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Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones

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Abstract

We previously reported that a high soya diet improved memory and frontal lobe function in young volunteers, and since soya isoflavones are agonists at oestrogen receptors, they may improve these functions in postmenopausal women. Thirty-three postmenopausal women (50–65 years) not receiving conventional hormone replacement therapy (HRT) were randomly allocated in a double-blind parallel study to receive a soya supplement (60 mg total isoflavone equivalents/day) or placebo for 12 weeks. They received a battery of cognitive tests and completed analogue rating scales of mood and sleepiness, and a menopausal symptoms questionnaire before the start of treatment and then after 12 weeks of treatment. Those receiving the isoflavone supplement showed significantly greater improvements in recall of pictures and in a sustained attention task. The groups did not differ in their ability to learn rules, but the isoflavone supplement group showed significantly greater improvements in learning rule reversals. They also showed significantly greater improvement in a planning task. There was no effect of treatment on menopausal symptoms, self-ratings of mood, bodily symptoms or sleepiness. Thus, significant cognitive improvements in postmenopausal women can be gained from 12 weeks of consumption of a supplement containing soya isoflavones that are independent of any changes in menopausal symptoms, mood or sleepiness.

Keywords: Isoflavones; Phytoestrogens; Memory; Attention; Frontal lobe function; Mood; Menopause

1. Introduction

A soya diet containing high levels of isoflavone phytoestrogens significantly improved memory and frontal lobe function in young healthy male and female volunteers compared with volunteers receiving a soya-free diet for 10 weeks (File et al., 2001a). Isoflavones are nonsteroidal oestrogens (for review, see Wiseman, 2000); daidzin and genistin and their glucoconjugates are the predominant isoflavone forms in soya foods and supplements (Wiseman et al., 2002). They are hydrolysed in the large intestine to daidzein and genistein and these act as weak agonists at oestrogen receptors (Pike et al., 1999). There are two types of oestrogen receptor (ERα and ERβ) and both are expressed in the brain (Kuiper et al., 1998). The isoflavones have a greater affinity for ERβ (Kuiper et al., 1998), and it is possible that they can reach the brain in sufficient concentrations to activate these receptors, since high plasma concentrations are reached after daily consumption of a textured soya protein burger for 2 weeks (Rowland et al., 2000). ERβ mRNA is prevalent in the hippocampus, frontal cortex and thalamus (Shughrue et al., 1997; Pau et al., 1998; Petersen et al., 1998; McEwen and Alves, 1999; Shughrue et al., 2000; Gundlah et al., 2000; Osterlund et al., 2000), and thus it is likely that the ERβ receptors will play an important role in cognition.

Soya phytoestrogens have been shown to act like oestrogen in the brain, which has a number of direct and indirect receptor-mediated genomic effects that could affect cognition, such as influencing cell survival, growth and neuroplasticity (McEwen, 2001). Phytoestrogens have been shown to increase choline acetyltransferase and mRNA levels of neurotrophins in the frontal cortex and hippocampus (Pan et al., 1999a,b). In addition, there are receptor-mediated nongenomic effects of oestrogen that could be of

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great importance to cognition. For example, at physiological levels of glutamate, oestrogen potentiates glutamate-induced calcium signalling by acting at the NMDA receptors (Nilsen and Brinton, 2002). Other rapid actions of oestrogens are protection of neurones from damage by excitotoxins and free radicals (Nilsen and Brinton, 2002; Toran-Allerand et al., 1999; Kelly and Levin, 2001). Thus, although it is unlikely that phytoestrogens will mimic all the CNS effects of oestrogens, there are sufficient potential mechanisms that could mediate cognitive improvement. Furthermore, it is possible that phytoestrogens could have effects that are not seen with oestrogens since, for example, there is a splice variant of ERβ in the hippocampus, to which oestradiol does not bind (Price et al., 2000).

In several well-controlled experimental studies, short-term oestrogen replacement therapy has been found to significantly improve episodic memory in postmenopausal women (e.g., Phillips and Sherwin, 1992; Jacobs et al., 1998; Duka et al., 2000). However, improved memory has only been found in 50% of experimental studies in which women have been randomly assigned to the treatments (see Hogervorst et al., 2000). Most of the cross-sectional epidemiological studies have reported improved memory in users of hormone replacement therapy (HRT) compared with nonusers, but these studies always carry a risk that the women who chose HRT might be younger or have higher levels of IQ, education or socioeconomic class than the nonusers (see Yaffe et al., 1998; Le Blanc et al., 2001; Hogervorst et al., 2000). Improvements in semantic memory have also been found in postmenopausal women treated with tibolone, which is only a weak oestrogen agonist (Albertazzi et al., 2000; Fluck et al., 2002).

There is considerably less evidence on the effects of HRT on frontal lobe function. In a very small cross-sectional study, Keenan et al. (2001) found that women on HRT performed better than those who had never used HRT. However, in a study in which treatment was randomly assigned, no improvement in frontal function was found after 3 weeks of oestradiol treatment in postmenopausal women (Duka et al., 2000). There is even evidence that very long-term treatment (10 years) with HRT can impair frontal functions (Fluck et al., 2002; File et al., 2002). These impairments were found both after oestradiol implants, which give rise to very high circulating levels of oestrogen, and after treatment with tibolone, which has oestrogenic potency only 1/50 of that of oestradiol.

The purpose of the present study was to evaluate the effects of 12 weeks of treatment with a dietary supplement that contained an extract of soya isoflavones on the cognitive performance of postmenopausal women. Improvements in memory were shown in our previous study after 10 weeks with a higher concentration of isoflavones (100 mg total isoflavones equivalents/day) consumed as part of the dietary matrix (File et al., 2001a). The cognitive test battery was the same as that used in our previous study looking at the effect of a 10-week period of dietary intervention with high and low soya diets (File et al., 2001a). The dose used in this study (60 mg total isoflavone equivalents/day) was chosen since 56 mg total isoflavone equivalents/day in a dietary matrix was shown to be effective in reducing in vivo lipid peroxidation in a previous study (Wiseman et al., 2000). The treatment period used in this study was chosen since 12 weeks consumption of 60 mg/day isoflavones in the diet showed beneficial effects on risk factors for cardiovascular disease and osteoporosis in postmenopausal women (Scheiber et al., 2001). In addition, we examined the effects on mood and menopausal symptoms in order to determine whether the supplement would be of more general benefit to this group of women.

2. Methods

2.1. Subjects

Thirty-six postmenopausal women aged 50–65 were recruited by circular e-mail at King's College London or from a database of those who had previously participated in a study on bone mineral density at Guy's Hospital. King's College Ethics Committee approved the study and all subjects gave written informed consent. All subjects were healthy and defined as postmenopausal if they had not menstruated in the previous 12 months. In the Solgen group, 78% of subjects were naturally menopausal and 22% were surgically menopausal; in the placebo group, 87% were naturally menopausal and 13% were surgically menopausal. Exclusion criteria were use of HRT in the previous 12 months, use of antibiotics in the previous 3 months, current illness or use of psychoactive medication. All subjects were nonsmokers. Three subjects were excluded during the course of treatment, two because they started treatment with amitryptiline and one because she started HRT.

2.2. Dietary supplement and assessment

The soya isoflavone supplement used was Solgen 40 (Solbar Plant Extracts, Ashdod, Israel). Each capsule contained 30 mg total isoflavone equivalents. The subjects took two capsules a day for 12 weeks (one in the morning and one in the evening) providing 60 mg total isoflavones equivalents/day. Those allocated to the placebo group took two identical looking capsules daily containing colour-matched lactose for 12 weeks.

The subjects were asked to avoid consuming soya foods and food products containing soya for the duration of the study and for 2 weeks prior to the commencement of the study.

They were asked to fill in a 7-day food diary (Bingham et al., 1995) during the last 7 days of the treatment period. Subjects were asked to record everything they ate and drank using standard household measures. Two subjects in the placebo group did not complete and return their food diary.

Food consumption data were entered and nutrient intakes were calculated using Integrated Dietary Analysis.

2.3. Procedure

The subjects were randomly allocated, 18 each to the Solgen and placebo groups. The three women who were excluded during the trial because of concurrent medication all came from the placebo group. Subjects were required to attend two sessions in the Metabolic Unit in the Nutrition and Dietetics Department, King's College London, for cognitive testing. The first test session (baseline) was before the start of supplement treatment and the other session was at the end of 12 weeks of supplements. On the baseline test day, all subjects were given a practice session to familiarise them with the computerised test battery. An estimate of verbal IQ was obtained using the National Adult Reading Test—revised version (NART-R, Nelson and Willison, 1991). Thereafter, the battery of tests was the same on the baseline test day and on the test day after 12 weeks of supplement intervention.

The Hospital Anxiety and Depression Scale (HAD) (Zigmond and Snaith, 1983) was used to determine anxiety and depression. The Greene Climacteric Scale (Greene, 1998) was used to assess menopausal symptoms yielding three main independent symptom measures: psychological, somatic and vasomotor symptoms. Sleepiness was assessed using the Stanford Sleepiness Scale (Hoddes et al., 1973), which was given at the start of testing and then again at the end, and the Epworth Sleepiness Scale (Johns, 1991, 1992), which was given at the end of the test session.

2.4. Episodic memory

There were three tests of episodic memory. In a test of immediate memory, a short story was read to the subject (from the Weschler Memory Scale—revised, Weschler, 1987); with 25 units of information read at the rate of 1 unit per second, the subjects were told to try and remember the story as closely as possible. They were asked immediately to recall the story, and the number of correctly recalled units was scored. Different stories were used on the baseline and 12-week test days. The test of short-term nonverbal memory was the Delayed Matching To Sample Test (DMTS) from the Cambridge Neuropsychological Test Automated Battery (CANTAB CeNeS, Cambridge), which has been shown to activate the temporal cortex (Owen et al., 1995). In this test, a sample complex pattern is displayed on the computer screen and the task is to select one of four similar patterns that matches the sample. The four patterns are either displayed simultaneously with the sample or appeared after a delay of 0, 4 or 12 s. The number of correct responses and the median response times were scored for five patterns at each delay. Long-term episodic memory was measured by presenting a set of 20 pictures of common objects, each picture was presented for 5 s, and then 20 min later, the subject was asked to recall as many of these as possible. Different sets of pictures were used on the two test occasions.

2.5. Category generation

In this task, the subjects were given 1 min to name all the animals they could think of, and every 20 s they were given a different category of animals—house, farm and jungle. This task has elements of verbal fluency and semantic memory.

2.6. Tests of frontal lobe function

Two tests of frontal lobe function were selected from the CANTAB. A test of rule learning and reversal (the first five stages of the IDED test) provided a measure of mental flexibility controlled by the frontal cortex (Owen et al., 1991). In this test, a series of pairs of patterns was presented on a computer screen and the task was to learn the rule that determined which pattern was correct. Once the rule was correctly learned, this rule was reversed. The time taken to complete each stage (simple discrimination, simple reversal, compound discriminations 1 and 2, compound reversal) was recorded. Planning ability was measured using the Stockings of Cambridge (SoC) Test, which has also been shown to be a measure of frontal lobe function (Owen et al., 1990). The computer screen displayed two sets of three coloured balls that could be housed in three stockings. The task was to move the balls in the lower part of the screen so that the pattern exactly matched that shown in the upper part. The task varied in difficulty so that the match could be obtained in two to four moves. The easy parts of the task were used for training, and then the time to correctly complete the four-move task was recorded.

2.7. Sustained attention

The Paced Auditory Serial Addition Test (PASAT) was used to measure sustained attention (Spreen and Strauss, 1991). This involved adding together successive pairs of digits read from a list of 61 numbers, presented at different speeds from one digit every 2.7 s to one every 1.2 s. This is a difficult test to master and the first two tape speeds (2.7 and 2.0 s) were used as practice. The two fastest speeds, 1.6 and 1.2 s, were used to assess performance. The total number of correct responses (maximum 60) was recorded for each of these two trials.

2.8. Mood ratings

Self-ratings of mood and bodily symptoms were taken at the start of the test session and then again after completing the cognitive tests. Visual analogue rating scales were used to assess bodily symptoms, aggression and mood (Bond and Lader, 1974, 1986). For the bodily symptoms, the scale went from ''no symptoms'' to ''very severe'' and was

Table 1
Mean ± S.E.M. age, estimated IQ (based on the NART), years in secondary education, time since the menopause (months), scores on the Hospital Anxiety (HAD_A) and Depression (HAD_D) scales, weekly caffeine consumption (ml) and weekly alcohol intake (units) of subjects allocated to the placebo or Solgen treatment

	Placebo ($n=15$)	Solgen ($n=18$)
Age (year)	56.8 ± 1.0	58.8 ± 1.1
IQ	118.1 ± 2.3	115.3 ± 2.3
Years of secondary education	7.5 ± 0.8	7.9 ± 0.7
Months menopausal	84.5 ± 14.6	109.3 ± 15.6
HAD_A	4.0 ± 0.9	5.2 ± 0.8
HAD_D	2.0 ± 0.4	2.0 ± 0.4
Weekly caffeine (ml)	1848.0 ± 153.8	1669.8 ± 137.3
Weekly alcohol (units)	8.9 ± 1.9	3.4 ± 0.7 *

* $P<.05$ compared with placebo.

separated by a 100-mm line. For the aggression and mood scales, each item consisted of a pair of opposite adjectives separated by a 100-mm line. The subject indicated how she felt at the time by placing a perpendicular mark at the appropriate place along each line. Previous studies have found changes in anxiety and aggressive mood after a similar battery of cognitive tests, including the PASAT (File et al., 2001b, 2002; Fluck et al., 2002).

2.9. Statistics

The data from the episodic memory tests were first analysed by a two-way multivariate analysis of variance (MANOVA), which permits analysis of the effects of the supplement, while controlling for intercorrelations between measures, thus reducing the risk of false positives from a series of univariate tests. The two factors were the between-group factor of experimental group and the repeated-measures factor of test day. Because the first test day was at baseline, before any treatments were given, the effect of the Solgen treatment can only be assessed by the Group × Day interaction. The MANOVA was followed by a series of univariate two-way analyses of variance (ANOVAs) for each of the individual measures. Similar ANOVAs were used to analyse the data from the other cognitive tests, the Greene Climacteric Scale and the Epworth scale. Nonparametric tests were used for the Stanford scale, Wilcoxon t tests to test if there was a change after cognitive testing and Mann–Whitney U tests to test between the Solgen and placebo groups. The ratings of aggressive mood were analysed by a three-way MANOVA with treatment as the between-group factor and day (baseline and week 12) and time (before and after cognitive testing) as repeated measures. The bodily symptoms were likewise analysed by a three-way MANOVA and the mood factors by three-way ANOVAs. Where effects reached significance, both F values and probability levels are quoted. Where results did not reach significance, only the F ratios are presented, and nonsignificance is indicated (ns). The statistical package used was SPSS version 10.1 for windows.

3. Results

It can be seen from Table 1 that the groups did not differ in their age, IQ, years of secondary education, time since the menopause, trait anxiety or depression or in their weekly caffeine intake [$F(1,31)<1.8$, ns in all cases]. They did differ significantly in their weekly alcohol intake, with the placebo group consuming significantly more [$F(1,31)=8.0$, $P<.01$]. However, the level of alcohol consumption was very low in both groups with a maximum consumption of 26 units per week. The dietary nutrient intakes of the subjects are shown in Table 2. The Solgen group had a significantly higher intake of saturated fatty acids [$F(1,29)=4.2$, $P=.05$] and higher intakes of sugar and calcium, although these did not reach significance [$F(1,29)=3.8$ and 3.9, respectively, $P=.06$]. There were no other differences in dietary nutrient intake [$F(1,29)<2.6$, ns in all cases].

3.1. Episodic memory

There was a significant effect of test day on the factor measuring episodic memory [MANOVA, day effect $F(4,28)=12.8$], reflecting the fact that both groups showed a significant practice effect. However, there was also a significant Group × Day interaction [$F(4,28)=3.0$, $P<.03$] because the Solgen group is showing significantly greater improvement than the placebo group. This treatment effect was also reflected in two of the individual measures of memory, with the Solgen group showing significantly

Table 2
Mean ± S.E.M. of dietary nutrient intakes of subjects allocated to the placebo or Solgen treatment

	Placebo	Solgen
Energy (MJ)	7.0 ± 0.4	7.8 ± 0.3
Total fat (% of energy)	31.9 ± 1.7	34.3 ± 1.2
Monounsaturated	10.3 ± 0.8	10.4 ± 0.5
Polyunsaturated	6.1 ± 0.7	5.2 ± 0.4
Saturated	11.1 ± 0.6	12.7 ± 0.5 *
Protein (% of energy)	15.4 ± 0.5	15.1 ± 0.5
Carbohydrate (% of energy)	45.3 ± 2.2	47.4 ± 1.4
Total sugars (% of energy)	21.1 ± 1.5	24.1 ± 1.2
Total sugar (g)	95.7 ± 9.9	119.4 ± 7.5 **
Starch (g)	102.6 ± 7.4	110.7 ± 6.0
Cholesterol (mg/day)	225.9 ± 22.3	228.7 ± 17.4
Non-starch polysaccharide (g/day)	14.0 ± 1.4	14.6 ± 1.0
Calcium (mg)	761.1 ± 42.6	899.42 ± 51.9 **
Iron (mg)	11.6 ± 1.0	11.8 ± 0.6
Copper (μg)	1.1 ± 0.2	1.1 ± 0.1
Zinc (mg)	7.5 ± 0.4	8.4 ± 0.4
Selenium (μg)	45.6 ± 3.4	46.3 ± 4.0
Iodine (μg)	106.9 ± 8.6	129.5 ± 13.8
Carotene equivalents (μg)	2129.2 ± 396.7	2327.6 ± 336.4
Vitamin B12 (μg)	4.4 ± 1.0	4.9 ± 0.6
Folic acid (μg)	276.1 ± 21.7	304.4 ± 20.5
Vitamin C (mg)	107.2 ± 13.2	106.1 ± 10.3
Vitamin E (alpha-tocopherol equivalents) (mg)	8.5 ± 1.2	7.2 ± 0.8

* $P=.05$.
** $P=.06$, compared with placebo group.

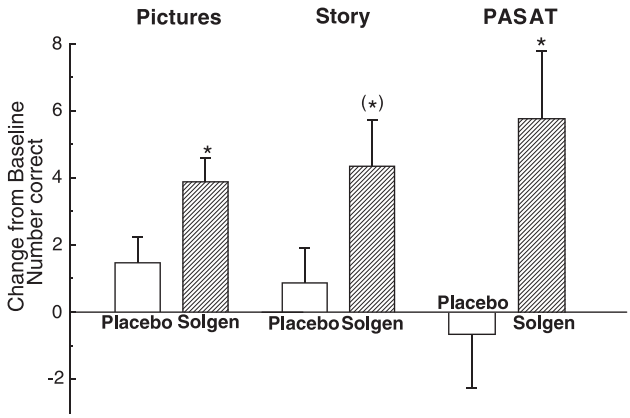

Fig. 1. Mean ± S.E.M. change in performance from baseline day in the numbers of correct responses in recall of pictures and story and in the difference in correct responses at 1.6 and 1.2 s speeds of presentation in PASAT, in subjects after 12 weeks on placebo treatment (clear columns) or Solgen treatment (filled columns). * $P < .05$, (*) $P < .06$, compared with placebo.

greater improvement in delayed recall of pictures [Group × Day interaction, $F(1,31) = 5.1$, $P < .03$] and greater improvement in immediate story recall [Group × Day interaction, $F(1,31) = 3.8$, $P < .06$]. To illustrate this interaction term, Fig. 1 shows these effects as the change in performance from the baseline test day to the test at 12 weeks. Post hoc tests showed that the groups did not differ in their baseline scores on recall of pictures (placebo = 9.8 ± 0.7, Solgen = 8.7 ± 0.6) or story (placebo = 15.9 ± 1.5, Solgen = 12.7 ± 1.0).

In the DMTS task (see Table 3), there were significant day effects on the number of correct responses [$F(1,31) = 4.6$, $P < .05$] and the median response times [$F(1,31) = 10.8$, $P < .01$], reflecting practice effects. However, there were no group differences in the extent of this [Group × Day interaction, $F(1,31) < 1.0$, ns, for the number of correct responses; $F(1,31) = 1.2$, ns, for median response times]. Thus, Solgen was without effect in this task.

3.2. Category generation

There was no practice effect in this task (day, $F < 1.0$) or any effect of treatment (Group × Day interaction, $F < 1.0$, ns, see Table 3).

3.3. Tests of frontal lobe function

The groups did not differ in their baseline scores in the IDED test of mental flexibility, but 12 of the women were unable to complete this test. The data were therefore analysed only for those who were able to do the task ($n = 11$ in the placebo group, $n = 10$ in the Solgen group). In Stage 1, learning the rules for simple discrimination, there was a significant practice effect [day, $F(1,19) = 4.9$, $P < .05$] and the Solgen treatment was without effect on this [Group × Day interaction, $F(1,19) = 1.2$, ns]. In Stage 2, learning the reversal of the simple discrimination rule, there was no overall practice effect (day, $F < 1.0$, ns), but there was a significant effect of the Solgen treatment [Group × Day interaction, $F(1,19) = 4.1$, $P = .05$, see Table 3]. There was a significant practice effect in learning the first compound discrimination [day, $F(1,19) = 9.0$, $P < .01$], but not in learning the second one [day, $F(1,19) = 3.4$, ns]. There was no effect of the Solgen treatment in learning these compound discrimination rules [Group × Day interaction, $F(1,19) < 1.0$ in both cases, ns]. There was a significant day effect in learning the reversal of the compound discrimination [$F(1,19) = 7.2$, $P < .05$], but it can be seen from Fig. 2 that this was solely due to the improved performance in the Solgen group [Group × Day effect in the time to learn reversal, $F(1,19) = 11.9$, $P < .003$]. To illustrate this interaction, i.e. the treatment effect, Fig. 2 shows the change in scores from the baseline day. Post hoc tests showed that the groups did not differ in their baseline scores (placebo = 1285.1 ± 12.9 ms, Solgen = 1858.3 ± 23.8 ms).

Table 3
Mean ± S.E.M. of median response latencies (ms) in the DMTS task, number correct in the DMTS and category generation tasks, times (ms) to learn simple and complex discriminations and to reverse the simple discrimination rule in the IDED task, scores for psychological, somatic and vasomotor symptoms on the Greene Climacteric Scale, and the Epworth Sleepiness Scale for subjects at baseline and after 12 weeks of placebo or Solgen treatment

	Placebo		Solgen	
	Baseline	12 weeks	Baseline	12 weeks
DMTS latency	2926.9 ± 222.9	2647.7 ± 123.9	3560.5 ± 210.9	3006.1 ± 153.9
DMTS no. correct	17.5 ± 0.4	18.3 ± 0.4	17.1 ± 0.5	17.8 ± 0.4
Category generation	23.6 ± 1.0	24.6 ± 0.9	25.3 ± 1.6	25.4 ± 1.9
Simple discrimination	1381.9 ± 68.4	1793.7 ± 214.3	1629.3 ± 135.2	1772.4 ± 113.1
Simple reversal *	1122.8 ± 49.0	1212.7 ± 65.6	1391.2 ± 117.7	1210.4 ± 39.2
Compound discrimination 1	1943.1 ± 166.2	1591.9 ± 140.7	2046.1 ± 213.3	1739.4 ± 106.1
Compound discrimination 2	1543.3 ± 100.0	1469.1 ± 117.2	1703.7 ± 161.8	1494.4 ± 108.5
Psychological	5.0 ± 0.8	5.9 ± 0.8	5.4 ± 0.9	6.3 ± 0.9
Somatic	1.7 ± 0.4	2.0 ± 0.4	2.8 ± 0.5	2.6 ± 0.6
Vasomotor	1.5 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.0 ± 0.3
Epworth	6.8 ± 1.6	5.9 ± 1.6	6.6 ± 2.2	5.3 ± 2.0

* $P = .05$ significant effect of Solgen treatment (day × group interaction ANOVA), see text for details.

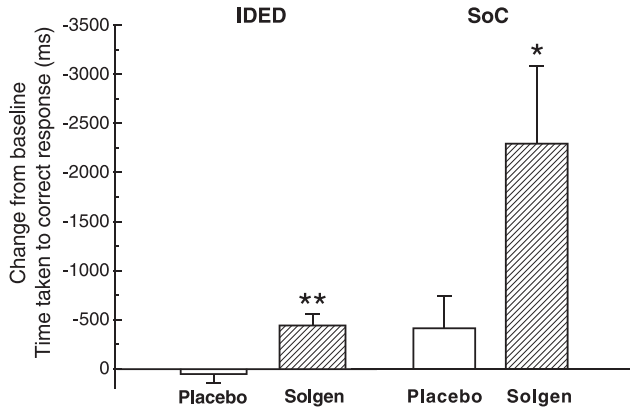

Fig. 2. Mean ± S.E.M. change in performance from baseline day in the time (ms) taken to learn complex rule reversals in the test of mental flexibility (IDED), in the time (ms) taken to complete the four-move task in the test of planning (SoC) by subjects after 12 weeks on placebo treatment (clear columns) or Solgen treatment (filled columns). Decreases in response times indicate better performance. * $P < .05$, compared with placebo, ** $P < .003$.

In the test of planning, there was no practice effect in the initial response time [day, $F(1,31) < 1.0$, ns] nor was there any effect of treatment [Group × Day interaction, $F(1,31) < 1.0$, ns]. In the time taken to complete the task, there was a significant practice effect [day, $F(1,31) = 8.6$, $P < .01$] and this was significantly greater in the Solgen group than in the placebo [Group × Day interaction, $F(1,31) = 4.2$, $P = .05$, see Fig. 2]. Post hoc tests showed that the groups did not differ significantly in their times to complete this task on the baseline day (placebo = 1829.6 ± 32.6 ms; Solgen = 3575.0 ± 57.6 ms).

3.4. Sustained attention

In the PASAT test of sustained attention, there was a significant Treatment × Day × Speed interaction [$F(1,30) = 6.1$, $P = .02$] because although the groups did not differ at the fastest speed (1.2 s), the Solgen group showed greater improvement than the placebo group at 1.6 s. Fig. 2 shows the difference in scores between these tape speeds between baseline and Week 12. Post hoc tests showed that the groups did not differ in their scores at baseline (placebo = 6.7 ± 1.6, Solgen = 8.8 ± 2.3).

3.5. Menopausal symptoms

There were no significant effects of treatment on any of the menopausal symptoms assessed by the Greene Climacteric Scale (Group × Day, $F < 1.0$, ns in all cases, see Table 3). However, there was a low incidence of symptoms in both groups.

3.6. Sleepiness

There were no significant effects of treatment on the rating of sleepiness, as assessed by the Epworth Sleepiness Scale [Group × Day, $F(1,31) < 1.0$, ns, Table 2]. On the Stanford scale, the subjects rated themselves as more tired after completing the test battery than before starting it, both on baseline day ($z = 3.2$, $P < .001$) and at Week 12 ($z = 2.4$, $P < .02$). There was no difference between the treatment groups in the change in sleepiness ratings either at baseline or at week 12 ($z < 1.2$ in both cases, ns, Table 4).

3.7. Mood ratings

Three mood factors (anxiety, sedation and well-being) can be extracted from the Bond and Lader Mood Scale (Bond and Lader, 1974). There were no significant effects of treatment on any of these factors (Group × Day, $F < 1.0$, ns in all cases). Furthermore, the two groups did not differ in their response to the stress of testing (Group × Day × Time, $F < 2.2$, ns, see Table 3). There were significant time effects on the three mood factors, showing that both groups became more anxious [$F(1,31) = 38.9$, $P < .001$], more sedated [$F(1,29) = 19.9$, $P < .001$] and more discontented [$F(1,31) = 24.0$, $P < .001$] as a result of testing (Table 4).

There was no effect of treatment on the factor measuring aggressive mood [MANOVA, Group × Day, $F(13,19) < 1.0$, ns] and no effect of treatment in response to the stress of cognitive testing [MANOVA, Group × Day × Time, $F(13,19) < 1.0$, ns]. However, there was a significant effect of time, with both groups feeling more aggressive at the end of testing [MANOVA, $F(13,19) = 4.6$, $P < .001$, data not shown].

The bodily symptoms provide measures of somatic anxiety, and on this factor, there was no significant effect of

Table 4
Mean ± S.E.M. scores on the Stanford Sleepiness Scale and scores for the factors of anxiety, sedation and well-being derived from the Bond and Lader Mood Scale, in subjects before (pre) and after (post) cognitive testing at baseline and after 12 weeks of treatment with placebo or Solgen

	Placebo				Solgen			
	Baseline		12 weeks		Baseline		12 weeks	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Stanford	2.4 ± 1.0	3.0 ± 1.0	2.5 ± 1.0	3.1 ± 0.9	2.5 ± 1.0	3.1 ± 1.0	2.2 ± 1.0	2.6 ± 1.0
Anxiety	48.0 ± 3.4	64.6 ± 4.4	46.2 ± 3.8	61.7 ± 4.7	55.6 ± 3.6	63.5 ± 4.6	46.8 ± 3.7	63.2 ± 4.1
Sedation	54.3 ± 2.7	65.6 ± 2.2	52.8 ± 3.0	59.0 ± 3.7	55.4 ± 3.3	60.6 ± 2.9	50.9 ± 2.5	55.6 ± 3.2
Well-being	53.8 ± 2.9	41.7 ± 2.8	54.6 ± 2.6	46.1 ± 3.1	51.2 ± 2.8	43.9 ± 3.5	55.3 ± 2.0	49.6 ± 3.1

$P < .001$ pre–post testing difference for all groups, ANOVA.

treatment [MANOVA, Group × Day, $F(14,18) < 1.0$, ns] and no difference between the groups in response to cognitive testing [MANOVA, Group × Day × Time, $F(14,18) < 1.0$, ns]; however, the symptoms did increase after the stress of cognitive testing [MANOVA, time $F(14,18) = 2.2$, $P < .06$, data not shown].

4. Discussion

The present study was a relatively small size one, and although volunteers were randomly assigned to the treatment groups, they could have differed in some of the measures. The only significant difference was found in alcohol consumption, with the group allocated to the Solgen treatment drinking significantly less than the group allocated to placebo treatment. However, the levels of alcohol consumption in both groups were very low and most unlikely to have caused neuronal damage. Furthermore, the groups did not differ significantly in their baseline scores in any of the cognitive tests. Importantly, the two groups in the present study were well matched in age, IQ and years in secondary education, thus effectively ruling out differences in demographic characteristics as a plausible basis for the treatment differences that were found in these tests after 12 weeks. Because the design of the experiment was double blind, we can also exclude the possible contribution of attitudes and expectancies. We attempted to minimise dietary intake of food containing soya isoflavones by asking the volunteers to refrain from eating these during the study. While, of course, we could not police this, their food diaries indicated good compliance and this population would not normally have a high soya intake from their diet. Any intake of dietary soya could have only served to increase the variance within groups and decrease the difference between groups. The group size for this study was calculated on the basis of the group sizes that showed significant effects of a soya diet in young adults (File et al., 2001a) and conventional HRT in postmenopausal women (Fluck et al., 2002; File et al., 2002).

However, our sample size was sufficient to detect some significant improvements in cognition. There were no differences between the groups in menopausal symptoms, mood or sleepiness and therefore the improvements that we did find cannot be secondary to any of these effects. Our results show that taking a dietary supplement of soya isoflavones for 12 weeks resulted in significant improvements in the long-term recall of pictures, in mental flexibility (as measured by the ability to learn rule reversals), in planning and in sustained attention. Although the MANOVA showed that the episodic memory factor was significant, of the three tests, only performance in the picture recall reached significance. Picture recall was tested several minutes after acquisition, whereas in the story and DMTS tests memory was tested within a few seconds. While it is possible that the improvement was mainly in long-term episodic memory, this would not seem very likely since the effects on story recall only just missed significance. There are several possible reasons for the failure to detect any effects of Solgen treatment in the DMTS task. It is possible that the Solgen treatment improved memory only in tasks in which it was possible to verbally code the information. The pictures were of everyday objects and could be readily coded verbally. Alternatively, the lack of effect in the DMTS test could be because it uses recognition rather than recall, and recognition is an easier process. Task difficulty may well be the most important reason and it can be seen from the baseline scores that performance in the DMTS was close to ceiling in this test (17 out of possible 20 correct answers). In a factor analysis of tests of frontal lobe function, including those from the CANTAB, Robbins et al. (1994) found that verbal fluency and visual recognition memory loaded on an independent factor from the other tests. Thus, the failure to find effects in the DMTS test and in category generation, which relies on verbal fluency, could be explained by lack of effects on this factor. The improvements that we did find in the frontal lobe tasks might also be related to task difficulty. While there were no effects of treatment in the ability to learn rules, indicating no differences in ability to attend to specific attributes of a stimulus, there was an effect on the mental flexibility required to learn that the rule had been reversed. In the SoC test, only the time to complete the task was significantly affected by the treatment and not the time to make the initial response. This is precisely the pattern of results found in patients with frontal lobe lesions (Owen et al., 1990). This may well be because of the way in which our subjects performed this task. They tended to make an initial response and then pause and think through the subsequent moves. Thus, it was the completion time that reflected their planning ability. Finally, we found that the Solgen group showed greater improvements in the PASAT, which is a sustained attention task that involves the frontal cortex as well as the thalamus. This is because it has a major working memory component and the necessity to ignore distracting items. Thus, it is quite possible that the improved performance in this task is also due to enhanced frontal lobe function.

The cognitive improvements found in this study are of interest since it has been demonstrated in a group that frequently expresses concern about deteriorating memory (File et al., 2002) and in which there is evidence that inefficient frontal lobe functioning might contribute to age-related memory impairments (Daum et al., 1996). It is also an age group in which there is a clear age-related decline in tests of attention (Fluck et al., 2001). Improvements in verbal episodic memory in postmenopausal women treated with HRT have been shown in several well-controlled studies (Phillips and Sherwin, 1992; Jacobs et al., 1998; Duka et al., 2000; Hogervorst et al., 2000). Fewer studies have investigated the effects of HRT on frontal lobe function, but two have found positive effects (Keenan et al., 2001; Duff and Hampson, 2000). However, there is evidence that long-term treatment (10 years) with HRT may impair frontal functions (Fluck et al., 2002; File et al., 2002).

At this stage, we can only speculate about the possible mechanisms underlying the cognitive effects of the soya isoflavones. Since postmenopausal women have low levels of exogenous oestrogen, it is possible that the isoflavone phytoestrogens are binding to free oestrogen receptor sites and providing a weak oestrogenic effect. The cognitive improvements that we found are most likely to be mediated by the hippocampal memory circuit and the frontal cortex. ERβ mRNA is prevalent in the hippocampus and frontal cortex (Shughrue et al., 1997, 2000; Gundlah et al., 2000; Pau et al., 1998), and since isoflavones show preferential binding to ERβ receptors, it is likely that the improvements in memory are the result of the interaction of the isoflavones with ERβ receptors. It is also possible that the isoflavones are binding to an isoform of ERβ at which oestradiol has no affinity (Price et al., 2000). It is quite possible that more than one mechanism may be contributing to the improved cognition, but it is unlikely to be the result of antioxidant activity (Wiseman et al., 2000) since plasma concentrations of an F_2-isoprostane, 8-*epi*-prostaglandin $F_{2\alpha}$, were unaffected by treatment (Duffy et al., 2002). At present, it is not known how many of the effects of oestrogen will be shared by phytoestrogens. Oestrogen exerts both genomic and non-genomic effects on brain tissue and there are many actions of oestrogen that could underlie improved memory. Possible actions include induction of synaptogenesis, increased formation of CA1 neurones in the hippocampus, direct effects on excitatory amino acids, enhancement of cholinergic and glutamatergic neurotransmitter systems and modulation of neurotrophins (see McEwen, 2001; McEwen and Alves, 1999; Toran-Allerand et al., 1999; Lee and McEwen, 2001).

We previously found cognitive benefits of 10 weeks of a high soya diet in young volunteers (File et al., 2001a). The results of the present study suggest that benefits from a soya isoflavone supplement can also be found. Animal studies have also found that both means of administration can improve cognitive performance. Performance of the radial arm maze by ovariectomised rats was significantly better after administration of a diet high in soya isoflavones (Lund et al., 2001) or oral administration of soya phytoestrogens (Pan et al., 2000). Our results also showed that soya isoflavones can improve cognition in an age group in which there is a marked decline in attention, memory and frontal lobe function (West, 1996; File et al., 1999; Fluck et al., 2001). Direct comparisons between our two studies cannot be made since the form in which soya isoflavones were administered was different. Furthermore, there is a great paucity of information on the pharmacokinetics of isoflavones and no studies comparing old and young subjects. Clearly, there is a need for studies to further characterise the precise nature of the cognitive enhancement caused by soya isoflavones, but our results suggest that oral administration of a supplement could be an effective and practical means of administration. Phytoestrogens are currently being extensively investigated to determine their likely therapeutic potential in chronic degenerative diseases such as cardiovascular disease and cancer (for review, see Wiseman, 2000). In the light of our present results, it would be important to include cognitive function and to determine whether the benefits persist after long-term treatment.

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