

# Acute cognitive effects of standardised Ginkgo biloba extract complexed with phosphatidylserine

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Recent data suggest that the complexation of standardised Ginkgo biloba extract (GBE) with soy-derived phospholipids enhances the bio-availability of GBE's active components. The current study therefore aimed to assess the comparative cognitive and mood effects of a low dose of GBE and products complexing the same extract with either phosphatidylserine or phosphatidylcholine.

The study utilised a placebo-controlled, multi-dose, double-blind, balanced-crossover design. Twenty-eight healthy young participants received 120 mg GBE, 120 mg GBE complexed with phosphatidylserine (Virtiva<sup>TM</sup>), 120 mg GBE complexed with phosphatidylcholine and a matching placebo, on separate days 7 days apart. Cognitive performance was assessed using the Cognitive Drug Research (CDR) computerised test battery and Serial Subtraction tasks immediately prior to dosing and at 1, 2.5, 4 and 6 h thereafter. The primary outcome measures were the four aspects of cognitive performance, which have previously been derived by factor analysis of CDR subtests. Levels of terpenoids (bilobalide, ginkgolide A and ginkgolide B) were concomitantly assessed in plasma samples taken pre-dose and at 3 and 6.5 h post-dose.

In keeping with previous research utilising the same methodology, 120 mg of GBE was not associated with markedly improved performance on the primary outcomes. However, administration of GBE complexed with phosphatidylserine resulted both in improved secondary memory performance and significantly increased speed of memory task performance across all of the post-dose testing sessions. Enhancement following GBE complexed with phosphatidylcholine was restricted to a modest improvement in secondary memory performance which was restricted to one post-dose time point. All three treatments were associated with improved calmness. There were no significant differences in post-dose levels of terpenoids between the Ginkgo containing treatments, although this latter finding may be attributable to methodological factors.

Complexation with phosphatidylserine appears to potentiate the cognitive effects associated with a low dose of GBE. Further research is required to identify whether this effect is due to the complexation of the extracts, their mere combination, or the separate psychopharmacological actions of the two extracts. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS — Ginkgo; phosphatidylserine; phosphatidylcholine; memory; attention; mood

## INTRODUCTION

Extracts of Ginkgo biloba leaf are sold both 'over the counter', and on prescription in a number of countries. The active constituents, a range of species specific flavonoids and the terpenoids—bilobalide and ginkgolides A,B,C and J (Kleijnen and Knipschild, 1992)—are believed to be responsible for a number of physiological effects potentially relevant to the

enhancement of cognition. These include: specific antagonism of platelet activating factor (e.g. Smith *et al.*, 1996; Akiba *et al.*, 1998; Stromgaard *et al.*, 2002), scavenging and inhibition of free radicals (e.g. Droy-Lefaix 1997; Hibatallah *et al.*, 1999; Siddique *et al.*, 2000), modulation of a number of neurotransmitter systems (e.g. Taylor, 1986; Ramassamy *et al.*, 1992; Huguet *et al.*, 1994; Kristoikova and Klaschka, 1997), beneficial effects on blood circulation (e.g. Jung *et al.*, 1990; Koltringer *et al.*, 1993; Kriegelstein *et al.*, 1986; Chung *et al.*, 1999; Topp *et al.*, 2001), both *in vitro* and *in vivo* protection against hypoxic challenges (e.g. Oberpichler *et al.*, 1988; Klein *et al.*, 1997; Jannsens *et al.*, 1999) and *in vivo* neuro-protective

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properties (e.g. Attella *et al.*, 1989; Bruno *et al.*, 1993; Tadano *et al.*, 1998; Lee *et al.*, 2002).

Accumulating evidence suggests that chronic administration of Ginkgo biloba may be effective in the enhancement of cognition. Whilst the evidence is not entirely unequivocal (e.g. Solomon *et al.*, 2002), an accumulating evidence base suggests that Ginkgo may ameliorate the cognitive declines associated with ageing and dementia (e.g. see the Cochrane review by Birks *et al.*, 2002), and improve cognitive performance in healthy older (Mix and Crews, 2000, 2002) and younger adults (Stough *et al.*, 2001). Several studies have also demonstrated improved performance following acute doses of Ginkgo in healthy adults (Hindmarch, 1986; Warot *et al.*, 1991; Rigney *et al.*, 1999; Scholey and Kennedy, 2002; Kennedy *et al.*, 2002a).

Of particular relevance here, in a double-blind, placebo-controlled, balanced cross-over study utilising the CDR computerised assessment battery, Kennedy *et al.* (2000) demonstrated linear, dose-dependent increases in speed of attention task performance in 20 young participants administered single doses of 120 mg, 240 mg and 360 mg of a standardised Ginkgo biloba extract (GBE). Whilst not affecting attention, there was some evidence of improved secondary memory performance following the lowest dose.

Administration of the endogenously occurring phospholipids phosphatidylcholine and phosphatidylserine has also been suggested as potential cognition enhancing candidates in pathological groups (Amenta *et al.*, 2001). In the case of phosphatidylcholine the rationale for its putative efficacy is in its role as a direct donor of the precursors of acetylcholine (ACh) (Amenta *et al.*, 2001). Research in rodents has indeed provided some evidence that comparatively high doses of phosphatidylcholine, which is readily derived from a normal diet, can increase brain choline and ACh concentrations (e.g. Chung *et al.*, 1995; Blusztajn *et al.*, 1987) although findings in this area could not be described as unequivocal (Amenta *et al.*, 2001). However, research in humans has failed to demonstrate any clear evidence of nootropic potential. As an example, Higgins and Flicker (2003), in a Cochrane review, assessed twelve randomised trials of phosphatidylcholine's efficacy in dementia and cognitive impairment that reached their inclusion criteria. They concluded that there was no evidence to support such a role for phosphatidylcholine.

Phosphatidylserine is also naturally derived from the diet. Research suggests that, whilst it is quantitatively modest, it is ubiquitous, making up a

small percentage of the phospholipid content of all cell membranes (Blusztajn *et al.*, 1987). However, phosphatidylserine is unique amongst phospholipids in that it plays a role in regulating the function of key cell membrane proteins (Pepeu *et al.*, 1996) and acts as an essential enzyme co-factor for a number of cellular proteins (Vance and Steenbergen, 2005). It is therefore essential not only for the general homeostasis and maintenance of the cell, including entry of nutrients to the cell, but also for a number of other cellular functions, such as nerve transmitter release, signal transduction (e.g. Bruni and Toffano, 1982; Yoshimura and Sone, 1990; Cohen and Mueller, 1992; Moynagh and Williams, 1992) and determination of neuronal membrane surface potential (Blusztajn *et al.*, 1987). Whilst it is present as a component of the membrane matrix of all cells, phosphatidylserine is particularly richly represented in neural tissue (Kidd, 1999). Phosphatidylserine has also been shown to exert a number of other physiological effects. These include a greater effect on ACh biosynthesis and release than phosphatidylcholine (see: Pepeu *et al.*, 1996; Giovannini *et al.*, 1997; Amenta *et al.*, 2001), modulation of a number of neurotransmitter systems (e.g. Toffano *et al.*, 1978; Argentiero and Tavalato, 1980; Samson, 1987; Casamenti *et al.*, 1991) and attenuation of age associated dendritic-spine loss (Nunzi *et al.*, 1987).

Animal behavioural studies suggest memory improvements in rodents following phosphatidylserine (e.g. Blokland *et al.*, 1999; Claro *et al.*, 1999; Alves *et al.*, 2000). However, trials in humans are sparse, with the pattern of cognitive improvements in cognitively challenged population at best being described as modest (Amenta *et al.*, 2001; McDaniel *et al.*, 2003).

Products that feature the complexation of either phosphatidylcholine or phosphatidylserine with GBE have recently been developed. In the case of the GBE/phosphatidylcholine complex an initial study in rats demonstrated cardio-protective activity and plasma antioxidant capacity in excess of that seen following the non-complexed extract (Carini *et al.*, 2001). The authors suggest that the embedding of phenolic antioxidants within the lipophilic carrier increases their bio-availability. This possibility is supported by data showing increased urinary metabolites of ginkgolides, and increased levels of plasma bilobalide and ginkgolides A and B following oral administration of the conjugate to healthy adults.

The current study therefore assessed whether the complexation of GBE with either phosphatidylcholine or phosphatidylserine would lead to improvements in

bio-availability and cognitive performance, over and above those seen following the non-complexed extract. In order to adequately assess the potential additive or synergistic effects, the specific dose of GBE (120 mg) was chosen on the basis that in a previous study (Kennedy *et al.*, 2000), using the same testing paradigm, it was both the lowest dose, and proved the least potent in terms of cognitive enhancement.

In the current double-blind, placebo-controlled, counter-balanced experiment, the cognitive and mood effects of single doses of: GBE (120 mg); GBE (120 mg) complexed with phosphatidylserine; GBE (120 mg) complexed with phosphatidylcholine and placebo were assessed in 28 healthy participants utilising the Cognitive Drug Research (CDR Ltd) computerised assessment battery, Serial Subtraction tasks and Bond-Lader visual analogue mood scales. In order to assess potential differential time course effects, testing took place pre-dose and at 1, 2.5, 4 and 6 h thereafter, and to allow a sufficient 'wash-out' between treatments, testing was conducted at 7-day intervals. Concomitant blood sampling was undertaken to assess whether the complexes modulated the bio-availability of GBE's active principles.

## MATERIALS AND METHODS

### *Participants*

10 male and 18 female participants took part in the study. The mean age of the cohort was 20.4 years (SD 1.2). The study was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority and was carried out in accordance with the Declaration of Helsinki. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter' or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Smokers were excluded from the study. All participants abstained from alcohol for a minimum of 12 h prior to the first testing session of the morning.

### *Treatment formulations*

Standardised GBE (containing 24% Ginkgo-flavonol glycosides and 6% terpene lactones) was prepared by Indena SpA. (Milan) according to the procedure described in the patent EP0360556. Treatments

comprising GBE complexed with soy-derived phosphatidylcholine and phosphatidylserine were prepared according to the procedures described respectively in the patents EP0275005 and WO 2005/074956.

All three treatments were encapsulated in conventional hard-gelatin capsules containing 60 mg of GBE, plus phospholipids in complexed form where applicable. Identical placebo capsules were prepared using inert packaging material.

### *Evaluation of plasma levels of terpenoids*

Evaluation of plasma levels of terpenoids was undertaken using the methods described by Mauri *et al.* (1999, 2001, 2003). Blood samples were collected in vacutainer tubes containing sodium-heparin. Plasma was separated by centrifugation at 10 000 g for 1 min and stored at  $-80^{\circ}\text{C}$ . Frozen samples were then sent to the Institute of Biomedical Technologies (ITB-CNR, Segrate, Italy) for analysis.

Bilobalide, ginkgolide A and ginkgolide B were analysed in plasma using liquid chromatography/atmospheric pressure chemical ionisation mass-spectrometry (LC/APCI-ITMS).

The plasma samples (0.3 mL) were extracted with the same volume of ethyl acetate and, after centrifugation at 2000 g for 8 min, the supernatant was evaporated to dryness under vacuum. The residue, dissolved in 150  $\mu\text{L}$  of 10% methanol, was injected into the LC/APCI-ITMS system.

A Spectra Series HPLC (Thermoquest, Milan, Italy) equipped with an autosampler was used. Separation of ginkgolides was performed using a C18 Hypersil column (100  $\times$  3 mm, 5  $\mu\text{m}$ ) and a methanol gradient (eluent A, water; eluent B, methanol; 0–1 min 30%B, 1–7 min from 30 to 45%B, 7–10 min 45% B). The flow rate was 0.55 mL/min and the volume injected was 50  $\mu\text{L}$ .

Detection of terpene lactones was performed by means of an LCQ ion trap mass spectrometer (Thermoquest, Milan, Italy) equipped with an atmospheric pressure chemical ionisation (APCI) interface. APCI parameters were optimised by flow injection of ginkgolides and bilobalide standard solutions. LC/MS analyses were carried out in the negative ion scale mode from  $m/z$  200–700. The following instrumental parameters were used for APCI-MS detection of terpenoids: discharge current, 4.0 mA; discharge voltage, 1750 V; capillary voltage,  $-10$  V; capillary temperature,  $190^{\circ}\text{C}$ ; sheath gas, 70 (arb); aux gas, 7 (arb); vaporiser temperature,  $450^{\circ}\text{C}$ .

For calibration curves, ginkgolides and bilobalide standards were dissolved in methanol (about 1 mg/mL) and stored at  $0^{\circ}\text{C}$ . Aliquots of terpenoids standard

solutions (dissolved in 10% methanol) in the range 5–2,000 ng/mL were injected into the HPLC apparatus. Peak areas were integrated and plotted against the corresponding masses of injected standards.

### *Cognitive and mood measures*

**Cognitive Drug Research (CDR) computerised assessment battery.** The CDR computerised assessment battery has been used in over 500 European and North American drug trials.

The tailored version of the CDR battery utilised here, including a detailed description of the constituent tasks, is described in detail by Kennedy *et al.* (2000, 2001a, 2001b, 2002b, 2003). This battery has previously been found to be sensitive to modulation of cognitive function as a consequence of acute ingestion of herbal extracts, including *Melissa officinalis* (Kennedy *et al.*, 2002b, 2003), *Ginkgo biloba* (Kennedy *et al.*, 2000, 2002a) and *Panax Ginseng* (Kennedy *et al.*, 2001a, 2002b), and acute and chronic administration of a *Ginkgo biloba*/*Panax ginseng* combination (Wesnes *et al.*, 1987, 2000; Kennedy *et al.*, 2001b, 2002a). The selection of computer-controlled tasks from the system was administered with randomly ordered parallel forms of the tests being presented at each testing session. Presentation was via desktop computers with high-resolution VGA colour monitors, and, with the exception of written word recall tests, all responses were recorded via two-button (YES/NO) response boxes. The entire selection of tasks took approximately 20 min to perform.

**Primary cognitive outcome measures.** As with the previous studies assessing herbal treatments, the single task outcomes from the CDR battery were collapsed into the five cognitive outcome factors derived from the battery by a factor analysis conducted and described by Wesnes *et al.* (2000). The factor composition is described briefly below.

‘Speed of Attention’ factor: derived by combining the reaction times of the three attentional tasks—simple reaction time, choice reaction time and digit vigilance (units are summed milliseconds for the three tasks).

‘Speed of Memory’ factor: derived by combining the reaction times of numeric working memory, spatial memory, delayed word recognition and delayed picture recognition (units are summed milliseconds for the four tasks).

‘Accuracy of Attention’ factor: derived by calculating the combined percentage accuracy across the choice reaction time and Digit vigilance tasks. 100% accuracy across the two tasks would generate a maximum score of 100.

‘Secondary Memory’ factor: derived by combining the percentage accuracy scores from delayed word recognition, delayed picture recognition, immediate word recall and delayed word recall tasks. One hundred per cent accuracy across the four tasks would generate a maximum score of 400 on this index.

‘Working Memory’ factor: derived by combining the percentage accuracy scores from the two working memory tests—spatial working memory and numeric working memory. One hundred per cent accuracy across the two tasks would generate a maximum score of 200 on this index.

‘Quality of Memory’: a global measure of mnemonic performance derived by combining scores from the ‘Secondary Memory’ and ‘Working Memory’ factors.

### *Other measures*

**‘Serial Threes’ and ‘Serial Sevens’ subtraction tasks.** A modified computerised version of the Serial Sevens test was utilised. The original verbal Serial Sevens test (Hayman, 1942) has appeared in a number of forms, including as part of the Mini-Mental State Examination (Folstein *et al.*, 1975). It has been used to assess cognitive impairment during hypoglycaemia (e.g. Taylor and Rachman, 1987), and has also been used to investigate the relationship between increased blood glucose levels and cognitive performance (Kennedy *et al.*, 2000; Scholey *et al.*, 2001; Scholey, 2001). In the current studies, computerised versions of serial subtractions were implemented (see Scholey *et al.*, 2001 for details), here using tests of 2-min duration. For the Serial Sevens task a standard instruction screen informed the participant to count backwards in sevens from the given number, as quickly and accurately as possible, using the numeric keypad to enter each response. Participants were also instructed verbally that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each three-digit response was entered via the numeric keypad with each digit being represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the



screen. The task was scored for total number of subtraction and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

The Serial Threes task was identical to Serial Sevens, except that it involved serial subtraction of threes.

**Subjective mood measure.** The Bond-Lader Visual Analogue Scales (Bond and Lader, 1974), consisting of 16 100 mm visual analogue scales anchored by antonyms (e.g. Alert-Drowsy, Lethargic-Energetic, etc.) were combined as recommended by the authors to form three mood factors: alertness, calmness and contentedness.

### *Treatments*

On each study day participants received two hard-gelatin capsules that were of identical appearance on each occasion. The counterbalanced order of presentation of the treatments was dictated by random allocation of the participant to a position on a Latin Square. In total, the two capsules contained either:

- (a) an inert placebo;
- (b) 120 mg GBE;
- (c) 120 mg GBE, complexed with 360 mg phosphatidylcholine;
- (d) 120 mg GBE, complexed with 360 mg phosphatidylserine [Virtiva<sup>TM</sup>].

All treatments were supplied coded by the manufacturer (Indena SpA, Milan). A disinterested third party was responsible for preparing the individual participants' treatments in identical containers, as per the study's Latin Square. The codes remained unbroken until initial statistical analysis had been completed. All treatments were identical in appearance and scent.

### *Procedure*

Each participant was required to attend a total of five study days that were conducted 7 days apart, to ensure a sufficient wash-out between conditions. Testing took place, commencing at the same time on each day, in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day, participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four to allow familiarisation with the test battery and procedure. However, no treatment (active or placebo) was offered, nor were any pharmacokinetic samples obtained. Data from the five sessions of this practice day were not included in any analysis.

Each active study day comprised five identical cognitive/mood testing sessions. The first was a pre-dose testing session that established baseline performance for that day, and was immediately followed by the day's treatment on days 2 to 5. Further testing sessions began at 1, 2.5, 4 and 6 h following consumption of the day's treatment. Each testing session comprised completion of the Bond-Lader visual analogue scales, and the CDR test battery.

Blood samples were taken immediately following the baseline testing session, the 2.5- and the 6-h testing sessions (i.e. pre-dose, and 3 and 6.5 h post-dose).

### *Statistics*

**Blood level of terpenoids.** Data from the 13 participants providing a full data set from the 2nd and 3rd blood samples on the days that they received GBE, GBE/phosphatidylcholine and GBE/phosphatidylserine were analysed by two-way repeated measures ANOVA (condition X 2nd/3rd reading) to assess the differential effects of complexation on terpenoid levels in the blood. Data from the pre-dose baseline and placebo conditions were not entered into the analysis. A lack of terpenoids in these samples was confirmed by descriptive analysis.

### *Cognitive and mood data*

Scores on the individual task outcomes, the four primary factors and the two memory sub-factors were analysed as 'change from baseline' using the Minitab statistical package.

Prior to carrying out planned comparisons, an ANOVA (General Linear Model), with terms fitted to the model for dose, visit, dose x visit and subject, was carried out to identify main effects and interaction effects on change from baseline data for each measure. The primary statistical analysis of the 'change from baseline' data for each measure was carried out using planned comparisons, utilising *t* tests with the mean squares for 'Dose\*Time\*Subjects' from an omnibus ANOVA as an error term (Keppel, 1991), with *t* evaluated at the degrees of freedom associated with the interaction. At each time point (1, 2.5, 4 and 6 h post-dose) data from the placebo condition was compared to that for each of the three treatments

(GBE, GBE/phosphatidylcholine, GBE/phosphatidylserine). To ensure the overall Type I error protection level, only those planned comparisons associated with measures that generated a significant main effect or interaction effect on the initial ANOVA are reported. Furthermore, all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

## RESULTS

### *Blood levels of terpenoids*

Thirteen participants provided a full data set from the 2nd and 3rd blood samples. Two-way repeated-measures ANOVA (condition  $\times$  2nd/3rd blood sample) showed that there was no significant difference in the post-dose levels of bilobalide, ginkgolide A or ginkgolide B between the three active conditions (GBE, GBE/phosphatidylcholine, GBE/phosphatidylserine). There were no interactions between condition and blood sample (2nd/3rd). There was, however, a significant main effect of blood sample, with both bilobalide and ginkgolide A levels decreasing between

the 3 and 6.5 h measurements ( $[F(1, 24) = 7.13, p = 0.02]$  and  $[F(1, 24) = 6.53, p = 0.025]$ , respectively). Mean terpenoid levels are shown in Figure 1.

### *Cognitive assessment*

**Baseline scores.** Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, GBE, GBE/phosphatidylcholine, GBE/phosphatidylserine) for each outcome (single task outcomes, cognitive factor scores and mood scale scores) were subjected to a one-way, repeated-measures ANOVA followed, for measures generating a significant result, by planned comparisons as per the cognitive outcome data. Several single task outcomes generated significant pre-dose baseline differences on the planned comparisons. Prior to ingestion of the days treatment participants in the GBE/phosphatidylserine condition performed significantly less accurately [ $t(81) = 2.32, p = 0.022$ ] and more slowly [ $t(81) = 2.75, p = 0.007$ ] than placebo on the Digit vigilance task. The same condition performed significantly slower on the Word recognition task [ $t(81) = 2.11, p = 0.036$ ]. No other planned comparisons proved significant for any other measure.

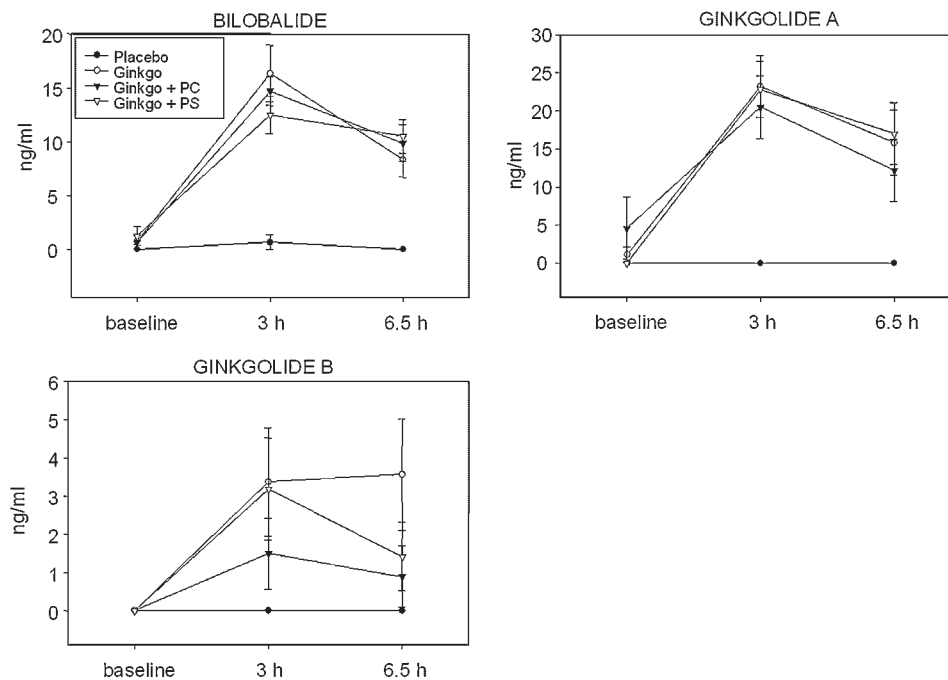


Figure 1. Mean levels of bilobalide and ginkgolids A and B in serum samples taken at baseline, 3 and 6.5 h post-dose. The figure represents data from the 13 participants that provided a full set of samples

**Individual task outcome measures.** Task outcomes in the order they were undertaken, and performance data from the individual task outcome measures are presented in the table and graphs of Figure 2. Results of planned comparisons of individual task outcomes that generated a significant result on the initial ANOVA (statistic not reported) are described in relationship to the overall factor to which they contribute below.

### Cognitive factor outcome measures

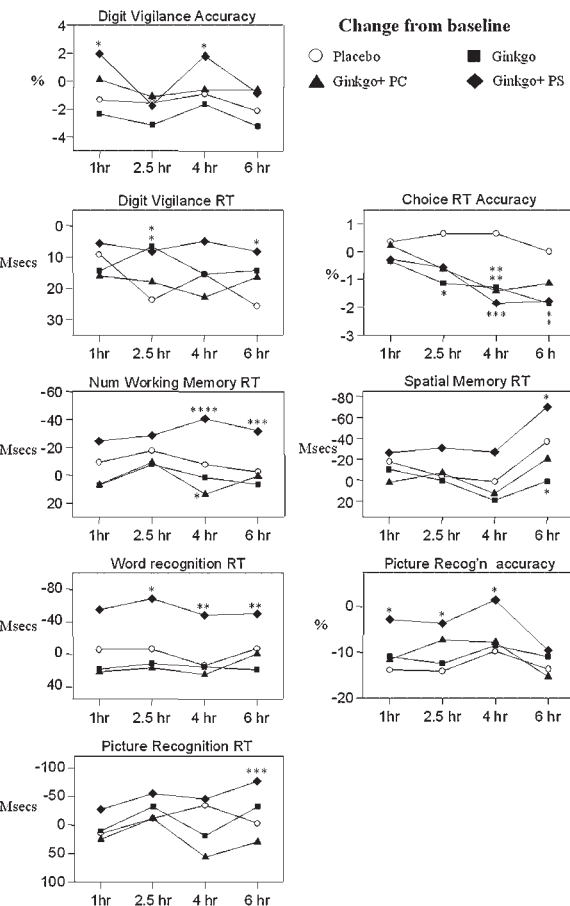
Mean raw baseline scores and change from baseline scores for each condition across each session are presented in the tables and graphs of Figure 3.

**Speed of attention factor.** Whilst there was no significant difference on the primary outcome factor, a single component task outcome (digit vigilance reaction time) generated significant differences on the initial ANOVA and subsequent planned comparisons. Both GBE [ $t(243) = 2.45, p = 0.015$ ] and GBE/phosphatidylserine [ $t(243) = 2.25, p = 0.026$ ] performed significantly faster than placebo at 2.5 h post-dose with GBE/phosphatidylserine also performing faster at 6 h [ $t(243) = 2.5, p = 0.013$ ].

**Accuracy of attention factor.** Whilst there was no significant difference on the primary outcome factor, two single component task outcomes generated

Measure		Pre-dose Baseline score	1 hr post-dose	2.5 hr post-dose	4 hr post-dose	6 hr post-dose
Immediate Word Recall (% accuracy)	placebo	51.90 $\pm$ 3.12	-4.05 $\pm$ 2.76	-5.71 $\pm$ 3.33	-6.43 $\pm$ 2.22	-9.76 $\pm$ 3.07
	Ginkgo	51.43 $\pm$ 3.33	-3.81 $\pm$ 2.33	-6.31 $\pm$ 2.71	-6.19 $\pm$ 2.19	-8.33 $\pm$ 3.19
	Ginkgo+PC	47.74 $\pm$ 2.66	-4.29 $\pm$ 3.82	-2.26 $\pm$ 3.07	-9.17 $\pm$ 2.91	-5.12 $\pm$ 3.41
	Ginkgo+PS	47.86 $\pm$ 2.23	-3.21 $\pm$ 2.57	-5.71 $\pm$ 2.29	-2.62 $\pm$ 2.81	-7.26 $\pm$ 3.18
Simple Reaction time (msec)	placebo	297.34 $\pm$ 4.47	9.60 $\pm$ 3.40	2.21 $\pm$ 3.33	2.36 $\pm$ 7.40	-0.42 $\pm$ 4.84
	Ginkgo	283.93 $\pm$ 11	10.90 $\pm$ 3.04	20.59 $\pm$ 21.11	14.56 $\pm$ 6.31	27.92 $\pm$ 4.97
	Ginkgo+PC	290.59 $\pm$ 28	5.83 $\pm$ 3.93	2.06 $\pm$ 4.22	15.10 $\pm$ 6.50	10.82 $\pm$ 6.68
	Ginkgo+PS	294.16 $\pm$ 49	12.52 $\pm$ 5.41	11.13 $\pm$ 9.93	10.24 $\pm$ 6.30	19.73 $\pm$ 9.60
Digit Vigilance Accuracy (%)	placebo	95.48 $\pm$ 0.85	-1.35 $\pm$ 1.39	-1.59 $\pm$ 0.99	-0.95 $\pm$ 0.99	-2.14 $\pm$ 1.40
	Ginkgo	95.87 $\pm$ 0.72	-2.38 $\pm$ 1.36	-3.17 $\pm$ 1.21	-1.67 $\pm$ 1.01	-3.25 $\pm$ 1.93
	Ginkgo+PC	94.76 $\pm$ 1.00	0.08 $\pm$ 1.31	-1.11 $\pm$ 1.02	-0.63 $\pm$ 1.29	-0.63 $\pm$ 1.17
	Ginkgo+PS	92.45 $\pm$ 1.67	1.94 $\pm$ 1.39	-1.75 $\pm$ 1.37	1.73 $\pm$ 1.31	-0.87 $\pm$ 1.69
Digit Vigilance False alarms (number)	placebo	0.93 $\pm$ 0.20	0.04 $\pm$ 0.27	0.88 $\pm$ 0.45	0.29 $\pm$ 0.32	0.43 $\pm$ 0.25
	Ginkgo	1.07 $\pm$ 0.21	0.18 $\pm$ 0.37	-0.29 $\pm$ 0.20	-0.04 $\pm$ 0.21	0.68 $\pm$ 0.65
	Ginkgo+PC	0.86 $\pm$ 0.22	-0.04 $\pm$ 0.31	0.18 $\pm$ 0.28	0.36 $\pm$ 0.33	0.00 $\pm$ 0.27
	Ginkgo+PS	1.21 $\pm$ 0.21	-0.04 $\pm$ 0.32	-0.18 $\pm$ 0.30	-0.21 $\pm$ 0.32	0.71 $\pm$ 0.55
Digit Vigilance Reaction time (msec)	placebo	434.45 $\pm$ 4.46	9.24 $\pm$ 6.16	23.77 $\pm$ 5.82	15.55 $\pm$ 6.86	25.78 $\pm$ 5.62
	Ginkgo	435.88 $\pm$ 6.69	14.43 $\pm$ 5.74	6.84 $\pm$ 8.66	15.67 $\pm$ 5.40	14.38 $\pm$ 8.23
	Ginkgo+PC	426.13 $\pm$ 6.65	16.06 $\pm$ 4.92	17.97 $\pm$ 6.15	22.89 $\pm$ 5.50	16.57 $\pm$ 5.25
	Ginkgo+PS	453.16 $\pm$ 10.27	5.63 $\pm$ 8.88	8.25 $\pm$ 6.74	4.94 $\pm$ 7.68	8.44 $\pm$ 6.99
Choice reaction time accuracy (%)	placebo	92.45 $\pm$ 1.67	0.36 $\pm$ 0.19	0.64 $\pm$ 0.16	0.64 $\pm$ 0.16	0.00 $\pm$ 0.67
	Ginkgo	94.86 $\pm$ 0.86	-0.36 $\pm$ 0.72	-1.14 $\pm$ 0.71	-1.29 $\pm$ 0.96	-1.86 $\pm$ 0.78
	Ginkgo+PC	95.14 $\pm$ 0.67	0.21 $\pm$ 0.61	-0.64 $\pm$ 0.77	-1.43 $\pm$ 0.70	-1.14 $\pm$ 0.65
	Ginkgo+PS	94.71 $\pm$ 0.89	-0.29 $\pm$ 0.88	-0.57 $\pm$ 0.86	-1.88 $\pm$ 0.78	-1.79 $\pm$ 0.85
Choice reaction time (msec)	placebo	419.05 $\pm$ 0.05	-3.87 $\pm$ 6.92	5.70 $\pm$ 7.53	-2.50 $\pm$ 6.62	-2.69 $\pm$ 6.07
	Ginkgo	413.55 $\pm$ 0.91	12.43 $\pm$ 7.37	-1.57 $\pm$ 5.93	4.22 $\pm$ 7.72	7.82 $\pm$ 7.45
	Ginkgo+PC	419.04 $\pm$ 0.40	1.36 $\pm$ 6.09	-4.53 $\pm$ 9.90	-1.89 $\pm$ 6.02	4.02 $\pm$ 9.19
	Ginkgo+PS	432.03 $\pm$ 12.06	-3.22 $\pm$ 14.89	-13.93 $\pm$ 7.77	-11.80 $\pm$ 9.00	-3.95 $\pm$ 9.90
Spatial Memory (%>chance)	placebo	50.93 $\pm$ 5.75	-10.3 $\pm$ 4.60	-11.6 $\pm$ 5.05	-9.40 $\pm$ 3.70	-4.73 $\pm$ 3.92
	Ginkgo	90.18 $\pm$ 0.99	-6.74 $\pm$ 0.90	-6.83 $\pm$ 0.29	-5.54 $\pm$ 2.20	-2.81 $\pm$ 0.60
	Ginkgo+PC	90.54 $\pm$ 2.25	-0.89 $\pm$ 2.97	-5.85 $\pm$ 0.94	-13.7 $\pm$ 5.93	-8.84 $\pm$ 0.83
	Ginkgo+PS	89.33 $\pm$ 1.00	-4.60 $\pm$ 2.70	-2.77 $\pm$ 2.62	-5.67 $\pm$ 2.21	-3.26 $\pm$ 2.81
Spatial memory Reaction time (msec)	placebo	509.93 $\pm$ 5.75	-17.7 $\pm$ 11.11	-35.0 $\pm$ 11.82	1.77 $\pm$ 8.41	-36.6 $\pm$ 7.70
	Ginkgo	487.69 $\pm$ 7.52	-9.87 $\pm$ 8.78	0.81 $\pm$ 10.48	19.36 $\pm$ 10.59	1.50 $\pm$ 15.98
	Ginkgo+PC	488.98 $\pm$ 12.71	2.35 $\pm$ 10.94	-6.89 $\pm$ 10.56	13.03 $\pm$ 8.65	-19.8 $\pm$ 11.42
	Ginkgo+PS	528.05 $\pm$ 17.38	-24.7 $\pm$ 12.62	-30.5 $\pm$ 13.26	-26.9 $\pm$ 16.20	-49.8 $\pm$ 16.68
Numeric Working Memory (%>chance)	placebo	89.05 $\pm$ 1.50	-3.41 $\pm$ 1.82	-5.16 $\pm$ 1.79	-5.72 $\pm$ 1.49	-4.76 $\pm$ 1.69
	Ginkgo	87.86 $\pm$ 1.45	-2.22 $\pm$ 1.49	-2.14 $\pm$ 1.07	-3.09 $\pm$ 1.41	-5.48 $\pm$ 0.68
	Ginkgo+PC	89.13 $\pm$ 1.50	-4.92 $\pm$ 1.83	-3.73 $\pm$ 0.82	-3.81 $\pm$ 1.61	-5.16 $\pm$ 1.78
	Ginkgo+PS	85.56 $\pm$ 2.01	-1.03 $\pm$ 1.40	-2.46 $\pm$ 0.92	-3.33 $\pm$ 1.99	-3.18 $\pm$ 0.60
Numeric Work'g Memory RT (msec)	placebo	519.67 $\pm$ 6.59	-9.12 $\pm$ 8.64	-17.5 $\pm$ 10.56	-7.55 $\pm$ 9.90	-2.41 $\pm$ 10.21
	Ginkgo	509.01 $\pm$ 14.55	7.42 $\pm$ 5.89	-7.45 $\pm$ 7.05	1.60 $\pm$ 8.89	6.85 $\pm$ 10.92
	Ginkgo+PC	513.06 $\pm$ 16.22	6.71 $\pm$ 10.66	-8.14 $\pm$ 10.17	13.78 $\pm$ 12.72	0.90 $\pm$ 12.28
	Ginkgo+PS	540.21 $\pm$ 28.20	-24.7 $\pm$ 12.62	-28.5 $\pm$ 14.17	-40.2 $\pm$ 20.34	-31.9 $\pm$ 21.49
Delayed Word Recall (% accuracy)	placebo	38.69 $\pm$ 3.55	-5.40 $\pm$ 2.80	-12.2 $\pm$ 3.91	-12.10 $\pm$ 6.70	-16.7 $\pm$ 4.30
	Ginkgo	37.14 $\pm$ 4.01	-6.19 $\pm$ 2.50	-11.52 $\pm$ 7.09	-9.64 $\pm$ 2.97	-13.8 $\pm$ 2.61
	Ginkgo+PC	35.60 $\pm$ 2.58	-7.62 $\pm$ 3.54	-7.14 $\pm$ 2.05	-13.1 $\pm$ 2.92	-9.64 $\pm$ 2.01
	Ginkgo+PS	33.93 $\pm$ 3.40	-7.02 $\pm$ 3.27	-11.3 $\pm$ 2.61	-8.93 $\pm$ 2.90	-12.6 $\pm$ 2.68
Word Recognition (%>chance)	placebo	65.00 $\pm$ 4.09	-13.8 $\pm$ 2.92	-12.9 $\pm$ 3.12	-14.5 $\pm$ 3.97	-16.7 $\pm$ 4.30
	Ginkgo	55.95 $\pm$ 4.44	-6.43 $\pm$ 3.06	-7.86 $\pm$ 4.95	-7.14 $\pm$ 4.30	-12.1 $\pm$ 4.41
	Ginkgo+PC	55.24 $\pm$ 3.52	-8.34 $\pm$ 4.55	-7.38 $\pm$ 4.99	-9.05 $\pm$ 3.75	-11.7 $\pm$ 4.68
	Ginkgo+PS	55.43 $\pm$ 4.09	-13.1 $\pm$ 4.44	-11.4 $\pm$ 2.26	-12.4 $\pm$ 5.06	-16.2 $\pm$ 4.07
Word Recognition Reaction time (msec)	placebo	662.94 $\pm$ 4.28	-5.76 $\pm$ 13.36	-6.82 $\pm$ 10.47	13.68 $\pm$ 24.40	-6.92 $\pm$ 15.57
	Ginkgo	642.37 $\pm$ 2.59	17.86 $\pm$ 19.39	11.03 $\pm$ 15.51	15.74 $\pm$ 21.27	19.28 $\pm$ 15.80
	Ginkgo+PC	646.92 $\pm$ 24.16	21.82 $\pm$ 22.15	16.54 $\pm$ 18.64	25.16 $\pm$ 25.81	-0.39 $\pm$ 22.82
	Ginkgo+PS	710.53 $\pm$ 48.18	-55.2 $\pm$ 21.99	-68.2 $\pm$ 23.79	-48.2 $\pm$ 18.27	-49.6 $\pm$ 21.57
Picture Recognition (%>chance)	placebo	60.00 $\pm$ 4.43	-13.9 $\pm$ 3.91	-14.1 $\pm$ 5.04	-9.82 $\pm$ 4.28	-13.7 $\pm$ 3.68
	Ginkgo	60.18 $\pm$ 4.82	-11.4 $\pm$ 6.66	-12.5 $\pm$ 4.18	-8.57 $\pm$ 6.34	-11.2 $\pm$ 3.96
	Ginkgo+PC	61.23 $\pm$ 2.32	-11.6 $\pm$ 4.78	-7.32 $\pm$ 6.03	-7.89 $\pm$ 4.22	-15.4 $\pm$ 4.10
	Ginkgo+PS	51.95 $\pm$ 1.78	-11.6 $\pm$ 4.78	-7.32 $\pm$ 6.03	-7.89 $\pm$ 4.22	-15.4 $\pm$ 4.10
Picture recognition Reaction time (msec)	placebo	755.06 $\pm$ 1.98	15.11 $\pm$ 1.21	-11.0 $\pm$ 2.72	-33.7 $\pm$ 21.24	-2.20 $\pm$ 12.12
	Ginkgo	755.94 $\pm$ 16.27	11.23 $\pm$ 16.46	-30.4 $\pm$ 14.29	19.67 $\pm$ 25.65	-32.4 $\pm$ 40.40
	Ginkgo+PC	747.12 $\pm$ 24.19	24.98 $\pm$ 13.54	-10.8 $\pm$ 14.12	56.22 $\pm$ 19.04	29.96 $\pm$ 20.47
	Ginkgo+PS	796.49 $\pm$ 62.26	-26.4 $\pm$ 48.45	-54.27 $\pm$ 7.70	-45.2 $\pm$ 55.24	-76.2 $\pm$ 58.06

Figure 2. Effects of the treatments on the individual task outcome measures from the CDR battery. Mean baseline and change from baseline scores (with standard errors) are presented. Tasks are displayed in order of completion, with a graphical representation of measures generating significant differences on the initial ANOVA and subsequent planned comparisons (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.005$ ; \*\*\*\*,  $p < 0.001$  compared to placebo). Ginkgo + PC = Ginkgo complexed with phosphatidylserine; Ginkgo + PS = Ginkgo complexed with phosphatidylserine



Factor		Pre-dose Baseline score	Change from baseline score			
			1 hr post-dose	2.5 hr post-dose	4 hr post-dose	6 hr post-dose
Speed of Attention	placebo	1150.8 <sup>22.45</sup>	14.96 <sup>11.54</sup>	31.68 <sup>14.07</sup>	15.41 <sup>14.65</sup>	22.67 <sup>11.98</sup>
	Ginkgo	1133.4 <sup>19.59</sup>	37.75 <sup>13.16</sup>	25.86 <sup>18.60</sup>	34.45 <sup>11.76</sup>	50.12 <sup>21.07</sup>
	Gink+PC	1145.8 <sup>22.98</sup>	23.26 <sup>10.06</sup>	15.50 <sup>12.07</sup>	36.07 <sup>12.11</sup>	31.40 <sup>16.18</sup>
	Gink+PS	1179.3 <sup>27.94</sup>	14.93 <sup>22.69</sup>	5.45 <sup>17.53</sup>	4.16 <sup>16.39</sup>	24.32 <sup>25.90</sup>
Accuracy of Attention	placebo	88.75 <sup>0.74</sup>	-0.46 <sup>0.90</sup>	-1.07 <sup>0.61</sup>	-0.39 <sup>0.80</sup>	-1.39 <sup>0.88</sup>
	Ginkgo	89.50 <sup>0.75</sup>	-1.43 <sup>0.85</sup>	-1.71 <sup>1.08</sup>	-1.36 <sup>0.81</sup>	-3.07 <sup>1.59</sup>
	Gink+PC	89.36 <sup>0.64</sup>	0.18 <sup>0.78</sup>	-1.00 <sup>0.68</sup>	-1.36 <sup>0.73</sup>	-0.86 <sup>0.64</sup>
	Gink+PS	87.75 <sup>1.22</sup>	0.75 <sup>0.59</sup>	-0.89 <sup>0.99</sup>	0.07 <sup>1.01</sup>	-2.00 <sup>1.17</sup>
Speed of Memory	placebo	2438.2 <sup>62.43</sup>	-17.08 <sup>42.94</sup>	-38.66 <sup>45.28</sup>	-25.80 <sup>36.97</sup>	-48.19 <sup>41.34</sup>
	Ginkgo	2395.0 <sup>62.72</sup>	26.63 <sup>34.86</sup>	-27.05 <sup>32.99</sup>	56.38 <sup>29.69</sup>	-5.00 <sup>38.42</sup>
	Gink+PC	2396.1 <sup>62.43</sup>	55.86 <sup>41.25</sup>	-10.10 <sup>36.77</sup>	108.19 <sup>42.30</sup>	10.64 <sup>49.99</sup>
	Gink+PS	2574.2 <sup>125.63</sup>	-132.2 <sup>65.01</sup>	-181.3 <sup>97.35</sup>	-160.5 <sup>79.46</sup>	-227.3 <sup>98.14</sup>
Quality of Memory	placebo	397.77 <sup>11.50</sup>	-50.99 <sup>9.53</sup>	-62.47 <sup>11.11</sup>	-57.98 <sup>7.69</sup>	-66.34 <sup>11.41</sup>
	Ginkgo	382.74 <sup>12.25</sup>	-37.36 <sup>9.96</sup>	-47.19 <sup>9.81</sup>	-40.18 <sup>10.26</sup>	-54.54 <sup>11.81</sup>
	Gink+PC	379.49 <sup>10.25</sup>	-37.66 <sup>14.07</sup>	-33.69 <sup>12.85</sup>	-56.68 <sup>12.71</sup>	-55.78 <sup>12.75</sup>
	Gink+PS	364.11 <sup>12.12</sup>	-31.82 <sup>8.45</sup>	-37.19 <sup>9.79</sup>	-31.50 <sup>9.94</sup>	-52.50 <sup>9.46</sup>
Secondary Memory	placebo	215.59 <sup>10.82</sup>	-37.26 <sup>8.05</sup>	-45.65 <sup>10.42</sup>	-42.80 <sup>7.45</sup>	-56.85 <sup>10.19</sup>
	Ginkgo	204.70 <sup>12.18</sup>	-28.39 <sup>9.41</sup>	-38.21 <sup>9.37</sup>	-31.55 <sup>10.42</sup>	-46.25 <sup>8.87</sup>
	Gink+PC	199.82 <sup>8.81</sup>	-31.85 <sup>12.56</sup>	-24.11 <sup>10.70</sup>	-39.17 <sup>10.32</sup>	-41.79 <sup>10.64</sup>
	Gink+PS	189.23 <sup>11.50</sup>	-26.19 <sup>6.78</sup>	-31.96 <sup>8.17</sup>	-22.50 <sup>8.81</sup>	-46.07 <sup>8.32</sup>
Working Memory	placebo	182.18 <sup>2.43</sup>	-13.73 <sup>4.87</sup>	-16.81 <sup>4.99</sup>	-15.18 <sup>3.45</sup>	-9.50 <sup>4.00</sup>
	Ginkgo	178.04 <sup>2.50</sup>	-8.96 <sup>4.10</sup>	-8.97 <sup>3.77</sup>	-8.63 <sup>2.68</sup>	-8.29 <sup>4.12</sup>
	Gink+PC	179.66 <sup>3.37</sup>	-5.81 <sup>3.62</sup>	-9.58 <sup>4.40</sup>	-17.51 <sup>6.80</sup>	-14.00 <sup>6.75</sup>
	Gink+PS	174.89 <sup>3.67</sup>	-5.63 <sup>3.33</sup>	-5.23 <sup>3.53</sup>	-9.00 <sup>3.32</sup>	-6.43 <sup>5.06</sup>

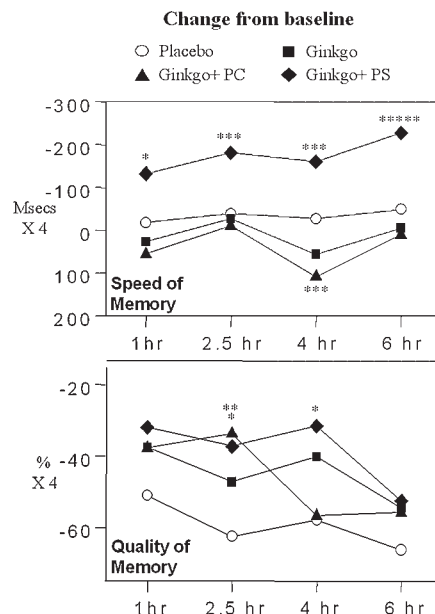


Figure 3. Effects of the treatments on the primary outcome cognitive factors derived from the CDR battery outcomes. Mean baseline and change from baseline scores (with standard errors) are presented, with a graphical representation of the factors generating significant differences on the initial ANOVA and subsequent planned comparisons (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.005$ ; \*\*\*\*,  $p < 0.0005$  compared to placebo). Ginkgo + PC = Ginkgo complexed with phosphatidylserine; Ginkgo + PS = Ginkgo complexed with phosphatidylserine

significant differences on the initial ANOVA and subsequent planned comparisons. Accuracy of performing the Digit vigilance task was significantly improved for the GBE/phosphatidylserine condition at both 1 h [ $t(243) = 2.58$ ,  $p = 0.011$ ] and 4 h [ $t(243) = 2.14$ ,  $p = 0.034$ ] post-dose. In contrast to this all three doses evinced decrements in the accuracy of performing the Choice reaction time task with this effect being evident for GBE at 2.5 h [ $t(243) = 2.46$ ,  $p = 0.015$ ], 4 h [ $t(243) = 2.66$ ,  $p = 0.009$ ] and 6 h [ $t(243) = 2.56$ ,  $p = 0.011$ ] post-dose, for GBE/phosphatidylcholine at 4 h post-dose [ $t(243) = 2.86$ ,  $p = 0.005$ ], and for GBE/phosphatidylserine at 4 h [ $t(243) = 3.45$ ,  $p = 0.001$ ] and 6 h [ $t(243) = 2.46$ ,  $p = 0.015$ ] post-dose.

**Speed of memory factor.** The initial ANOVA showed that there was a significant main effect of the treatment on the Speed of Memory factor [ $F(3, 405) = 15.76$ ,  $p < 0.001$ ]. Planned comparisons showed that performance was enhanced at all time points following GBE/phosphatidylserine (1 h [ $t(243) = 2.58$ ,  $p = 0.011$ ], 2.5 h [ $t(243) = 3.19$ ,  $p = 0.002$ ], 4 h [ $t(243) = 3$ ,  $p = 0.003$ ] and 6 h [ $t(243) = 4$ ,  $p < 0.001$ ] post-dose). In contrast to this, performance was

significantly slowed for the GBE/phosphatidylcholine condition at 4 h post-dose [ $t(243) = 3$ ,  $p < 0.003$ ].

Reference to the ANOVAS of the single outcomes contributing to this factor showed that performance was significantly modulated on all four tasks. Planned comparisons showed that following GBE/phosphatidylserine performance was faster on: the Spatial memory task at 6 h [ $t(243) = 2.23$ ,  $p < 0.027$ ], with trends towards the same at 2.5 h [ $t(243) = 1.8$ ,  $p = 0.071$ ] and 4 h [ $t(243) = 1.92$ ,  $p = 0.056$ ]; the Numeric working memory task at 4 h [ $t(243) = 3.29$ ,  $p = 0.001$ ] and 6 h [ $t(243) = 2.944$ ,  $p = 0.004$ ]; the Word recognition task at 1 h [ $t(243) = 2.2$ ,  $p = 0.028$ ], 2.5 h [ $t(243) = 2.75$ ,  $p = 0.006$ ] and 4 h [ $t(243) = 2.77$ ,  $p = 0.006$ ], with a trend towards the same at 6 h post-dose [ $t(243) = 1.91$ ,  $p = 0.057$ ]; and the Picture recognition task at 6 h [ $t(243) = 3.12$ ,  $p = 0.002$ ], with trends towards the same at 1 h [ $t(243) = 1.75$ ,  $p = 0.082$ ] and 2.5 h [ $t(243) = 1.82$ ,  $p = 0.071$ ].

In contrast to these marked improvements both of the other conditions were associated with the occasional decrement, with GBE evincing slowed performance on the Spatial memory task at 6 h [ $t(243) = 2.56$ ,  $p = 0.011$ ] and the Picture recognition task at 4 h [ $t(243) = 2.25$ ,  $p = 0.026$ ], and GBE/phosphatidylcholine evincing slowed performance on



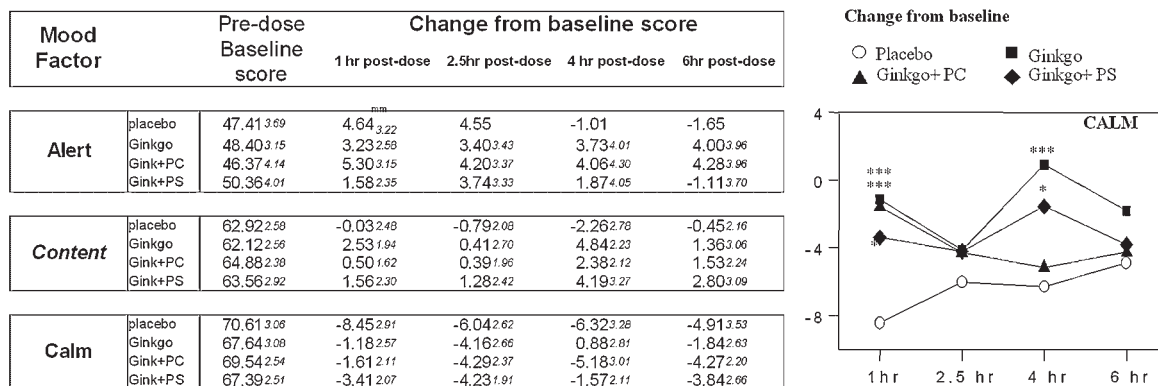


Figure 4. Effects of the treatments on the mood factors derived from the Bond-Lader mood scales. Mean baseline and change from baseline scores (with standard errors) are presented, with a graphical representation of the factor that generated significant differences on the initial ANOVA and subsequent planned comparisons (\*,  $p < 0.05$ ; \*\*\*,  $p < 0.005$  compared to placebo). Ginkgo + PC = Ginkgo complexed with phosphatidylserine; Ginkgo + PS = Ginkgo complexed with phosphatidylserine

the Numeric working memory task [ $t(243) = 2.15$ ,  $p = 0.033$ ] and the Picture recognition task [ $t(243) = 3.8$ ,  $p < 0.001$ ] at 4 h post-dose.

**Quality of memory measure.** The initial ANOVA showed that there was a significant main effect of treatment on the global Quality of Memory measure [ $F(3, 405) = 3.33$ ,  $p = 0.02$ ]. Planned comparisons showed that GBE/phosphatidylserine resulted in improved change from baseline performance at 2.5 h [ $t(243) = 2.35$ ,  $p = 0.02$ ] and 4 h [ $t(243) = 2.46$ ,  $p = 0.015$ ] with a trend towards the same at 1 h [ $t(243) = 1.78$ ,  $p = 0.077$ ] post-dose. Performance was also enhanced for GBE/phosphatidylcholine at 2.5 h [ $t(243) = 2.67$ ,  $p = 0.008$ ].

Reference to the contributing single task outcomes showed that performance was only significantly improved on one individual task outcome, with GBE/phosphatidylserine outperforming placebo at 1 h [ $t(243) = 2.39$ ,  $p = 0.018$ ], 2.5 h [ $t(243) = 2.24$ ,  $p = 0.027$ ] and 4 h [ $t(243) = 2.43$ ,  $p = 0.016$ ] on the Picture recognition task.

**Secondary memory factor.** The initial ANOVA suggested that this factor was not significantly affected by the treatment.

**Working memory factor.** The initial ANOVA suggested that this factor was not significantly affected by the treatment.

**Serial 7s and Serial 3s subtraction tasks.** The initial ANOVA suggested that these tasks were not significantly affected by the treatments.

### Mood assessment

Mean raw baseline scores and change from baseline scores for the three mood factors (alert, content, calm) are presented in the tables and graph of Figure 4.

**Bond-Lader mood scales.** The initial ANOVA suggested that there was only a main effect of treatment on the 'Calm' factor derived from the visual analogue scales [ $F(3, 405) = 2.95$ ,  $p < 0.032$ ].

Planned comparisons revealed that all three treatments resulted in enhanced 'calmness' in comparison to placebo. This effect was evident for Ginkgo at 1 h [ $t(243) = 3.05$ ,  $p = 0.003$ ] and 4 h [ $t(243) = 3.02$ ,  $p = 0.003$ ], GBE/phosphatidylcholine at 1 h [ $t(243) = 2.87$ ,  $p = 0.005$ ], and GBE/phosphatidylserine at 1 h [ $t(243) = 2.1$ ,  $p = 0.036$ ] and 6 h [ $t(243) = 1.99$ ,  $p = 0.048$ ] post-dose.

### DISCUSSION

The results of the current study suggest that, in keeping with previous research (Kennedy *et al.*, 2000), a single dose of 120 mg GBE (GK501) failed to markedly enhance cognitive performance in healthy young volunteers. Similarly, other than modest evidence of enhanced accuracy but slowed performance on memory tasks, GBE complexed with phosphatidylcholine failed to elicit performance benefits. However, GBE complexed with phosphatidylserine led to significantly improved performance both in terms of improved accuracy of memory task performance and, most notably, in increased speed of memory task performance, with the latter evident

across all of the timed memory tasks in the battery. All of the treatments proved to be well tolerated, with no instances of adverse events recorded for any of the treatments.

The pharmacokinetic measures incorporated in the study failed to confirm any bio-availability advantage for the complexed products. However, it should be noted that the analysis was undertaken on a much reduced dataset due to participant withdrawal (from blood sampling) and inconclusive data.

Previous research in humans has failed to demonstrate any clear evidence of nootropic potential for chronic regimes of phosphatidylcholine in humans (Higgins and Flicker 2003). Phosphatidylserine, on the other hand, exhibits a wider range of potentially psychopharmacological properties in animals (Tofano *et al.*, 1978; Argentiero and Tavolato 1980; Samson, 1987; Casamenti *et al.*, 1991; Pepeu *et al.*, 1996; Giovannini *et al.*, 1997; Amenta *et al.*, 2001). Evidence also suggests that it can engender memory improvements in rodents (e.g. Blokland *et al.*, 1999; Claro *et al.*, 1999; Alves *et al.*, 2000), and modest cognitive improvements in cognitively challenged population of humans (e.g. Amenta *et al.*, 2001; McDaniel *et al.*, 2003) with these effects including somewhat fragile memory effects in elderly population and those with pre-existing memory problems (reviewed in: Benton *et al.*, 2005). No studies have as yet addressed the acute effects of either phospholipid in cognitively intact humans. The clear benefit seen in the current study for GBE/phosphatidylserine could therefore be attributable either to its concomitant administration with GBE, or alternatively to the effects of phosphatidylserine alone. Similarly, if the effects are attributable to the co-administration, they may be due to increased bio-availability of the active constituents of Ginkgo biloba due to complexation, or mere combination, or they may be due to the simple addition or interaction of the psychopharmacological properties of the two components. Further research designed to delineate the comparative contributions of GBE and phosphatidylserine, and the relative benefits of complexation versus combination in any behavioural effects is therefore called for.

The current study, as a proof of concept trial, merely assessed the cognitive and mood properties of single doses of GBE/phosphatidylcholine and GBE/phosphatidylserine utilising healthy young volunteers and a testing paradigm that has previously been found to be sensitive to modulation by herbal products. The possibility exists that the effects of chronic dosage or administration to cholinergically challenged population of these treatments may have a somewhat

different profile, particularly as the rationale for administering phosphatidylcholine and phosphatidylserine alone (i.e. protection of membrane integrity) would specifically anticipate no acute effects and increased benefits with increasing length of administration. It may well be the case that co-administration of either may also potentiate the benefits of *Ginkgo biloba* seen in cognitively challenged groups (see: Birks *et al.*, 2002). Again, this possibility requires further research consideration.

In conclusion, acute administration to healthy young humans of a product complexing, a standardised GBE with phosphatidylserine was found to significantly improve memory task performance throughout multiple assessments made over the 6 h following administration. No such benefits were evident for either the low dose of Ginkgo biloba, or the product complexing Ginkgo biloba with phosphatidylcholine. Whilst the investigation of bio-availability parameters was inconclusive in terms of differential patterns between treatments, there were no adverse events recorded for any of the treatments suggesting that they are well tolerated.

The relative contributions of Ginkgo biloba and phosphatidylserine, and the differential effects of complexation versus combination, and acute versus chronic administration require further research.

## ACKNOWLEDGEMENTS

Materials and a contribution to the direct costs of the study were provided by Indena SpA (Milan) the manufacturer of the products under investigation. The results from the current study were included in the portfolio that Indena SpA submitted in support of patent WO 2005/074956. None of the authors have any financial interests in Indena SpA or any of their products.

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