significant when corrected for multiple comparisons.

Changes in the normalized results for cognitive function tests between baseline assessments and the assessments at the end of 6 months of treatment with isoflavone supplementation or placebo are presented in Table 3.

The subjects who received isoflavone supplementation demonstrated a trend for greater improvement in the block design test (+12%) compared to subjects who received placebo (–3%) ($p = 0.034$). In contrast, subjects who received

Table 3 Changes in standardized cognitive function test results from baseline after 6 months of dietary supplementation with isoflavones or placebo ($n = 14$ in each group). Values are the mean change ± standard deviation. Positive t values indicate a greater improvement during isoflavone therapy. Statistical comparisons between the groups were performed using Student's independent t test. The level for statistical significance was $p < 0.003$ (Bonferroni correction for multiple comparisons)

	Change in test results			
	Isoflavone	*Placebo*	*t*	*p Value*
Digit recall test	–0.6 ± 2.2	1.2 ± 1.9	–2.29	0.029
Arithmetic test	0.6 ± 1.7	0.2 ± 1.3	0.75	0.461
Trail A test	0.8 ± 2.6	0.7 ± 2.3	0.07	0.939
Trail B test	0.7 ± 1.3	0.6 ± 1.9	0.23	0.817
Memory 1 test	0.7 ± 4.6	1.5 ± 3.4	–0.51	0.611
Memory 2 test	1.1 ± 2.8	2.2 ± 2.6	–0.96	0.344
Verbal memory 1 test	1.0 ± 2.7	1.3 ± 2.3	–0.30	0.765
Verbal memory 2 test	0.14 ± 2.3	2.4 ± 2.5	–2.41	0.023
Visual memory 1 test	1.4 ± 2.7	0.7 ± 2.3	0.79	0.435
Visual memory 2 test	2.3 ± 2.3	2.4 ± 2.9	–0.12	0.909
Block design test	1.1 ± 1.3	–0.3 ± 2.0	2.34	0.034
Digit symbol test	1.3 ± 1.5	0.6 ± 1.4	1.31	0.202
Boston naming test	0.2 ± 0.8	0.3 ± 0.6	–0.32	0.748
FAS test	0.1 ± 0.8	0.3 ± 0.4	–1.36	0.182
Animals naming test	–0.2 ± 1.0	0.3 ± 0.5	–1.58	0.124
Similarities test	0.6 ± 1.6	1.4 ± 2.6	–0.95	0.347

isoflavones had a tendency for a deterioration of digit recall (–6%), compared to subjects receiving placebo who improved by +12% ($p = 0.029$). In addition, there was a trend for a greater improvement in the second verbal memory tests in subjects receiving placebo (+29%) compared to the subjects receiving isoflavones (+1%) ($p = 0.023$). However, none of these differences achieved statistical significance when adjusted for multiple comparisons. There were no notable changes from baseline between the subjects who received isoflavones compared to those who received placebo for the other cognitive function tests studied. Analysis of the raw (non-normalized) data produced similar results. Analysis of differences between the isoflavone and placebo groups for the specified combinations of tests revealed no statistically significant differences.

Body weight, blood pressure, heart rate and plasma lipoprotein cholesterol were not significantly affected by isoflavone supplementation.

Adverse events

No adverse events or side-effects were reported during the study other than the death of one patient from pancreatic cancer. This patient was receiving placebo.

DISCUSSION

This study is the first to report the results of a randomized, controlled trial of the effects of isoflavone supplementation on cognitive function in postmenopausal women. Isoflavone supplementation appeared to be associated with a small improvement in the block design tests and a slightly worse performance in the digit recall and second verbal memory tests, compared to placebo. Although these differences were associated with p values of less than 0.05, they did not achieve statistical significance when adjusted for multiple comparisons. The apparent differences between isoflavone and placebo therapy observed in the present study are therefore consistent with chance findings.

Estrogen replacement therapy has been reported to be associated with improvements in cognitive function, particularly for tests of memory[1–4]. However, the long-term benefits of estrogen on cognitive function have been disputed by the findings of adverse effects of estrogen plus progestogen therapy on cognitive function in the Women's Health Initiative study[5]. It is possible that the inclusion of progestogen therapy in the Women's Health Initiative study may have contributed to differences in outcome between these studies. Higher plasma levels of estrogen have been associated with poorer performance in tests of visual memory[14]. The effects of hormone replacement therapy on tests of spatial memory and orientation have, in general, demonstrated no significant change[1,2].

The consumption of tofu, which has a high content of isoflavones, has been associated with an increased incidence of dementia and low brain weight in a large epidemiological study[15]. It has been hypothesized that this effect may be due to tyrosine kinase inhibition by the isoflavone genistein[16]. The *in vitro* inhibition of tyrosine kinase by genistein in rat hippocampus has been shown to inhibit the induction of long-term potentiation of synaptic transmission, a mechanism believed to be involved in memory formation[16,17]. Inhibition of tyrosine kinase by genistein in 4-day old chicks has been shown to significantly impair memory[18].

While isoflavones could affect cognitive function via partial agonist effects[6], they could also affect cognitive function by exerting a partial antagonist effect on estrogen receptors[7] or by inhibiting enzymes involved in endogenous estrogen synthesis[19]. Nonetheless, dietary isoflavone supplementation using soy protein, which contains genistein and daidzein, has been shown to improve working memory in ovariectomized rats[20].

The present study investigated the effects of isoflavone supplementation over a 6-month period. It is possible that longer periods of supplementation of isoflavone at different doses may produce different results. Nonetheless, hormone replacement therapy using estrogen has been reported to produce significant improvements in memory following periods of therapy as short as 2 months[4].

In summary, the results of the present study suggest that isoflavone supplementation, using an extract from red clover that predominantly contains formononetin, does not lead to major changes in cognitive function in postmenopausal women over a 6-month period. Further studies are required to determine if longer-term effects or relatively smaller effects on cognitive function are associated with isoflavone supplementation.

Conflict of interest Nil.

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References

1. Sherwin B. Hormones, mood, and cognitive functioning in postmenopausal women. *Obstet Gynecol* 1996;87:20–6
2. Le Blanc ES, Janowsky J, Chan BK, Nelson HD. Hormone replacement therapy and cognition: systematic review and meta-analysis. *J Am Med Assoc* 2001;285:1489–99
3. Maki P, Zonderman A, Resnick S. Enhanced verbal memory in nondemented elderly women receiving hormone-replacement therapy. *Am J Psychol* 2001;158:227–33
4. Linzmayer L, Semlitsch HV, Saletu B, *et al*. Double-blind, placebo-controlled psychometric studies on the effects of a combined estrogen/progestin regimen versus estrogen alone on performance, mood and personality of menopausal syndrome patients. *Arzneimittel-Forschung* 2001;51:238–45
5. Rapp SR, Espeland MA, Shumake SA, *et al*. WHIMS investigators. Effect of estrogen plus progestogen on global cognitive function in postmenopausal women. *J Am Med Assoc* 2003;289:2663–72
6. Setchell KD. Soy isoflavones – benefits and risks from nature's selective estrogen receptor modulators (SERMs). *J Am Coll Nutr* 2001;20(Suppl 5):S354–62
7. Rice MM, Graves AB, McCurry SM, Larson EB. Estrogen replacement therapy and cognitive function in postmenopausal women with and without dementia. *Am J Med* 1997;103:26–35S
8. Howes JB, Waring M, Huang L, Howes LG. Long term pharmacokinetics of an extract of isoflavones from red clover. *J Alt Complement Med* 2002;8:135–42
9. Wechsler D, Stone CP. *Wechsler Memory Scale-Revised* (WAMS-R). San Antonio, Texas: The Psychological Corporation, 1987
10. Spreen O, Strauss E. *A Compendium of Neuropsychological Tests: Administration, Norms and Commentary*, 2nd edn. Oxford: Oxford University Press, 1998
11. Wechsler D. *Adult Intelligence Scale-Revised (WAIS-R) with Australian Supplement*. San Antonio, Texas: The Psychological Corporation, 1985
12. Ivnik RJ, Malec JF, Smith GE. Neuropsychological Tests' Norms Able Age 55: COWART, BNT, MAE Token, WRAT-R Reading, AMNART, STROOP, TMT, and JLO. *Clin Neuropsychol* 1996;10:262–78
13. Ivnik RJ, Smith GE, Glenn E, *et al*. Free and cued reminding tests: MOANS. *J Clin Exp Neurol Psychol* 1997;19:676–91
14. Drake EB, Henderson VW, Stanczyk FZ, *et al*. Associations between circulating sex steroid hormones and cognition in normal elderly women. *Neurology* 2000;54:599–603
15. White LR, Petrovitch H, Webster R. Brain aging and midlife tofu consumption. *J Am Coll Nutr* 2000;19:242–55
16. Huang CC, Hsu KS. Protein tyrosine kinase is required for the induction of long-term potentiation in the rat hippocampus. *J Physiol* 1999;3:783–96
17. O'Dell TJ, Kandel ER, Grant SG. Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Nature* 1991;353:558–60
18. Whitechurch RA, Ng KT, Sedman GL. Tyrosine kinase inhibitors impair long-term memory formation in day-old chicks. *Cog Brain Res* 1997;6:115–20
19. Wang CK, Keung WM. Bovine adrenal 3 beta-hydroxy steroid dehydrogenase (E.C.1.1.1.145)/5-ene-4-ene isomerase (E.C.5.3.3.1): characteristics and its inhibition by isoflavones. *J Steroid Biochem Mol Biol* 1999;71:191–202
20. Pan Y, Anthony M, Watson S, Clarkson TB. Soy phytoestrogens improve radial arm maze performance in ovariectomized retired breeder rats and do not attenuate benefits of 17beta-estradiol treatment. *Menopause* 2000;7:230–5