Soya isoflavone supplementation enhances spatial working memory in men

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Females perform better in certain memory-related tasks than males. Sex differences in cognitive performance may be attributable to differences in circulating oestrogen acting on oestrogen β receptors (ERβ) which are prevalent in brain regions such as the hippocampus, frontal lobe and cortex that mediate cognitive functions. Since soya isoflavones are known to activate ERβ, chronic isoflavone supplementation in males may improve cognitive performance in memory-related tasks. A 12-week double-blind, placebo-controlled cross-over trial was conducted in thirty-four healthy men to investigate the effect of isoflavone supplementation on cognitive function. Volunteers were randomised to take four capsules/d containing soya isoflavones (116 mg isoflavone equivalents/d: 68 mg daidzein, 12 mg genistein, 36 mg glycitin) or placebo for 6 weeks, and the alternate treatment during the following 6 weeks. Assessments of memory (verbal episodic, auditory and working), executive function (planning, attention, mental flexibility) and visual-spatial processing were performed at baseline and after each treatment period. Isoflavone supplementation significantly improved spatial working memory ($P = 0.01$), a test in which females consistently perform better than males. Compared with placebo supplementation, there were 18 % fewer attempts ($P = 0.01$), 23 % fewer errors ($P = 0.02$) and 17 % less time ($P = 0.03$) required to correctly identify the requisite information. Isoflavones did not affect auditory and episodic memory (Paired Associate Learning, Rey's Auditory Verbal Learning Task, Backward Digit Span and Letter-Number Sequencing), executive function (Trail Making and Initial Letter Fluency Task) or visual-spatial processing (Mental Rotation Task). Isoflavone supplementation in healthy males may enhance cognitive processes which appear dependent on oestrogen activation.

Phyto-oestrogens: Cognitive performance: Diet: Sex differences

Sex differences in cognitive ability are a widely studied and controversial topic. Women have consistently performed better on tests of language ability (word fluency, spelling, grammatical usage)[1,2] and certain memory tasks (verbal recall of information), while men appear to be superior at mechanical and visual-spatial tasks[3,4].

Social scientists have attributed these differences between sexes to our evolution in which complementary roles as hunters and gatherers placed different selection pressures on specific aspects of cognition[5]. There is now compelling evidence that the sex hormone oestrogen and the location of oestrogen β receptors (ERβ) in brain areas such as the hippocampus, frontal lobe and cortex, which play pivotal roles in memory (verbal and visual) and learning function[6–8], are responsible for the differentiation and maintenance of dimorphic sex differences in cognitive function[9–11].

Hormone replacement therapy, in which oestrogen is exogenously supplied, has been consistently shown to enhance and protect memory function in postmenopausal women[12,13]. Evidence that it may be possible to enhance the execution in men of cognitive tasks that are naturally performed better by women first arose from a study involving genetically male transsexuals who had been undergoing hormone replacement therapy for several months whilst awaiting gender reassignment surgery[14]. The authors of the study reported that, compared with those not on any hormone therapies, men receiving oestrogen showed improved verbal memory performance, a task at which women are generally superior. Visual-spatial processing, a task at which men naturally excel, was also preserved in those undergoing therapies, indicating that oestrogen may provide similar cognitive benefits in men as in women without detrimental effects on male-related cognitive processes. However, the use of oestrogen therapy to improve cognitive function in otherwise healthy males is not advocated.

Soya isoflavones such as daidzein and genistein mimic many of the physiological actions of oestrogens due to their high binding affinity for ERβ without inducing the development of female secondary sex characteristics. Animal studies[15–17] have confirmed that isoflavones, with their similar chemical structure to endogenous oestrogen, can improve cognition via ERβ-mediated events that lead to enhanced brain cell survival, growth and neuroplasticity. Lee *et al.* specifically demonstrated the ability of isoflavones to improve cholinergic enzyme activity in the brain and increase neuron density, resulting in improved cognitive performance[15]. Hence, there has been considerable interest in whether the

regular consumption of these isoflavones can provide similar benefits for cognitive performance in males as has been reported for oestrogen therapy.

While some studies in postmenopausal women not undergoing oestrogen therapy indicate that soya isoflavones improve cognitive performance[18–20], others have failed to reproduce the same effects[20–22]. Whether isoflavones exert a beneficial effect in males is also debatable. White et al.[23] examined relationships between tofu consumption by healthy middle-aged Japanese-American men living in Oahu and their cognitive performance and brain weight later in life. They observed greater brain atrophy and deterioration in brain function in men who consumed more than two servings of tofu per week. However, the design of their study is open to several criticisms. Even though it provided no direct evidence that brain atrophy and deterioration in cognitive function was due to the isoflavone content of the tofu, it was implied that isoflavones were a contributing factor. Several rodent studies support the claim of White et al. that isoflavone supplementation may be detrimental to cognitive performance in males[17,24], specifically worsening traits such as visual conceptualisation at which males are naturally predisposed to perform better than females.

Of the few human studies that have investigated the effects of soya consumption on cognition in males, all have been conducted using young, healthy populations[25–27], with only one[27] directly correlating effects with isoflavone consumption. This particular study by File et al. found that, compared with a control diet, consumption of a high-soya diet enriched with 100 mg total isoflavones/d for 10 weeks improved immediate and long-term episodic memory, mental flexibility and planning ability in males. However, improvements in cognition were not consistent across sexes, with females improving more at certain cognitive tasks after supplementation and males showing impaired performance in others.

In view of the limited number of studies undertaken to assess the impact of isoflavone supplementation on cognition in males, we aimed to investigate whether regular consumption of isoflavones by healthy men could improve their performance of cognitive tasks that appear dependent on oestrogen, such as memory, without impairing performance of other cognitive tasks in which men are naturally proficient.

Experimental methods

Subjects

Forty healthy men aged 30–80 years were recruited from the general community by media, print and electronic advertisements. Eligibility was initially assessed via a phone interview and by a diet and lifestyle screening questionnaire. Volunteers were excluded if they were habitual soya consumers (more than two serves of soya foods per week), suffered from known cognitive impairments or took prescribed medications or over-the-counter supplements that could influence cognition, mood, depression or anxiety.

Study design

A double-blind, placebo-controlled cross-over supplement intervention trial of 12 weeks' duration was undertaken at the Nutritional Physiology Research Centre, a joint affiliation of the University of South Australia and the University of Adelaide. The protocol for the intervention was approved by, and followed in accordance with, ethical standards of each institution's Human Research Ethics Committee. All participants provided written informed consent before taking part.

Volunteers were block-matched into two groups, balanced for age, and randomised to commence treatment with either an active isoflavone or placebo supplement for the first 6 weeks of the intervention. They then crossed over to the alternate treatments for the remaining 6 weeks. Cognitive assessments were made at baseline and at the end of each treatment phase.

Supplementation

Encapsulated supplements were produced by Frutarom (Nutrilab B.V., Giessen, The Netherlands) and donated by Soy Health Pty Ltd (Sydney, Australia). Placebo supplements were manufactured as 500 mg capsules and designed to contain 30 mg of raftilose and fibre. Subjects on the active treatment phase consumed four 500 mg capsules of Soy Life 40® per d, comprising 116 mg total isoflavones of which 68 mg was daidzein, 12 mg was genistein and 36 mg glycitin. Subjects on the placebo treatment phase consumed four matching placebo capsules/d, comprising 30 mg of fibre and raftilose. They were instructed to consume two capsules in the morning and two at night. All capsules were provided at the commencement of each treatment phase. Volunteers were provided with information on foods and beverages that contain soya and/or isoflavones and advised to abstain from consuming them during the course of the intervention.

Supplementation adherence was determined by counting the number of capsules returned after each 6-week treatment phase.

Cognitive assessments

All three cognitive testing sessions were conducted after a minimum 6 h fast from food and minimum 2 h fast from stimulants (caffeine, alcohol) and other fluids (except water).

Subjects consumed a standardised meal (plain sweet 60 g cookie and 250 ml orange juice) before commencing the cognitive assessments. The baseline session was designed to familiarise subjects with the test battery which was then repeated after the completion of each 6-week treatment phase. All tests were conducted for a subject at the same time of the day (morning or afternoon) in a quiet environment by an experienced study investigator trained by a research psychologist.

Battery of cognitive tests

As isoflavones are shown to specifically affect those areas of the brain (hippocampus, frontal lobe and cortex) that control executive mental function, memory and visual-spatial processing, tests that measure these cognitive processes were selected for the intervention. These included tasks on which females perform better than males. For instance, women are repeatedly shown to excel at the Novel Spatial Working Memory Task[28], Paired Associate Learning Task (PAL)[29] and the Rey Auditory Verbal Learning Test (RAVLT)[30,31]

in comparison with men. These tests appear to be directly influenced by oestrogen, for which women have naturally higher circulating levels, rather than by differences in the integrity of the prefrontal cortex and hippocampal formation which is responsible for executing these tasks[32]. Furthermore, to investigate whether performance on male-related tasks was reduced by the intervention, a test of visual-spatial processing was included.

Therefore, the test battery incorporated five memory based tests (the RAVLT[31], PAL[32], Backwards Digit Span[33], Letter-Number Sequencing[33] and Novel Spatial Working Memory[26]), two tests of executive mental function (Initial Letter Fluency[34,35] and the Trail Making Test[31,36]) and one test of visual-spatial processing (the Mental Rotation Task[37]).

Summarised in Table 1 are the eight cognitive tests which were performed, a brief description of the specific cognitive function being assessed, the specific region of the brain that pertains to that function and whether the test exhibited any known sex bias.

Tests of memory

The RAVLT[31] and PAL[32] measure verbal recall memory. In the RAVLT, responders are required to immediately recall a fifteen-word list read aloud by the examiner with five repetitions, then after hearing a distracter list of fifteen words, and again after a 20 min delay. The PAL similarly tests immediate and delayed auditory recall of words, although additionally assesses the ability to associate unrelated word pairs together.

Spatial working memory was assessed using a novel multi-trial task developed by Duff & Hampson[28]. In the Novel Spatial Working Memory Task, testees are required to progressively discover the location of ten pairs of coloured dots concealed in an array of twenty cards (five × four), in as few choices as possible and in the shortest amount of time possible. Proficient performance in the task is dependent on the ability to maintain and continually update representations of the spatial locations where coloured dots had previously been found in order to identify the location of coloured pairs.

Backwards Digit Span and Letter-Number Sequencing, subsets of the Wechsler Adult Intelligence Scale (WAIS III)[33], were performed to assess auditory working memory. Backwards Digit Span requires testees to recall, in reverse order, a sequence of numbers read aloud to them. In the Letter-Number Sequencing Task, letters and numbers are provided verbally in random order, with responders required to recall first the numbers and then the letters, both in an ascending order.

Tests of executive mental function

Mental flexibility, attention and planning ability were assessed using Initial Letter Fluency[34,35] and the Trail Making Test[36]. The Trail Making Test is administered in two parts. In part A, testees are presented with a piece of paper containing randomly placed numbered (1–25) circles and asked to connect as quickly as possible the circled numbers in their consecutive order, i.e. 1 to 2 to 3, etc. In part B, testees are given a piece of paper that has both randomly placed numbered (1–25) and lettered (A–Y) circles and are asked to link the numbered and lettered circles in consecutive order and alternately between the two sequences, i.e. link A to 1 to B to 2 to C, etc. The time taken to successfully complete part A and part B with no errors was recorded (in s) and the difference score (part B − part A) was calculated to remove the element of speed from the evaluation.

Initial Letter Fluency, a shortened version of the Controlled Oral Word Association Test, requires testees to state as many words as possible that begin with a specific letter of the alphabet in 60 s. Testees are instructed that the words must be composed of four or more letters, may not begin with a capital letter (which means excluding words such as people's names, places, brand names, etc), must not be a number and must not be a word they may have previously stated but with a different ending. Scores are based on the number of words recited within 60 s that comply with the test rules.

Test of visual-spatial processing

The Mental Rotation Task is a test commonly used to evaluate an individual's ability to mentally rotate visual forms. For the present study, Hay's online Mental Rotation Task was used[33]. Participants were shown two different rotations of a multi-faceted object and asked to identify whether the second object was a correct rotation of the first. The number of correct responses and speed of responding (in s) were recorded.

Table 1. Battery of cognitive tests used in the intervention

Cognitive test	Brain area	Cognitive function	Test duration (min)	Sex bias
Novel Spatial Working Memory Task	Prefrontal cortex	Object and spatial location recall, working memory	10	Female
Paired Associate Learning Task	Hippocampus	Declarative memory, learning, auditory memory recall	5–10	Female
Backwards Digit Span	Prefrontal cortex	Working memory	3–5	None
Letter-Number Sequencing Task	Prefrontal cortex	Working memory	5–10	None
Mental Rotation Task	Hippocampus, parietal lobe	Visual-spatial processing	5	Male
Rey Auditory Verbal Learning Test	Hippocampus	Learning, auditory memory recall	10–15	Female
Trail Making Test	Prefrontal cortex, parietal lobe	Planning ability	3–5	None
Initial Letter Fluency Task	Frontal lobe	Spontaneous mental flexibility, strategic retrieval of verbal information	3	None

Data analysis and statistical considerations

A potential confounder with the repetition of cognitive tests is improvement in performance due to learning or practice. The randomised, balanced cross-over design of the study should prevent any bias due to learning by eliminating order effects. Moreover, only the second and third assessments (the end of each treatment phase) were used for the cross-over comparison, thus eliminating any improvement from the initial baseline test. In any case, in the initial placebo arm, no significant improvement was detected in any test at the second and third assessments compared with the baseline assessment, indicating no evidence of a practice effect.

Data were analysed using STATISTICA for Windows software (version 5.1; StatSoft Inc., Tulsa, OK, USA). Effects of treatment on cognitive test scores were determined by one-way ANOVA with repeated measures using baseline scores as a fixed covariate. Where ANOVA showed significant main effects, differences between means were determined *post hoc* using Tukey's honestly significant difference test. For the Spatial Working Memory Task with multiple components in its assessment, a one-way multivariate ANOVA with repeated measures was performed to determine an overall effect of treatments on the test. Relationships between dependent variables (age and daily isoflavone intake) were determined using linear regression (SPSS software version 10; SPSS Inc., Chicago, IL, USA). Based on the results of an unpublished pilot study of twenty subjects (AA Thorp,

N Sinn, JD Buckley, AM Coates and PRC Howe, unpublished results), it was calculated that forty participants would give 80 % power to detect significant ($P < 0.05$) improvements in spatial working memory (reductions of 20 % in pairs viewed and 25 % in errors). Statistical significance was set at $P < 0.05$. All data are shown as mean values with their standard errors unless otherwise stated.

Results

Subjects

Of the forty men initially recruited, thirty-four (aged 49 (SEM 10) years) completed the intervention. Inability to comply with the study protocol (n 4), change in banned study medication (n 1) and surgery for a medical condition unrelated to the study (n 1) were the reasons cited by the six subjects who withdrew. Adherence to the active treatment was 92 (SEM 1) %, equating to a mean increase in daily isoflavone intake of 105 (SEM 9) mg.

Cognitive assessments

Table 2 describes the absolute and change scores for the eight cognitive tests performed during the active and placebo treatments. One-way multivariate ANOVA with repeated measures revealed a significant overall improvement in performance of the Spatial Working Memory Task while subjects were on the active treatment compared with the placebo ($P = 0.01$),

Table 2. Absolute scores for cognitive tests at the end of each treatment period and within-subject changes in scores for all subjects (n 34)

(Mean values with their standard errors)

Cognitive test	Active Mean	Active SEM	Placebo Mean	Placebo SEM	P†	Change Mean	Change SEM
Paired Associate Learning Task (no. of words)							
First recall‡	2·88	0·40	2·56	0·35	0·18	0·32	0·24
Total recall‡	19·41	1·46	18·06	1·46	0·16	1·35	0·94
Delayed recall‡	5·50	0·37	5·18	0·39	0·24	0·32	0·27
Novel Spatial Working Memory Task*							
Pairs viewed‡	56·44	2·75	68·44	5·08	0·01	12·00	4·62
Memory errors§	73·15	5·37	95·09	9·43	0·02	21·94	8·54
Time to complete task (s)§	344	24	413	37	0·03	69	31
Rey Auditory Verbal Learning Test (no. of words)							
First recall‡	7·62	0·43	7·29	0·38	0·40	0·32	0·38
Total recall‡	55·12	1·49	54·29	1·60	0·47	0·82	1·24
Immediate recall after distraction‡	10·82	0·57	10·97	0·51	0·71	− 0·15	0·39
Delayed recall (20 min)‡	10·62	0·65	10·29	0·58	0·48	0·32	0·45
Initial Letter Fluency Task (no. of words)‡	26·12	1·30	25·65	1·20	0·63	0·47	0·96
Backwards Digit Span (no. of words)‡	7·35	0·42	6·97	0·38	0·34	0·38	0·39
Trail Making Test (s)							
Trial A§	29	2	28	1	0·31	− 0·5	1
Trial B§	68	4	70	4	0·64	2	4
Total (B − A)§	39	4	41	4	0·74	2	4
Letter-Number Sequencing Task (no. correct)‡	10·38	0·36	10·71	0·40	0·37	− 0·32	0·35
Mental Rotation Task							
Number correct‡	24·65	0·60	24·62	0·64	0·94	0·03	0·40
Time to complete task (s)§	110	9	113	10	0·61	3	6

* All components of the Novel Spatial Working Memory Task were significantly improved after the isoflavone treatment compared with the placebo treatment ($P < 0.05$).
† Statistical significance was determined *post hoc* using Tukey's honestly significant difference test.
‡ Larger values reflect better performance.
§ Smaller values reflect better performance.

with each of the three test components individually shown to significantly improve. On average, males viewed 18 % fewer pairs ($P=0.01$), conducted 23 % fewer memory errors throughout the test ($P=0.02$) and took 17 % less time (in s) to complete the task ($P=0.03$). One-way ANOVA with repeated measures revealed no significant treatment effect with respect to any of the other cognitive tests.

Linear regression showed no significant within-subject correlation between change in age or change in mean daily isoflavone intake and changes in any of the cognitive test scores ($r < 0.26$; $P > 0.12$).

Discussion

The present intervention provides the first known evidence that isoflavone supplementation in men may significantly improve spatial working memory, a female-dominant cognitive process, without being detrimental to the performance of visual-spatial processing, a male-dominant cognitive task. Spatial working memory relates to one's ability to temporarily store and hold location information for an object in a readily assessable form, while other cognitive decisions or operations are taking place, and to manipulate that information or use it to guide action.

These findings support previous data from File et al. who reported improvements in cognitive performance when young males consumed isoflavones[27]. They also contradict controversial epidemiological data from White et al. who stated that isoflavones from tofu consumed during midlife can manifest cognitive impairment in males[23].

In the present study, the age of males ranged considerably from 30 to 80 years but was not shown to influence the degree to which their spatial working memory improved. This contradicts the recent findings of Hogervorst et al.[34] that isoflavones present in soya foods worsen memory performance of older but not younger adults.

The improvement observed in the Novel Spatial Working Memory Task may be due to increased binding of isoflavones to ERβ in the prefrontal cortex, an area of the brain which plays a critical role in working memory[35]. Neuroendocrine research confirms that the cortex is highly susceptible to the organisational and activation effects of sex hormones[36–38], with the prefrontal cortex shown to be one of the highest binding sites for oestrogen[39] and its activity associated with variations in female hormonal status[40]. Whilst it was beyond the scope of the intervention to assess the entry of isoflavones into specific cerebral regions, their ability to rapidly enter the lipophilic environment of the brain is well established. Gamache et al.[41] was the first to demonstrate that intra-peritoneal injections of genistein and daidzein to adult Sprague–Dawley rats resulted in increased levels in the brain tissue. Animal studies examining the relative isoflavone content of specific brain structures, after feeding an isoflavone-rich diet, have furthermore established that they are most concentrated in regions that are abundant in ERβ[24,42].

The Novel Spatial Working Memory Task used in the intervention was directly modelled on past tests measuring working memory in which prefrontal involvement has been determined unequivocally[43–46]. Women are traditionally shown to view fewer pairs, make fewer mental errors and require less time to complete the task compared with males, with no evidence to suggest that this sex difference in performance is attributable to differences in overall ability, attention, perceptual speed or speed of verbal access. In the present intervention, all of our male subjects viewed significantly fewer pairs (18 %), made fewer mental errors (23 %) and performed the task in a shorter amount of time (17 %) after receiving isoflavone supplementation.

In contrast to our predictions, the two other cognitive tasks (PAL and RAVLT) that are known to be performed naturally better by females did not improve. With only a small number of male subjects completing the trial (n 34), this sample size may have been underpowered to detect a significant effect in a healthy population. For instance, the Novel Spatial Working Memory Task is specifically devised to be sensitive enough to detect significant improvements in healthy adults, whereas the PAL and RAVLT tests were both developed to detect differences in populations who have known or severe cognitive impairments. Moreover, the sex difference in performance of the Spatial Working Memory Task is typically greater than the observed sex difference in other cognitive tests[28], so it is not surprising that this was the only test to show improvement in males in the present study.

Three tests in our cognitive battery known to have no sex difference (Letter-Number Sequencing Task, Initial Letter Fluency Task and the Backward Digit Span Task)[47–50] were also shown to be unaffected by isoflavone treatment, which is similar to the lack of effect seen in postmenopausal women consuming 99 mg isoflavone/d for 12 months[51] and 70 mg total isoflavones for 16 weeks[52]. Overall, the Trail Making Test is not considered to be sex biased. However, there is evidence that women may perform somewhat slower than men on part B[53]. Part B of the Trail Making Test is shown to be more sensitive to cognitive flexibility than part A and places increased demands on motor speed and visual search[54,55]. In the intervention, the time taken to complete part B of the Trail Making Test did not differ for subjects during each of the treatments, suggesting that the isoflavones were not detrimental to the men's performance of this task.

In visual-spatial processing tasks that require the ability to perceive, analyse, synthesise, and think with non-linguistic visual patterns and spatial configurations, researchers have consistently found that males outperform females[56–59]. The underlying mechanism for this difference is not fully understood though there is strong evidence to suggest that gonadal steroids modulate this sexually dimorphic ability, specifically testosterone through interneuronal conversion to oestradiol in the brain[60]. For instance, the castration of male rats is shown to inhibit visual-spatial performance, whilst treatment of intact and ovariectomised female rats with testosterone improves their spatial ability[61,62]. Several animal studies have investigated the effect of isoflavones on cognitive tasks that favour males, such as visual-spatial processing, using the Mental Rotation Task[63]. Two studies found that consumption of isoflavones was detrimental to performance of the Mental Rotation Task in male rats compared with females[17,24]. In contrast, elderly male rats consuming a high-isoflavone diet have been reported to exhibit improved spatial memory in a delayed matching-to-place water maze task[15].

In the present intervention, performance of the Mental Rotation Task was consistent across the treatments. A lack of difference indicates that our men's visual-spatial performance was not adversely affected by isoflavone supplementation and

contradicts previous evidence in animal models that isoflavones are harmful to this specific cognitive function in males.

Despite predicting sufficient power with forty male subjects, only one of the eight tests showed a significant improvement following supplementation of isoflavones. An explanation for this could be that the thirty-four males in the intervention had no apparent impairment in their cognitive function and a greater number of subjects would be needed to detect significant improvements in a healthy population. A further limitation of the intervention was our inability to control potential hormonal effects, with evidence that testosterone levels fluctuate during the day and can influence the cognitive performance of males[64–66]. Whilst subjects attended the clinic at the same time of day after each treatment, no blood samples were collected before testing. Subsequently it was not possible to quantify subjects' testosterone levels and use this as a covariate in our analysis of their cognitive performance. Compliance of participants in the study is also subject to criticism. Adopting a more robust method such as assessing 24 h urinary isoflavone levels as opposed to capsule counting would have improved the accuracy of the assessment.

In summary, when healthy men consumed isoflavones, an improvement in spatial working memory was observed. This type of cognitive process is traditionally performed better by females because of the sex hormone, oestrogen. The test used to assess this cognitive function was the Novel Spatial Working Memory Task. An improvement in all aspects of this multi-component test denotes an enhanced ability by the men to retain and continually update information based on visual cues. It is speculated that the ability of isoflavones to bind to ERβ and mediate transcriptional events that enhance neuronal growth, survival and synaptogenesis in the prefrontal cortex may have instigated the effect. Importantly, isoflavones were not shown to be detrimental to the performance of visual-spatial processing, a task at which males naturally excel.

Acknowledgements

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