

## *Ginkgo biloba*: no robust effect on cognitive abilities or mood in healthy young or older adults

Nicholas R. Burns<sup>1\*</sup>, Janet Bryan<sup>2</sup> and Ted Nettelbeck<sup>3</sup>

<sup>1</sup>Department of Psychology, University of Adelaide, Australia

<sup>2</sup>CSIRO, Health Sciences and Nutrition, Australia and Department of Psychology, University of Adelaide, Australia

<sup>3</sup>Department of Psychology, University of Adelaide, Australia

*Ginkgo biloba* extracts are commonly used to prevent or treat memory problems but evidence on the efficacy of ginkgo is equivocal. In any case, the psychological locus of ginkgo's effects is unknown. A 12-week, double-blind, placebo-controlled study assessed effects of ginkgo (120 mg per day) on a wide range of cognitive abilities, executive function, attention and mood in 93 healthy older adults (55–79 years) and in 104 young adults (18–43 years). For the older adult sample, longer-term memory assessed by associational learning tasks showed improvement with ginkgo ( $d = 0.52$ ,  $p = 0.04$ ). There was no statistically significant difference on any other measure. For the young adult group no measure showed statistically significant effects of ginkgo enhancement. There were no side effects unequivocally attributable to treatment with ginkgo and those reported by participants in the ginkgo groups were mild and similar to those reported elsewhere. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS — *Ginkgo biloba*; cognitive abilities; mood; young adults; older adults

### INTRODUCTION

Ginkgo, extracted from the leaves of *Ginkgo biloba* (maidenhair tree) is the most commonly sold herbal product in Germany (Grunwald, 1995) and one of the most popular herbals in the US where it is primarily a supplement to prevent or treat memory problems (Beaubrun and Gray, 2000). The major therapeutic components of the ginkgo extract (EGb 761), flavonoids and terpenoids, are held to have antioxidant, anti-inflammatory and neuroprotective effects. Ginkgo is thought to impact positively on neurological and cognitive function by increasing blood flow via vasoregulation (Santos *et al.*, 2003) and, because the platelet activating factor antagonism of ginkgolides improves cerebral metabolism, by pro-

tecting the brain against hypoxic damage (Kleinjen and Knipschild, 1992). Brain metabolic changes linked to ginkgo have been demonstrated as increased alpha EEG activity, accompanied by reduction in delta and theta activity in both young and older adults (Itil *et al.*, 1998). It has also been suggested that neural cell membrane damage is prevented by the antioxidant properties of ginkgo flavonoids (Kleijnen and Knipschild, 1992). Ginkgo may have acute effects on the brain by modulating neurotransmitter systems, notably the catecholamines (Ramassamy *et al.*, 1992) and the cholinergic system (Wirth *et al.*, 2000). Nathan (2000) reviewed evidence that ginkgo directly affects cholinergic systems and that it also has an indirect effect on them via modulation of the serotonergic system. He argued that ginkgo's pharmacological effects are likely due to a combination of platelet activating factor antagonism, free radical scavenging, and modulation of the cholinergic system.

Most studies assessing the efficacy of ginkgo on human cognitive performance have involved older participants with cognitive impairment, typically those in the early stages of Alzheimer's disease (AD; Curtis-Prior *et al.*, 1999). Oken *et al.* (1998) concluded that 3- to 6-month treatment with 120 mg

\*Correspondence to: Nicholas R. Burns, Department of Psychology, University of Adelaide, South Australia, Australia 5005. Tel: 61 8 8303 3965. Fax: 61 8 8303 3770. E-mail: nicholas.burns@adelaide.edu.au

Contract/grant sponsors: CSIRO Collaborative Grants Scheme and University of Adelaide and Faculty of Health Sciences Small Research Grant Schemes and Blackmores Ltd.

to 240 mg of ginkgo per day was efficacious in AD; their meta-analysis on four studies meeting minimally acceptable scientific criteria found an effect size of  $d = 0.4$ . However, many studies have shown methodological shortcomings, like small samples and inadequate description of randomisation procedures, patient characteristics, effect measurement and data presentation (Kleinjen and Knipschild, 1992; Oken *et al.*, 1998). Furthermore, many studies have used measures of cognitive *impairment* (e.g. Le Bars *et al.*, 1997)—often subjective patient and/or doctor-reports on degree of impairment—rather than objective measures of cognitive *performance*.

Two studies using objective measures of the cognitive performance of older adults produced mixed findings. Vesper and Hänsgen (1994) conducted a 12 week, double-blind, placebo-controlled study in 90 patients (average age, 62.7 years) with cerebral insufficiency. They administered 150 mg per day and reported positive results on attention and memory from the sixth week of treatment. These improvements were supported by patients' and significant others' self-reports. Van Dongen *et al.* (2000) reported on a 24-week randomised, double-blind, placebo-controlled study on 214 participants (average age, 83.9 years) with either dementia (AD or vascular dementia) or age-related cognitive impairment. Participants received either 160 mg or 240 mg ginkgo per day and the efficacy, dose-dependence and durability of ginkgo were evaluated at both 12 and 24 weeks. No beneficial effects of ginkgo on neuropsychological, psychopathological or behavioural outcomes were found. Importantly, however, a recent comprehensive review of the efficacy of ginkgo among individuals with cognitive impairment or dementia concluded that there is an evidence of improvement following ginkgo administration (Birks and Grimley Evans, 2002).

Very few studies have assessed the efficacy of ginkgo among healthy older adults. Mix and Crews (2000) conducted a six-week, randomised, double-blind, placebo-controlled study in 40 cognitively intact older adults (mean age 67.5 years). They assessed the efficacy of 180 mg ginkgo per day on neuropsychological outcomes, finding a beneficial effect on the colour-naming trial of the Stroop test but no significant effects on other cognitive abilities including memory, although the latter showed beneficial trends. However, more participants in the ginkgo group rated their ability to remember as better than did those in the placebo group. More recently, Solomon *et al.* (2002) reported on a six-week, double-blind, placebo-controlled and parallel-group trial on 130 healthy older adults (mean age 69.3 years). They administered 120 mg ginkgo

daily and found no effects on cognitive performance. Mix and Crews (2002) conducted a second study, this time on 262 older adults. From an extensive battery of tests, they reported statistically significant enhancement only for delayed recall and recognition sub-tests along with better self-rated memory performance in the ginkgo group compared with placebo.

Studies on the effects of ginkgo on the cognitive performance of healthy young adults have assessed shorter-term effects of higher doses of ginkgo. Subhan and Hindmarch (1984) reported positive effects on the Sternberg memory task 1 h after treatment with 600 mg of ginkgo. Rigney *et al.* (1999) examined the effects of four doses, ranging from 120 mg–300 mg ginkgo over two days, in 31 participants aged 30–59 years, on a range of cognitive outcome measures and found beneficial effects on reaction times for the Sternberg memory test, with the 120 mg dose producing the largest effect. They also found that cognitive enhancement was more apparent among their older (50–59 years) participants. Kennedy *et al.* (2000) reported short-term dose-dependent improvement on speed of attention following 240 mg and 360 mg doses of ginkgo after both 2.5 hr and 6 hr, and also on quality of memory following 120 mg ginkgo but after 1 hr. Moulton *et al.* (2001) found no effects of 120 mg per day ginkgo, compared with placebo, after five days of treatment among 60 healthy men (mean age around 20 years in both groups) on a range of cognitive outcomes including memory performance, reaction time and digit- and reading-span. In a longer-term study, Stough *et al.* (2001) investigated the effects of 120 mg per day ginkgo given over 30 days, compared with placebo, on the performance of a wide range of cognitive tests among 61 healthy adults aged 18–40 years. They found positive effects of ginkgo on working memory and delayed recall. In addition, participants receiving ginkgo also reported subjectively better cognitive clarity, memory and attention compared with those receiving placebo. Kennedy *et al.* (2002) used a 360 mg dose of ginkgo and found positive effects on their memory measures (i.e. tests combined on the basis of factor analytic outcomes) in 20 healthy undergraduates.

The current study aimed to extend previous research by examining the efficacy of 120 mg per day ginkgo, given over 12 weeks, to benefit the performance of healthy older and young adults on a comprehensive battery of cognitive outcomes. This administration period was chosen because it has commonly been used in previous studies and appears to be a minimum period required for assessing longer-term effects of ginkgo on cognition (Birks and Grimley

Evans, 2002), as opposed to studies on acute effects of higher doses. We assessed a wide variety of cognitive abilities using a battery of tests informed by the theory of fluid and crystallised abilities (Gf-Gc theory; see e.g. Horn and Noll, 1994). This theory identifies nine broad cognitive abilities, five of which were included here: fluid ability (Gf, basic processes of reasoning); crystallised ability (Gc, breadth of knowledge, experience, learning, and acculturation); short-term memory (Gsm, working memory, information processing, apprehension and retention); long-term storage and retrieval (Glr, fluency and breadth of retrieval of stored information) and cognitive processing speed (Gs, rapid cognitive processing of information). For the older adult sample, two tests for each of these constructs were taken from the Woodcock-Johnson Psycho-Educational Battery-Revised (WJ-R; Woodcock and Johnson, 1989) while for the young adult sample a largely computerised battery of tests measuring the same constructs was used. The reason for this procedural difference was that we judged that the older adults would be more comfortable with the traditional mode of testing. We also included measures of executive function, attention, vocabulary, general knowledge, reaction time (RT) and inspection time (IT). Finally, we measured subjective well-being using the Profile of Mood States (POMS; McNair *et al.*, 1971). Previous research has seen positive effects of ginkgo on mood as measured using the Bond-Lader scales (Kennedy *et al.*, 2002); specifically, they found improvements on the Alert factor and the Content factor. More recently, Trick *et al.* (2004) followed-up 1570 healthy older adults involved in a previous 4 month study on ginkgo for a further 6 months and found self-rated perceptions of mood and activities of daily living were better for those participants who had continued to take ginkgo.

If ginkgo enhanced cognitive performance, we expected to see evidence of effects on tests for Gf, Gsm and Gs, because these abilities are known to be influenced by a wide range of psychopharmacological agents and other circumstances; whereas Gc and Glr are known to be more stable and less vulnerable to even substantial insult to the brain (Horn and Noll, 1994).

## STUDY 1: OLDER ADULTS

### *Materials and method*

*Participants.* Ninety-three participants (50 males, 43 females) aged 55 to 79 years (mean age = 61.7,  $SD = 5.5$  years) were recruited from volunteers who responded to a television news report about study

plans. Volunteers were excluded if they were taking cardiovascular medication (such as imdur), had a known cardiovascular condition, were taking anti-coagulant medication (such as warfarin), were taking dietary supplements that have a blood-thinning effect (such as fish oil), or were taking medication likely to affect mental performance or mood (such as antidepressant or anti-anxiety medication) or who had suffered any injury (such as stroke) that might impair performance on the cognitive tests. Participants were also required to be proficient in English due to the language requirements of some tests.

*Cognitive abilities testing.* The following sub-tests from the WJ-R were completed (the hypothesised broad cognitive ability measured by each test is given in parentheses): Analysis-Synthesis (Gf; fluid ability), Concept-Formation (Gf), Picture-Vocabulary (Gc; crystallised ability), Oral Vocabulary (Gc), Memory for Sentences (Gsm; short-term memory), Memory for Words (Gsm), Visual Matching (Gs; processing speed) and Cross Out (Gs), Memory for Names (Glr; long term storage and retrieval) and Visual-Auditory Learning (Glr). In the delayed-recall versions of these Glr tests participants are tested on material learned in the earlier administration; they are not told that they will be retested. All tests were administered in accordance with manual instructions except that delayed-recall versions of the Glr tests were presented at the end of the testing session, rather than the recommended one-to-eight days later. Additional cognitive tests administered were Spot-the-Word (Vocabulary; Baddeley *et al.*, 1988), and Self-Ordered Pointing (executive function; Petrides and Milner, 1982).

*Chronometric testing.* Two additional tasks estimated speed of information processing. The participant sat facing either a custom display panel or a computer monitor at a viewing distance of approximately 0.7 m. The first task was an 'odd-man-out' reaction time (OMO) task (Frearson and Eysenck, 1986). Participants responded to different configurations of three lights in which one light was always further away from the other two (i.e. one light was the 'odd-man-out'). There were eight lights, each paired with a response button, equally spaced at 3.5 cm apart and in a semicircle equidistant (10 cm) from a home-button and light (Jensen and Munro, 1979). Participants initiated a trial by depressing the home button and, after a variable period of from 1 to 4 sec, three lights were activated. The participant depressed the key next to the target ('odd-man-out') light as quickly as possible. This task returned two dependent variables;

decision time (DT), measured from the onset of the stimulus array until the participant lifted the finger from the home button; and movement time (MT), from release of the home button until the target button was pressed.

The second task was inspection time (IT; Nettelbeck, 1987), a pattern backward masking task measuring the duration for which a visual stimulus must be exposed for a simple discrimination to be made reliably. IT has been shown to be moderately related to IQ, with shorter IT correlating with higher IQ. The target figure was two vertical lines, one markedly longer than the other, joined at the top by a horizontal line; the shorter line appeared on the left or right equiprobably. Following exposure of the target for the duration determined by the estimation algorithm (see below), it was immediately replaced by a mask consisting of two vertical lines shaped as lightning bolts, which completely covered the two vertical lines of the target. The participant indicated whether the shorter line in the target had appeared on the left or right in the figure by pressing the corresponding key on the response panel. The estimation procedure for critical exposure of the target (IT) started with an exposure of 315 ms; following three correct responses in a row, this was reduced by 16 ms; following any error the exposure duration was increased by 16 ms. This procedure continued for eight changes of direction in exposure duration and returned an estimate of IT as the duration at which the probability of a correct response was 79%.

*Subjective well-being.* The Profile of Mood States (POMS, McNair *et al.*, 1971) is intended to measure current mood states including the dimensions: tension/anxiety; depression/dejection; anger/hostility; fatigue/inertia; confusion/bewilderment; vigour/activity and total mood. Participants reported how they felt during the past week on a scale of 1 (not at all) to 5 (extremely) in response to 65 adjectives (e.g. 'tense', 'cheerful') both at the beginning and at the end of the study.

#### *Procedure*

When potential participants had registered interest in the study, the full protocol for which was approved by the Human Research Ethics Committees of the University of Adelaide and the CSIRO, they received an information package by mail. They completed and returned a medical questionnaire requesting details on medical history and current medications, both those prescribed by a medical practitioner and also herbal-type supplements. Two questions asked for a

rating on a 5-point scale ('Poor', 'Fair', 'Average', 'Good' and 'Excellent') of current health compared to 'Perfect Health' and compared to 'Others (i.e. age peers)'. These questionnaires were reviewed by our medical advisor. Those people determined as eligible to participate were then mailed the POMS to complete immediately prior to their first attendance at the laboratory. At this first session, written informed consent was obtained to proceed further. We also sought permission to inform each participant's medical practitioner that their patient was enrolling in the study. Baseline measures on the cognitive abilities and chronometric tests were obtained at this time.

The experiment was a 12-week, double-blind, fixed-dose, placebo-controlled parallel group design. Participants were randomly assigned to either the ginkgo group, or the placebo group and took three tablets daily, one after each of breakfast, lunch and the evening meal. We used a product, (trade name Ginkgoforte) containing ginkgo extract 40 mg, standardised (according to the manufacturer Blackmores Ltd., Balgowlah, NSW, Australia) to contain ginkgo-flavonglycosides 10.7 mg (24%) and ginkgolides 2.7 mg (6%). Placebo tablets appeared the same as the ginkgo tablets, both being small capsule shaped tablets, film-coated pale yellow-green; we believe the film-coating minimised any difference in taste between the ginkgo and placebo capsules. Random assignment was achieved by randomly assigning (without replacement) numbers between 1 and 200 to pairs of bottles each containing either 126 ginkgo or placebo capsules (i.e. exactly enough for 12-weeks administration of three tablets per day) and then providing participants with the bottle number corresponding to the order in which they were tested.

Tests were completed in the following order for all participants: Visual Matching, Memory for Names, Cross Out, Visual-Auditory Learning, Memory for Sentences, Analysis-Synthesis, Memory for Words, Concept-Formation, IT, OMO Reaction Time, Self-Ordered Pointing, Spot-the-Word, Memory for Names (delayed-recall), Picture Vocabulary, Visual Auditory Learning (delayed-recall) and Oral Vocabulary.

Scores for the WJ-R tests were expressed as both Rasch-type *W*-scores (these are a transformed *raw score* centred on 500 and with equal interval properties such that they are comparable across all tests and do not mask individual differences that are confounded with age, as do other normed scores) and age-normed IQ-type scores. The *W*-scores from the two tests of each broad ability construct were averaged to provide measures of five broad cognitive abilities, Gf, Gc, Gsm, Gs and Glr, as well as a measure of

delayed-recall for Glr. The average of the age-normed scores for the 10 cognitive abilities tests provided an overall IQ score. For Spot-the-Word, the score was the number of correct items minus the number of errors; for Self-Ordered Pointing, the number of errors over three parallel forms was recorded. The measures for OMO were median decision time (DT) and median movement time (MT) from 60 trials; for IT, the measure was the average of two consecutive estimations, all in milliseconds.

Testing took about 3 h including breaks as required. We took great care that participants were not too taxed or fatigued by the testing. At the end of the session participants were provided with the next numbered supply of ginkgo or placebo. For the next 12 weeks, participants were contacted on a weekly basis to monitor the presence of any side effects and to encourage compliance with the intervention regime. At the end of 12 weeks, participants completed the POMS immediately prior to their return to the laboratory. At this final laboratory session, which was held at the same time of day (i.e. either morning or afternoon) as the first testing session, the identical test protocol to that administered initially was completed. Participants did not take a capsule on the day of the second testing session. All tasks were exactly as at the first testing session. Parallel versions of tasks were not used because, for these tests, none exists; in any case, the diverse battery used and the fact that there were only two testing points obviated the need to consider the use of alternate forms. Any unused capsules were returned to the laboratory and their number noted. The median number of unused capsules was seven and, with non-compliance operationalised as more than 25 per cent of capsules unused, no participant was classified as non-compliant.<sup>1</sup>

### Results

Table 1 shows information on 93 participants at the beginning of the study. The two groups did not differ on any of these measures. Eighty participants attended the laboratory for the final laboratory session. Of the thirteen who did not return, two males (ginkgo group) and one female (placebo group) withdrew because of headaches and one male (ginkgo) withdrew because of sleep disturbance. Two female participants

(ginkgo) and one male (placebo) withdrew for medical reasons unrelated to their involvement in the study; two females and one male (ginkgo) withdrew without giving reasons; two males (ginkgo) and one male (placebo) withdrew for personal reasons. There were no statistically significant differences between the groups in terms of the numbers of individuals reporting any type of side effects. The side effects reported by members of the ginkgo group were typical of those seen in other studies, viz., headaches, sleep disturbances and gastrointestinal symptoms.

Table 2 shows the means and standard deviations on five measures of broad cognitive ability, a measure of delayed-recall for tests on long-term storage and retrieval (Glr), and measures of vocabulary, executive function and speed of processing, for both groups at pre- and post-test, along with test-retest reliabilities for all measures; these were all high except for errors on Self-Ordered Pointing. The outcomes on a one-way analysis of covariance of the post-test scores, with the pre-test scores used as covariate and group

Table 1. Characteristics of the older adult sample in two groups on entry into the study

	Placebo ( <i>n</i> = 47)	Ginkgo ( <i>n</i> = 46)
Gender		
Male	26 (55%)	24 (52%)
Female	21 (45%)	22 (48%)
Age ( <i>SD</i> ) in years	62.2 (5.3)	61.2 (5.7)
Years of education ( <i>SD</i> )	12.7 (4.4)	13.4 (4.6)
IQ ( <i>SD</i> ) <sup>a</sup>	104.9 (8.7)	106.6 (7.8)
Number of medical conditions ( <i>SD</i> )	1.0 (1.02)	0.78 (0.96)
Number of orthodox medications ( <i>SD</i> )	0.8 (1.26)	0.50 (0.91)
Number of non-orthodox medications ( <i>SD</i> )	0.2 (0.60)	0.63 (1.1)
Health rating compared with others <sup>b</sup>		
Poor	0 (0%)	0 (0%)
Fair	1 (2%)	0 (0%)
Average	4 (9%)	5 (11%)
Good	26 (55%)	23 (50%)
Excellent	16 (34%)	18 (39%)
Health rating compared with perfect health <sup>b</sup>		
Poor	2 (4%)	0 (0%)
Fair	1 (2%)	2 (4%)
Average	7 (15%)	16 (35%)
Good	33 (70%)	20 (44%)
Excellent	4 (9%)	8 (17%)

<sup>1</sup>An anonymous reviewer suggested we re-run analyses using a more stringent criterion for non-compliance, viz., greater than 10% unused capsules. We did this for both studies and found means and effect sizes to be near identical to those for the original analyses. In the interest of maximising statistical power, we retained the original criterion for non-compliance.

<sup>a</sup>IQ was estimated as the average age-normed scores on 10 tests of cognitive ability from the Woodcock-Johnson Psycho-Educational Battery-Revised (Woodcock and Johnson, 1989).

<sup>b</sup>Self-ratings of current health compared to others of one's own age and compared to perfect health on a 5-point scale (Poor-to-Excellent).

Table 2. Descriptive statistics and test-retest reliabilities for the cognitive abilities measures at pre- and post-intervention for ginkgo and placebo groups and results of ANCOVA on effect of group at post-intervention with pre-intervention scores as covariate where outcomes showed increased improvement by the ginkgo group compared to placebo group in the older adult sample

	Reliability <sup>d</sup>	Placebo		Ginkgo		ANCOVA results
		Pre-test	Post-test	Pre-test	Post-test	
Fluid ability (Gf)	0.88	494 (16.9)	501 (15.7)	497 (19.5)	506 (20.1)	$F(1, 76) = 0.1, p = 0.74$
Crystallised ability (Gc)	0.91	544 (15.6)	546 (16.8)	546 (19.6)	550 (15.2)	$F(1, 77) = 0.8, p = 0.36$
Short-term memory (Gsm)	0.84	508 (16.5)	512 (17.4)	505 (11.9)	511 (14.0)	$F(1, 77) = 0.0, p = 0.97$
Cognitive processing speed (Gs)	0.89	511 (11.5)	513 (10.7)	516 (11.0)	519 (10.9)	$F(1, 77) = 0.6, p = 0.45$
Long-term storage and retrieval (Glr)	0.83	490 (7.9)	496 (8.9)	490 (6.8)	500 (9.2)	$F(1, 75) = 4.4, p = 0.04$
Glr Delayed-recall	0.79	489 (8.7)	495 (10.9)	488 (9.0)	501 (10.1)	$F(1, 74) = 3.0, p = 0.09$
Spot-the-word (Vocabulary) <sup>a</sup>	0.88	39.5 (9.9)	40.4 (10.1)	41.3 (8.4)	43.4 (8.1)	$F(1, 75) = 0.0, p = 0.86$
Self-ordered pointing (Executive function) <sup>b</sup>	0.57	8.8 (3.5)	8.0 (3.4)	8.6 (2.8)	7.9 (2.8)	
Odd-man-out decision time (DT) <sup>c</sup>	0.90	758 (222)	723 (196)	700 (231)	690 (314)	
Odd-man-out movement time (MT) <sup>c</sup>	0.85	257 (61)	242 (57)	258 (61)	249 (60)	
Inspection time (IT) <sup>c</sup>	0.68	100 (24)	91 (17)	100 (30)	86 (17)	$F(1, 76) = 3.1, p = 0.08$

Note: Except as indicated, scores are *W*-scores for broad abilities measured by the Woodcock–Johnson Psycho-Educational Battery-Revised (Woodcock and Johnson, 1989). ANCOVA degrees of freedom vary because some participants did not complete all tests at 12 weeks.

<sup>a</sup>Number of correct items less number of errors.

<sup>b</sup>Total number of errors over three parallel forms.

<sup>c</sup>Milliseconds.

<sup>d</sup>Correlation between measures at pre-test and post-test for all participants.

membership as the independent variable, are also presented in Table 3 where there was differential improvement in performance favouring the ginkgo group. There was a differences between the groups with an associated probability  $p < 0.05$  on the broad measure of Glr only. Differences in favour of the ginkgo group on delayed-recall on Glr and inspection time (IT) approached statistical significance. Given the relative importance of protecting against Type II error in intervention studies, these three results merit closer examination. The effect sizes for these three measures were therefore calculated as the ratio of the difference between the groups in change scores (i.e. post-test minus pre-test) to the pooled standard deviation, across the four measures for each test (i.e. pre- and post-test for the two groups). For Glr and delayed-recall Glr,  $d = 0.52$  and  $0.54$ , respectively; for IT,  $d = 0.20$ . The effect sizes for the long-term storage and retrieval measures were consonant with those reported in the meta-analysis of the effects of ginkgo in cognitively impaired older adults (Oken *et al.*, 1998). There were no differences between the two groups on any of the measures derived from the POMS. The possibility that older individuals would be differentially sensitive to ginkgo enhancement was also thoroughly explored but no correlation was significantly different from zero.

## STUDY 2: YOUNG ADULTS

### Materials and method

**Participants.** One hundred and four male participants aged 18–43 years (mean age = 30.4,  $SD = 6.9$  years) were recruited from volunteers who responded to a television news report about study plans. Only males

Table 3. Characteristics of the young adult sample in two groups on entry into the study

	Placebo ( $n = 50$ )	Ginkgo ( $n = 54$ )
Age ( $SD$ ) in years	31.1 (6.9)	29.7 (6.9)
Years of education ( $SD$ )	13.9 (2.6)	13.0 (1.9)
Health rating compared with others		
Poor	0 (0%)	0 (0%)
Fair	0 (0%)	0 (0%)
Average	13 (27%)	18 (33%)
Good	27 (55%)	24 (45%)
Excellent	9 (18%)	12 (22%)
Health rating compared with perfect health		
Poor	1 (2%)	0 (0%)
Fair	15 (10%)	9 (17%)
Average	17 (35%)	18 (33%)
Good	22 (45%)	21 (39%)
Excellent	4 (8%)	6 (11%)

were included because the Human Research Ethics Committees considered the inclusion of females of child-bearing age problematic in the absence of any evidence on the effects of ginkgo taken during pregnancy. Other exclusion criteria were as for Study 1.

*Cognitive abilities testing.* A largely computerised battery of tests was completed (the hypothesised broad cognitive ability measured by each test is given in parentheses): Concept-Formation (from WJ-R, Gf), Raven's Progressive Matrices (Gf), Information (Gc), Digit Span (Gsm), Picture Recognition (Gsm), Visual Matching (from WJ-R, Gs), Digit Symbol (Gs), Memory for Names (from WJ-R, Glr) and Visual-Auditory Learning (from WJ-R, Glr). Additional attentional tests administered were a computerised version of PASAT (Gronwall and Wrightson, 1974) and the Stroop Colour Word Test (Golden, 1978).

*Chronometric testing.* Two additional tasks estimated speed of information processing and were administered as described for Study 1.

*Subjective well-being.* The Profile of Mood States (POMS, McNair *et al.*, 1971) was administered as described for Study 1.

### Procedure

Procedural details were as described for Study 1 with the following exceptions. Tests were completed in the following order for all participants: Visual Matching, Memory for Names, Digit Symbol, Visual-Auditory Learning, Digit Span, Picture Recognition, Concept-Formation, IT, OMO Reaction Time, PASAT, Stroop Colour Word Test, Memory for Names (delayed-recall), Information, Visual Auditory Learning (delayed-recall) and Raven's Progressive Matrices.

Scores for the WJ-R tests were expressed as Rasch-type *W*-scores. For Raven's Progressive Matrices, Information, Digit Span, Picture Recognition, Digit Symbol and PASAT, items correct were recorded. For the Stroop Colour Word Test, the interference score was used. The measures for OMO and IT were as described for Study 1.

Testing took about 3 h, including breaks as required. At the end of the session participants were provided with the next numbered supply of ginkgo or placebo. For the next 12 weeks, participants were contacted on a weekly basis to monitor the presence of any side effects and to encourage compliance with the intervention regime. At the end of 12 weeks, participants completed the POMS immediately prior to

their return to the laboratory. At this final laboratory session, the identical test protocol to that administered initially was completed. Any unused capsules were returned to the laboratory and their number noted. The median number of unused capsules was 19 and, with non-compliance operationalised as more than 25 per cent of capsules unused, four participants (three ginkgo group, one placebo group) were classified as non-compliant and excluded from subsequent analyses.

### Results

Table 3 shows information on 104 participants at the beginning of the study. The two groups did not differ on any of these measures. Eighty-three participants attended the laboratory for the final session but of these, four were classified non-compliant, as described above. Of the 21 who did not return, two (placebo group) notified their withdrawal, one on medical advice and one 'because he knew he was in the placebo group'. The remaining 19 participants (10 placebo, 9 ginkgo) who did not complete the protocol either failed to keep appointments or did not respond to repeated efforts to contact them and make appointments for the final session. There were no statistically significant differences between the groups in terms of the numbers of individuals reporting any type of side effects. The side effects reported by members of the ginkgo group were again typical of those seen in other studies.

Table 4 shows the means and standard deviations on all measures of cognitive ability, attention and speed of processing, for both groups at pre- and post-test, along with test-retest reliabilities for all measures; these were all high except for the delayed recall version of Visual Auditory Learning. There was only one difference between the groups indicating enhanced performance in the ginkgo group with an associated probability  $p < 0.10$  and this was for Digit Symbol; however, the effect size was small ( $d = 0.17$ ). There were no differences between the two groups on any of the measures derived from the POMS.

### DISCUSSION

The aim of this study was to assess the nootropic effects of ginkgo in cognitively intact, healthy young and older adults. A broad range of cognitive abilities was assessed, along with tests of basic speed-of-processing and self-reported mood. In a double-blind, placebo-controlled, random groups design, 120 mg ginkgo extract was administered daily in three 40 mg doses for 12 weeks. Sample sizes were adequate for

Table 4. Descriptive statistics and test-retest reliabilities for the cognitive abilities measures at pre- and post-intervention for ginkgo and placebo groups and results of ANCOVA on effect of group at post-intervention with pre-intervention scores as covariate where outcomes showed increased improvement by the ginkgo group compared to placebo group in the young adult sample

	Reliability	Placebo		Ginkgo		ANCOVA results
		Pre-test	Post-test	Pre-test	Post-test	
Concept formation ( <i>W</i> scores)	0.75	525 (12.9)	529 (11.1)	521 (14.1)	521 (12.0)	
Raven's matrices (items correct)	0.60	48.3 (6.3)	48.8 (8.6)	47.6 (5.7)	48.2 (6.0)	$F(1, 80) = 0.02, p = 0.89$
Information (items correct)	0.88	37.6 (4.8)	38.7 (4.5)	36.2 (6.0)	37.3 (5.6)	
Digit span (items correct)	0.61	26.6 (4.8)	27.7 (4.4)	24.1 (5.4)	25.3 (5.2)	$F(1, 77) = 1.75, p = 0.19$
Picture recognition (items correct)	0.59	21.2 (3.3)	21.2 (2.9)	19.9 (3.9)	21.1 (3.7)	$F(1, 80) = 1.31, p = 0.26$
Visual matching ( <i>W</i> scores)	0.85	537 (14.1)	539 (16.5)	529 (16.4)	532 (17.2)	$F(1, 80) = 0.05, p = 0.83$
Digit symbol (items correct)	0.93	84 (15.5)	89 (15.7)	74 (14.1)	81 (15.6)	$F(1, 80) = 2.95, p = 0.09$
Memory for names ( <i>W</i> scores)	0.78	502 (10.1)	519 (14.5)	501 (14.8)	513 (14.9)	
Visual auditory learning ( <i>W</i> scores)	0.75	506 (9.0)	511 (10.4)	506 (10.4)	508 (10.0)	
Memory for names— delayed recall	0.74	497 (15.2)	514 (16.3)	496 (15.6)	512 (16.8)	
Visual auditory learning— delayed recall	0.48	497 (9.0)	508 (19.7)	499 (8.4)	510 (9.9)	
Odd-man-out decision time (DT, msec)	0.71	587 (136)	553 (102)	583 (102)	539 (95)	$F(1, 79) = 0.33, p = 0.57$
Odd-man-out movement time (MT, msec)	0.86	213 (52)	206 (47)	228 (69)	219 (67)	$F(1, 79) = 0.14, p = 0.71$
Inspection time (IT, msec)	0.59	54 (12)	55 (14)	61 (12)	60 (15)	$F(1, 80) = 0.00, p = 0.95$
PASAT (items correct)	0.88	44 (8.7)	47 (9.3)	39 (9.8)	41 (10.6)	
Stroop interference score	0.68	3.9 (5.5)	5.7 (4.9)	4.7 (6.5)	5.9 (6.5)	

detecting medium-sized differential improvement of about half a standard deviation (Oken *et al.*, 1998). Compared with placebo, positive results were limited to a single cognitive measure, for the older participants only. This effect, equivalent to about half a standard deviation of differential improvement, was found on tests of long-term storage and retrieval (Glr) for the older group but these participants made no reliable improvement on any other measure. No ginkgo effect was found on any of the cognitive measures among the young participants. Outcomes will be discussed separately in more detail for the two participant groups.

For the older participant sample, the most relevant comparable studies are those of Mix and Crews (2000, 2002) and Solomon *et al.* (2002). These three studies were all six week interventions with 180 mg/day (both the Mix and Crews studies) or 120 mg/day (Solomon *et al.*). Mix and Crews used the Stroop Colour Word Test and the Trail Making Test along with the Wechs-

ler Memory Scales (WMS) in their first study; in their second study they used the Selective Reminding Test, Wechsler Block Design and Coding and WMS. They found positive effects on Colour Naming from Stroop in the first study and on delayed recognition and recall for WMS in the second study. Solomon *et al.* used California Verbal Learning Test, WMS, Wechsler Coding and Digit Span, along with tests of expressive language. They found no effect for ginkgo on any of their tests. Thus, our study administered ginkgo for twice the duration of these three studies and we saw effects for tests of long-term storage and retrieval but not on the delayed recall versions of these tests. Nonetheless, this outcome can be seen as consistent with that of Mix and Crews (2002). Thus, our result suggests that intermediate-term storage and retrieval among elderly persons can be differentially improved by taking ginkgo. These outcomes are also consistent with Vesper and Hänsgen's (1994) finding that visual

memory performance requiring learned associations between geometric patterns and words improved after six weeks of ginkgo, continuing up to 12 weeks when the intervention was completed.

A potential criticism of all these studies on older adults is that the types of tests used were not sensitive to pharmacological effects. In the case of our study, however, there is an evidence that some of the tests used are sensitive to such effects. Frearson *et al.* (1988) demonstrated effects of nicotine on Odd-Man-Out RT as did Thompson *et al.* (2002) for IT. Hutchison *et al.* (2001) studied the effects of donepezil on IT. For our young sample we included Stroop (see Mix and Crews, 2000) and PASAT which are also sensitive to pharmacological intervention. Our aim was to be able to define the psychological locus of any effects of ginkgo and that is why we used the range of tests chosen for this study. It may be that other tests would detect effects of ginkgo but potentially at the cost of defining which cognitive domain was involved.

There are no studies in healthy young adults directly comparable to ours. As noted in the Introduction, most such studies have focussed on acute effects of higher doses, or on shorter interventions than for the current study. Stough *et al.* (2001), in their 30-day trial in healthy young adults, used the same ginkgo preparation as used here. They reported positive effects on Digit Span Backwards, Working Memory Speed and Rey Auditory Verbal Learning Test Delayed. They also used IT and found a small but non-significant improvement in performance, as we did for our older sample. In most respects, the Stough *et al.* study and our study were similar except that we administered ginkgo for 12 weeks but found no statistically significant effects of ginkgo in healthy young adults. It is unfortunate that we did not assess effects at four and eight weeks.

To summarise, then, we acknowledge the possibility that the single positive outcome in the elderly sample represents a Type 1 error. Consistent with this, the statistically significant outcome was limited to the two associational learning tasks used to define Glr. These required only about 10 min to complete, so that information was stored for only short periods of time. Improvements on delayed-recall (the same tasks but administered without forewarning about 2 h following initial testing), and on IT (a marker for processing speed) were not statistically significant ( $0.10 > p > 0.05$ ; cf. Stough *et al.*, 2001). The same was true for Digit Symbol in the younger sample.

However, when considering the possibility that the current result is reliable, it is useful also to consider

what functional mechanisms might support a specific beneficial effect. In a recent review on the effects of ginkgo on cognition Gold *et al.* (2002) questioned whether ginkgo directly affects cognition, or whether indirect effects could arise from ginkgo affecting some other aspect of performance (e.g. motivation, attention, perceptual-motor performance and reduction in stress level). The current result would not support this however; the absence of any effect on any test of ability, perceptual-motor or executive performance or various self-report aspects of mood, all of which should be vulnerable to effects of motivation or attention, suggests that if the effect is reliable, it is highly specific and therefore direct.

Gold *et al.* (2002) also considered biological processes that may be sensitive to ginkgo and thereby contribute to cognitive improvement, including improved cerebral blood flow. Santos *et al.* (2003) reported benefits on general intelligence, visuospatial abilities, attentional processes and speed of processing. Moreover, they reported improved cerebral blood flow and decreased blood viscosity in their ginkgo group after 8 month intervention (80 mg/day). Similarly, improvements in glucose metabolism in the brain and reduction of glucocorticoid levels, which may be implicated in enhanced hippocampal function, are also plausible mechanisms for the enhancement of memory and associative learning. Nonetheless, in the context of the current study, one may query what it is about such mechanisms that would be so selective as to improve only the longer-term aspects of memory without delivering any benefit for short-term components. Although there was a hint in the data for the younger group of a general arousal effect, no such trend was found among older participants. Moreover, but contrary to Kennedy *et al.* (2002) and Trick *et al.* (2004), POMS subscales detected no such influence.

A limitation of the current study is that we have no information on the smoking status of our participants. This is unfortunate in the context of Nathan's (2000) argument on the possible cholinergic bases of ginkgo's effects. However, Australia is ranked lowest of all OECD countries in terms of prevalence of daily smokers. For the age groups of our samples, the prevalence of daily smokers in Australia in 2004 was 24% for males aged 20–40 years and 9% for people over 60 years (Australian Institute of Health and Welfare, 2005). This low incidence of smoking is because of public education campaigns, restrictive legislation and a severe taxation regime. Given that our participants were self-selected healthy volunteers, we feel justified in speculating that the incidence of smokers

in our samples was likely low. In summary, and as Gold *et al.* (2002) commented, further research in animals and humans is required to elucidate putative mechanisms of ginkgo's effects on cognition.

The present results raise the possibility of a positive effect of ginkgo on intermediate-term storage and retrieval, in normal, healthy older adults. Moreover, the effect size is consonant with that found in elderly adults with early Alzheimer's Disease (Oken *et al.*, 1998). On balance therefore, while conceding that this result may not be reliable, we would not wish to discourage further investigation of the possible efficacy of such a widely used herbal remedy, particularly since the dosage here was relatively short-term and at the lower end (120 mg/day) of the range yielding positive effects in some studies. Another issue to consider in contextualising our results is whether ginkgo can exert any effect on healthy, cognitively intact adults. Such individuals may be performing at ceiling on the cognitive tests used here. In effect, the situation can be likened to assessing the ability of aspirin to lower body temperature in people without fever.

We were concerned to assess the recommended dosage of a reputable product because we saw this as relevant to the way in which the product is used in our community. It is noteworthy that there were no side effects unequivocally attributable to treatment with ginkgo; those reported by participants in the ginkgo group were mild and similar to those reported elsewhere. There appeared to be no obvious harmful effects among either young or older adults. Clearly, dose-response effects tested in a cross-over design would provide more convincing evidence about the efficacy of ginkgo. Future research on ginkgo should also investigate effects on different aspects of memory performance of elderly people and seek to compare ginkgo with other interventions known to improve cognitive performance generally and memory specifically (see also Gold *et al.*, 2002).

#### ACKNOWLEDGEMENTS

This research was supported by University of Adelaide/CSIRO Collaborative Grants Scheme and University of Adelaide and Faculty of Health Sciences Small Research Grant Schemes funds awarded to the first two authors. Blackmores Ltd. supplied the Ginkgoforte and placebo tablets used in this study free-of-charge. All authors affirm that none of them has a conflict of interest with respect to material in this paper. Dr Peter Clifton generously provided medical advice throughout the course of the study. We thank Shara Kremer, Jason McPherson and Kathleen Mansfield

for managing the study day-to-day and for collecting the data. We gratefully acknowledge our participants.

#### REFERENCES

- Australian Institute of Health and Welfare. 2005. Statistics on drug use in Australia 2004. AIHW Cat. No. PHE 62. AIHW (Drug Statistics Series No. 15): Canberra.
- Baddeley A, Nimmosmith MI, Emslie H. 1988. Estimating premorbid intelligence. *J Clin Exp Neuropsychol* **10**: 326–326.
- Beaubrun G, Gray GE. 2000. A review of herbal medicines for psychiatric disorders. *Psychiatr Serv* **51**: 1130–1134.
- Birks J, Grimley Evans J. 2002. Ginkgo Biloba for cognitive impairment and dementia. *The Cochrane Database of Systematic Reviews*. Issue 4. Art No: CD003120. DOI: 10.1002/14651858.CD003120
- Curtis-Prior P, Vere D, Fray P. 1999. Therapeutic value of Ginkgo biloba in reducing symptoms of decline in mental function. *J Pharm Pharmacol* **51**: 535–541.
- Frearson W, Eysenck HJ. 1986. Intelligence, reaction-time (RT) and a new odd-man-out RT paradigm. *Pers Individ Differ* **7**: 807–817.
- Frearson W, Barrett P, Eysenck HJ. 1988. Intelligence, reaction-time and the effects of smoking. *Intelligence* **9**: 497–517.
- Gold PE, Cahill L, Wenk GL. 2002. Ginkgo biloba: a cognitive enhancer? *Psychol Sci Public Interest* **3**: 2–11.
- Golden CJ. 1978. *Stroop Color and Word Test*. Stoelting: Chicago.
- Gronwall DMA, Wrightson P. 1974. Delayed recovery of intellectual function after minor head injury. *Lancet* **7874**: 1452.
- Grunwald J. 1995. The European phytomedicines: market figures trends analyses. *Herbalgram* **34**: 60–65.
- Horn J, Noll J. 1994. A system for understanding cognitive capabilities: a theory and the evidence on which it is based. In *Current Topics in Human Intelligence*, Detterman DK (ed.). Ablex: Norwood, NJ; 151–203.
- Hutchison CW, Nathan PJ, Mrazek L, Stough C. 2001. Cholinergic modulation of speed of early information processing: the effect of donepezil on inspection time. *Psychopharmacol* **155**: 440–442.
- Itil TM, Eralp E, Ahmed I, Kunitz A, Itil KZ. 1998. The pharmacological effects of Ginkgo biloba, a plant extract, on the brain of dementia patients in comparison with tacrine. *Psychopharmacol Bull* **34**: 391–397.
- Jensen AR, Munro E. 1979. Reaction time, movement time, and intelligence. *Intelligence* **3**: 121–126.
- Kennedy DO, Scholey AB, Wesnes KA. 2000. The dose dependent cognitive effects of acute administration of Ginkgo biloba to healthy young volunteers. *Psychopharmacol* **151**: 416–423.
- Kennedy DO, Scholey AB, Wesnes KA. 2002. Modulation of cognition and mood following administration of single doses of *Ginkgo biloba*, ginseng, and a ginkgo/ginseng combination in healthy young adults *Physiol Behav* **75**: 739–751.
- Kleinjen J, Knipschild P. 1992. Ginkgo biloba for cerebral insufficiency. *Br J Clin Pharmacol* **34**: 352–358.
- LeBars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. 1997. A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. *JAMA* **278**: 1327–1332.
- McNair DM, Lorr M, Droppleman LF. 1971. *Profile of Mood States*. Educational and Industrial Testing Services: San Diego, CA.
- Mix JA, Crews WD. 2000. An examination of the efficacy of Ginkgo biloba extract EGb 761 on the neuropsychological functioning of cognitively intact older adults. *J Alternative Complementary Med* **6**: 219–229.

GINKGO BILOBA AND COGNITIVE ABILITIES

- Mix JA, Crews WD. 2002. A double-blind, placebo-controlled, randomized trial of *Ginkgo biloba* extract EGb 761 in a sample of cognitively intact older adults: neuropsychological findings. *Hum Psychopharmacol* **17**: 267–277.
- Moulton PL, Boyko LN, Fitzpatrick JL, Petros TV. 2001. The effect of *Ginkgo biloba* on memory in healthy male volunteers. *Physiol Behav* **73**: 659–665.
- Nathan P. 2000. Can the cognitive enhancing effects of *Ginkgo biloba* be explained by its pharmacology? *Med Hypotheses* **55**: 491–493.
- Nettelbeck T. 1987. Inspection time and intelligence. In *Speed of Information Processing and Intelligence*, Vernon PA (ed.). Ablex: Norwood, NJ; 295–346.
- Oken BS, Storzbach DM, Kaye JA. 1998. The efficacy of *Ginkgo biloba* on cognitive function in Alzheimer disease. *Arch Neurol* **55**: 1409–1415.
- Petrides M, Milner B. 1982. Deficits on subject-ordered tasks after frontal-lobe and temporal-lobe lesions in man. *Neuropsychologia* **20**: 249–262.
- Ramassamy C, Christen Y, Clostre F, Costentin J. 1992. The *Ginkgo biloba* extract, Egb761, increases synaptosomal uptake of 5-hydroxytryptamine: invitro and exvivo studies. *J Pharm Pharmacol* **44**: 943–945.
- Rigney U, Kimber S, Hindmarch I. 1999. The effects of acute doses of standardized *Ginkgo biloba* extract on memory and psychomotor performance in volunteers. *Phytother Res* **13**: 408–415.
- Santos RF, Galduroz JCF, Barbieri A, Castiglioni MLV, Ytaya LY, Bueno OFA. 2003. Cognitive performance, SPECT, and blood viscosity in elderly non-demented people using *Ginkgo biloba*. *Pharmacopsychiatry* **36**: 127–133.
- Solomon PR, Adams F, Silver A, Zimmer J, DeVaux R. 2002. *Ginkgo* for memory enhancement: a randomized controlled trial. *JAMA* **288**: 835–840.
- Stough C, Clarke J, Lloyd J, Nathan PJ. 2001. Neuropsychological changes after 30-day *Ginkgo biloba* administration in healthy participants. *Int J Neuropsychopharmacol* **4**: 131–134.
- Subhan Z, Hindmarch I. 1984. The psychopharmacological effects of *Ginkgo biloba* extract in normal healthy volunteers. *Int J Clin Pharmacol Res* **4**: 89–93.
- Thompson JC, Wilby G, Stough C. 2002. The effects of transdermal nicotine on inspection time. *Hum Psychopharmacol Clin Exp* **17**: 157–161.
- Trick L, Boyle J, Hindmarch I. 2004. The effects of *Ginkgo biloba* Extract (LI 1370) supplementation and discontinuation on activities of daily living and mood in free living older volunteers. *Phytother Res* **18**: 531–537.
- van Dongen MCJM, van Rossum E, Kessels AGH, Sielhorst HJG, Knipschild PG. 2000. The efficacy of *Ginkgo* for elderly people with dementia and age-associated memory impairment: new results of a randomized clinical trial. *J Am Geriatr Soc* **48**: 1183–1194.
- Vesper J, Hänsgen KD. 1994. Efficacy of *Ginkgo biloba* in 90 outpatients with cerebral insufficiency caused by old age. *Phytomedicine* **1**: 9–16.
- Wirth S, Stemmelin J, Will B, Christen Y, Di Scala G. 2000. Facilitative effects of EGb 761 on olfactory recognition in young and aged rats. *Pharmacol Biochem Behav* **65**: 321–326.
- Woodcock RW, Johnson MB. 1989. *Woodcock-Johnson Psycho-Educational Battery-Revised*. DLM Teaching Resources: Allen, TX.