

Research paper

Effects of a food supplement containing phosphatidylserine on cognitive function in Chinese older adults with mild cognitive impairment: A randomized double-blind, placebo-controlled trial

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ABSTRACT

Background: Phosphatidylserine (PS) and α -Linolenic acid (ALA), are positively associated with cognitive function, but their combination effects and possible mechanisms remain unclear. We aimed to explore the effects on cognition and potential mechanism of the supplements.

Methods: This randomized, double-blind, placebo-controlled trial recruited 190 MCI patients in Tianjin, China, and randomly assigned in intervention group and placebo group. Each group consumed two capsules every day for 12 months. Each capsule for intervention group contains 144 mg ALA, 31.5 mg PS and 3.6 mg Ginkgo total flavonoids as main functional components, with 0.48 mg Vitamin B₁ (as thiamine hydrochloride), 0.48 mg Vitamin B₆ (as pyridoxine hydrochloride) and 90 μ g folic acid as supplement. Capsules for placebo group were identical but contain no active ingredients. Cognitive function, serum n-3 polyunsaturated fatty acids (PUFAs) and neurotransmitters were assessed at baseline and 12 months. Linear mixed effects model and causal mediation analysis were conducted to explore the effects and potential mechanism of the intervention.

Results: A total of 190 participants (mean [SD] age, 67.95 [5.62] years; 70 (36.8 %) male and 120 (63.2 %) female) were randomized to the placebo group ($n = 95$) and intervention group ($n = 95$). Compared with placebo group, the intervention group had statistically significant improvements in arithmetic testing (β , 0.688; 95 % CI, 0.103–1.274), the similarity test (β , 1.070; 95 % CI, 0.472–1.667) and short-term memory (β , 0.600; 95 % CI, 0.399–0.800). Besides, the intervention group had statistically significant increases in serum ALA (β , 1.620; 95 % CI, 0.967–2.265), DHA (β , 2.797; 95 % CI, 1.075–4.532), EPA (β , 1.472; 95 % CI, 0.296–2.643), acetylcholine (β , 0.441; 95 % CI, 0.415–0.468), GABA (β , 0.009; 95 % CI, 0.001–0.016) and 5-HT (β , 0.160; 95 % CI, 0.081–0.238) compared to the placebo group. And the intervention may improve short-term memory by increasing serum ALA levels (average causal mediation effect = 0.132, 95 % CI, 0.053–0.225) with 19.7 % mediation proportion.

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Conclusions: This food supplement containing phosphatidylserine could improve different cognitive functions of MCI patients, especially short-term memory, and increase serum n-3 PUFAs and neurotransmitters levels. Serum ALA level might play a mediation role.

1. Introduction

Mild cognitive impairment (MCI) is a transitional cognitive state between normal cognitive aging and dementia, especially Alzheimer's disease (AD) (Petersen et al., 1999). In some cases, this state is reversible, with 10–12 % MCI patients progressing to AD annually (Petersen et al., 1999) and 15.8 % MCI patients reversing to normal cognition per year (Thomas et al., 2019), which makes MCI a critical window for intervention. However, there are currently no proven causative treatments for MCI available.

Both genetic factors and nutritional intake are related to the risk of developing MCI (Petersen et al., 2014; Scarmeas et al., 2018). Apolipoprotein E polymorphism $\epsilon 4$ (*APOE* $\epsilon 4$) and methylenetetrahydrofolate reductase (*MTHFR*) TT genotype are genetic risk factors for developing MCI (Rajagopalan et al., 2012; Jefferson et al., 2015). α -Linolenic acid (ALA) is an essential fatty acid and the substrate for the synthesis of longer-chain n-3 polyunsaturated fatty acids (PUFAs) (Baker et al., 2016). It is reported that ALA was associated with a significantly lower risk of dementia (Yamagishi et al., 2017; Sala-Vila et al., 2022). Phosphatidylserine (PS) is the major anionic phospholipid class and play an important role in activating key signaling pathways involved in stimulating neuronal survival, neurite growth and synaptogenesis. Brain PS has a high docosahexaenoic acid (DHA) content (Kim et al., 2014). Naturally, it's crucial for maintaining healthy cognitive functioning, and it has been proven to have beneficial effects on cognitive function in older adults with memory complaints but the mechanism was unclear (Richter et al., 2013).

Therefore, the main purpose of this trial was to explore the effectiveness and possible mechanisms of phosphatidylserine supplement in improving cognitive function of older adults with MCI. We hypothesized that the supplement would improve different cognitive functions and possible through n-3 PUFAs and neurotransmitters.

2. Material and methods

2.1. Study design

This randomized double-blind, placebo-controlled trial was conducted from April 20, 2022, to January 26, 2024 in Binhai New District, Tianjin, China. The study protocol was approved by the Ethics Committee of Tianjin Medical University (Approval number: TMUH-MEC2021012), and all participants provided written informed consent before participation. All our procedures followed the Consolidated Standards of Reporting Trial (CONSORT) reporting guideline. The study was registered at <https://clinicaltrials.gov/> (Register number: ChiCTR2200058031). The trial protocol and statistical analysis plan can be found in Supplement 1.

2.2. MCI assessment

The diagnostic criteria based on the standard of mild neurocognitive impairment in Diagnostic and Statistical Manual of Mental Disorders-IV criteria of the United States (Petersen, 2004): (1) subjective memory loss; (2) Mini-Mental State Examination (MMSE) score ≤ 17 points for illiterates, ≤ 20 points for primary school, and ≤ 24 points for secondary education and above, were classified as cognitive impairment; (3) life and social function have decreased: Activities of Daily Living Scale (ADL) score ≤ 18 ; (4) Global Deterioration Scale (GDS) rating of 2–3; (5) memory loss lasting >3 months; (6) do not meet the diagnostic criteria of dementia; (7) eliminate cognitive decline caused by special reasons.

Combined with the results of previous studies, the diagnostic criteria of MCI in this trial were further revised.

2.3. Participants

Participants should meet the following inclusion criteria: (1) community-based adults aged 65 years or older; (2) the presence of MCI; (3) capable of walking, vision and hearing to complete the trial; and (4) informed consent and voluntary participation. Exclusion criteria included: (1) cognitive impairment caused by other reasons, such as dementia, traumatic brain injury or concussion; (2) taking medications that affect cognitive function; and (3) participation in other trials.

2.4. Intervention

Participants were randomly assigned in intervention group and placebo group based on randomization codes generated by the specialist (Fig. 1). Participants in each group consumed two capsules every day for 12 months. Each capsule for intervention group contains 144 mg ALA, 31.5 mg PS and 3.6 mg Ginkgo total flavonoids as main functional components, with 0.48 mg Vitamin B₁ (as thiamine hydrochloride), 0.48 mg Vitamin B₆ (as pyridoxine hydrochloride) and 90 μ g folic acid as supplement. *Ginkgo biloba* leaves is a popular dietary supplement, and special extract EGb 761 is one of the most studied herb medications (Kulić et al., 2022). Besides, it has been proven that 240 mg EGb 761 per day improves cognition for people with AD and vascular dementia, and such extracts contain 22–27 % Ginkgo total flavonoids, which is far more than the amount used in this trial (Gauthier and Schlaefke, 2014; Tan et al., 2015). Therefore, the Ginkgo added in this trial has symbolic value, but is unlikely to contribute to the observed effects. According to the latest published “Dietary Reference Intake for China (2023 edition)” (Zheng et al., 2023), the intake of supplements Vitamin B₁, Vitamin B₆ and folic acid are far below dietary reference intakes (DRIs) (1.2–1.4 mg/day, 1.6 mg/day and 400 μ g dietary folate equivalence/day, respectively). However, the daily intake of Vitamin B₁, Vitamin B₆ and folic acid of Chinese older adults does not reach the DRIs (0.7 mg/day, 0.4 mg/d, 59.2 μ g/day, respectively) (Liu et al., 2019), so in this trial, appropriate amounts of these ingredients were added to the supplement and serum folate and homocysteine were used as the observational indicators. Capsules for placebo group were identical in appearance and taste to intervention group but contain no active ingredients. All capsules were produced by BYHEALTH Co., LTD, China. This study was unblinded after all statistical analyses were completed.

2.5. Assessment of cognitive function

The pre-defined primary outcome in this study was cognitive function determined by scores of the Chinese version of the Wechsler Adult Intelligence Scale-Revised in China (WAIS-RC) (Gong, 1992; Li et al., 2021). WAIS-RC included four verbal subtests: information, arithmetic, similarity and digit span, and three performance subtests: picture completion, block design and picture arrangement. Information was to test general knowledge, interest and long-term memory; arithmetic was to detect sequential reasoning, calculation and problem-solving skills, and attention; similarity was to test logical thinking and abstract generalization abilities; digit span was to test attention and immediate memory; picture completion was to test perceptual and visuospatial organization; block design was to test spatial relationships, spatial structure and visuomotor coordination; picture arrangement was to detect part-whole and logical association skills. Age-appropriate norms

from the Chinese standardization were used to calculate performance intelligence quotient (IQ), verbal IQ and full scale IQ. Short-term memory was determined by subtest scores of Wechsler Memory Scale-Revised in China (WMS-RC) (He et al., 2011; Gong, 1989).

2.6. Laboratory measurements

The pre-defined secondary endpoints were serum levels of ALA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), acetylcholine, γ -aminobutyric acid (GABA), dopamine and 5-hydroxytryptamine (5-HT) at 12 months. During the clinical examination, we collected participants' fasting venous blood at baseline and 12 months. Genotypes were determined via the Custom Taqman SNP Genotyping Assay by sequencing rs429358 and rs7412 at exon 4 of the *APOE* gene, and rs1801133 of the *MTHFR* gene with the technical support of Biowing Company (Shanghai, China). Serum ALA, DHA, EPA were determined by gas chromatography–mass spectrometry, serum acetylcholine, GABA, dopamine and 5-HT were detected by ultra-high liquid chromatography–mass spectrometry with the technical support of Applied Protein Technology Company (Shanghai, China). In addition, plasma amyloid β -protein (A β) 40 and A β 42 were measured by enzyme-linked immunosorbent assay kits (Jiangsu Meimian Industrial Co., Ltd., China). Serum folate concentration was measured by chemiluminescence enzyme immunoassay and serum homocysteine was measured by enzymatic cycling method.

Fasting blood glucose levels were measured using the glucose oxidase method, full blood counts including hemoglobin, platelet, white blood cell, neutrophil, lymphocyte, intermediated cell mass were analyzed by Olympus AU5811 clinical chemistry analyzer (Tokyo, Japan). Besides, serum glutamic pyruvic transaminase (ALT), glutamic

oxaloacetic transaminase (AST), total bilirubin, serum creatinine, blood urea nitrogen (BUN), total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by chemical precipitation with appropriate kits (Roche Cobas 8000 modular analyzer, Mannheim, Germany). These indicators were used as safety index.

2.7. Other variables

During the general physical examination, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice of both arms using Yuwell mercury sphygmomanometer after minutes of rest in a seated position. These indicators were also used as safety index. In addition, participants' height and weight were measured using a standard protocol and their body mass index (BMI) (kg/m^2) was calculated by dividing their weight (kg) by the square of the height (m^2).

A structured questionnaire was designed to collect the demographic characteristics (age, sex, educational duration, living alone), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities) and the use of nutritional supplements, and medical history (diabetes, hypertension, cardiovascular disease) was obtained by individual physical examination file. Meanwhile, mini-mental state examination (MMSE), activity of daily living scale (ADL), self-rating anxiety scale (SAS) and self-rating depression scale (SDS) were performed at baseline to collect potential confounders comprehensively.

2.8. Statistical analysis

A sample size of 95 per group was calculated, assuming 10 % dropout rate to achieve an 90 % statistical power and a two-sided level of

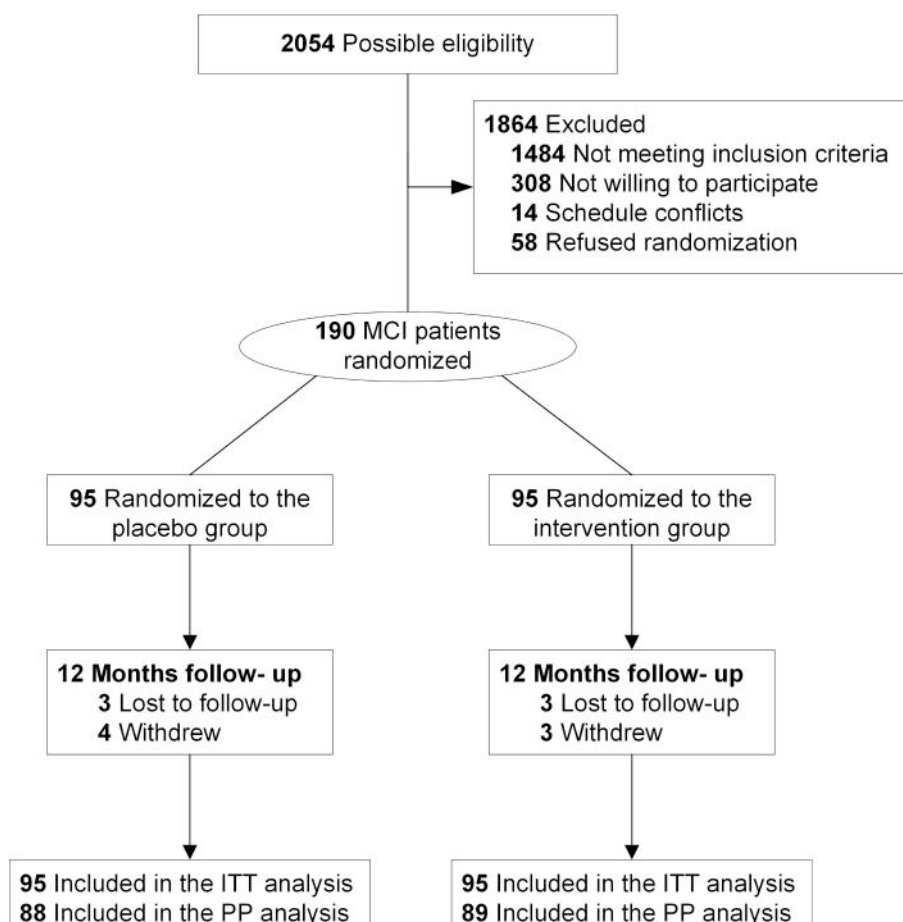


Fig. 1. Study flowchart. ITT, intention to treat; PP, per-protocol.

statistical significance at 5 % for the comparisons of the two groups. All participants were included in the intention-to-treat (ITT) analysis, and participants who adhered to the treatment protocol were included in the per-protocol (PP) analysis. Multiple imputations were performed for missing data after intervention.

Kolmogorov-Smirnov normality test was used to test the cumulative frequency distribution of continuous variables. Normally distributed variables were expressed as mean ± standard deviation (SD), non-normally distributed variables were presented as median (25th percentile, 75th percentile), and categorical variables were shown as frequencies and proportion percentages. Independent-samples *t*-test and Wilcoxon rank sum test were conducted for continuous variables based on their distribution, and *chi*-square test was used for categorical variables to compare characteristics between two groups. In addition, paired samples Wilcoxon test and paired samples *t*-test were performed to analyze changes in cognitive function, n-3 PUFAs and neurotransmitters within the groups before and after the intervention, and independent-samples *t*-test and Wilcoxon rank sum test to compare differences between groups.

A Linear mixed effects model was used to estimate the effect of interventions on cognitive functions, n-3 PUFAs and neurotransmitters based on maximum likelihood estimation, with and without adjustment for social demographics (age, sex, educational duration, living alone, BMI), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities), medical history (diabetes, hypertension, cardiovascular disease), nutritional supplements, MMSE score, ADL score, SAS score, SDS score, *APOE* ε4 and *MTHFR* C677T genotype. Besides, multiple linear regression was used to analyze longitudinal relationship between serum n-3 PUFAs, neurotransmitters and cognitive function based on the results of linear mixed effects models. Further, causal mediation analysis was conducted to explore the potential mechanism of the intervention on cognitive function. All analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing).

3. Results

3.1. Sociodemographic characteristics of participants

A total of 190 participants (mean [SD] age, 67.95 [5.62] years; 70 (36.8 %) male and 120 (63.2 %) female) were randomized to the placebo group (*n* = 95) and intervention group (*n* = 95) and included in the ITT analysis. 177 participants (88 for placebo group and 89 for intervention group) attended evaluations at 12 months were included in the PP analysis. The baseline demographic and clinical characteristics of participants are summarized in Table 1, and there was no statistical difference in all baseline characteristics between the two groups (all *P* values > 0.05), which indicated that the two groups are homogeneous and comparable.

3.2. Changes of cognitive function

After 12 months intervention, compared with placebo group, the intervention group had statistically significant improvements in arithmetic testing (β , 0.688; 95 % *CI*, 0.103–1.274), the similarity test (β , 1.070; 95 % *CI*, 0.472–1.667) and short-term memory (β , 0.600; 95 % *CI*, 0.399–0.800) after adjustment of potential confounders (Fig. 2). The change of cognitive scores is shown in Table S1 of supplement 2. In PP analysis, compared with placebo group, the intervention group had statistically significant improvements in arithmetic testing (β , 0.813; 95 % *CI*, 0.272–1.354), similarity test (β , 1.145; 95 % *CI*, 0.547–1.745), digit span (β , 0.581; 95 % *CI*, 0.164–0.998), performance IQ (β , 2.695; 95 % *CI*, 0.409–4.988), full scale IQ (β , 2.310; 95 % *CI*, 0.142–4.484) and short-term memory (β , 0.676; 95 % *CI*, 0.479–0.873) after adjustment of potential confounders (Table S6 of supplement 2). The change of cognitive scores is shown in Table S5 of supplement 2. Besides, plasma Aβ40, Aβ42 and Aβ42/Aβ40 were increased after intervention in

Table 1
Baseline characteristics of participants.

Characteristics	All participants (<i>n</i> = 190)	Placebo group (<i>n</i> = 95)	Intervention group (<i>n</i> = 95)	<i>P</i> value ^a
Social demographics				
Age (years)	67.95 ± 5.62	69.52 ± 4.57	69.99 ± 6.53	0.5629
Sex (<i>n</i> , %)				1.0000
Male	70 (36.8)	35 (36.8)	35 (36.8)	
Female	120 (63.2)	60 (63.2)	60 (63.2)	
Education duration (years)	12.00 (9.00, 12.00)	12.00 (9.00, 12.00)	9.00 (9.00, 12.00)	0.1661
Living alone (<i>n</i> , %)	15 (7.89)	5 (5.26)	10 (10.53)	0.2819
BMI (kg/m ²)	24.37 ± 3.14	24.63 ± 2.89	24.11 ± 3.37	0.5628
Lifestyles (<i>n</i> , %)				
Current smoker	19 (10.0)	9 (9.5)	10 (10.5)	1.0000
Drinking alcohol	33 (17.4)	18 (18.9)	15 (15.8)	0.7017
Exercise	125 (65.8)	66 (69.5)	59 (62.1)	0.3589
Participation in leisure activities	94 (49.5)	47 (49.5)	47 (49.5)	1.0000
Medical history (<i>n</i> , %)				
Diabetes	52 (27.4)	27 (28.4)	25 (26.3)	0.8707
Hypertension	128 (67.4)	62 (65.3)	66 (69.5)	0.6425
Cardiovascular disease	43 (22.6)	21 (22.1)	22 (23.2)	1.0000
Nutritional supplements (<i>n</i> , %)	46 (24.2)	24 (25.3)	22 (23.2)	0.8655
MMSE (scores)	23.00 (21.00, 24.00)	23.00 (21.00, 24.00)	24.00 (21.00, 24.00)	0.198
ADL (scores)	14.00 (14.00, 14.00)	14.00 (14.00, 14.00)	14.00 (14.00, 14.00)	0.5575
SAS (scores)	27.50 (25.00, 31.25)	27.50 (25.63, 30.00)	28.75 (25.00, 31.88)	0.4212
SDS (scores)	27.50 (26.25, 30.00)	27.50 (25.00, 29.38)	27.50 (26.25, 31.25)	0.1594
<i>APOE</i> ε4 carriers (<i>n</i> , %)	27 (14.2)	16 (16.8)	11 (11.6)	0.4059
<i>MTHFR</i> C677T (TT, <i>n</i> , %)	53 (27.9)	24 (25.3)	29 (30.5)	0.5176

^a *P* value showed comparison between two groups. Education duration, MMSE, ADL, SAS and SDS were presented as median (25th percentile, 75th percentile). MMSE, mini-mental state examination; ADL, activity of daily living scale; SAS, self-rating anxiety scale; SDS, self-rating depression scale.

placebo group but not in intervention group (Table S2 and Table S7 of supplement 2).

3.3. Changes of serum n-3 PUFAs and neurotransmitters

Table 2 illustrated that the intervention group had statistically significant increases in serum ALA (β , 1.620; 95 % *CI*, 0.967–2.265), DHA (β , 2.797; 95 % *CI*, 1.075–4.532), EPA (β , 1.472; 95 % *CI*, 0.296–2.643), acetylcholine (β , 0.441; 95 % *CI*, 0.415–0.468), GABA (β , 0.009; 95 % *CI*, 0.001–0.016) and 5-HT (β , 0.160; 95 % *CI*, 0.081–0.238) compared to the placebo group. The change of serum n-3 PUFAs and neurotransmitters is shown in Table S2 of supplement 2. In PP analysis, compared with placebo group, the intervention group had statistically significant increases in serum ALA (β , 1.577; 95 % *CI*, 1.006–2.142), DHA (β , 3.427; 95 % *CI*, 1.967–4.881), EPA (β , 2.050; 95 % *CI*, 1.101–2.998), acetylcholine (β , 0.447; 95 % *CI*, 0.422–0.471) and 5-HT (β , 0.170; 95 % *CI*, 0.096–0.242) after adjustment of potential confounders (Table S8 of supplement 2). The change of serum n-3 PUFAs and neurotransmitters is

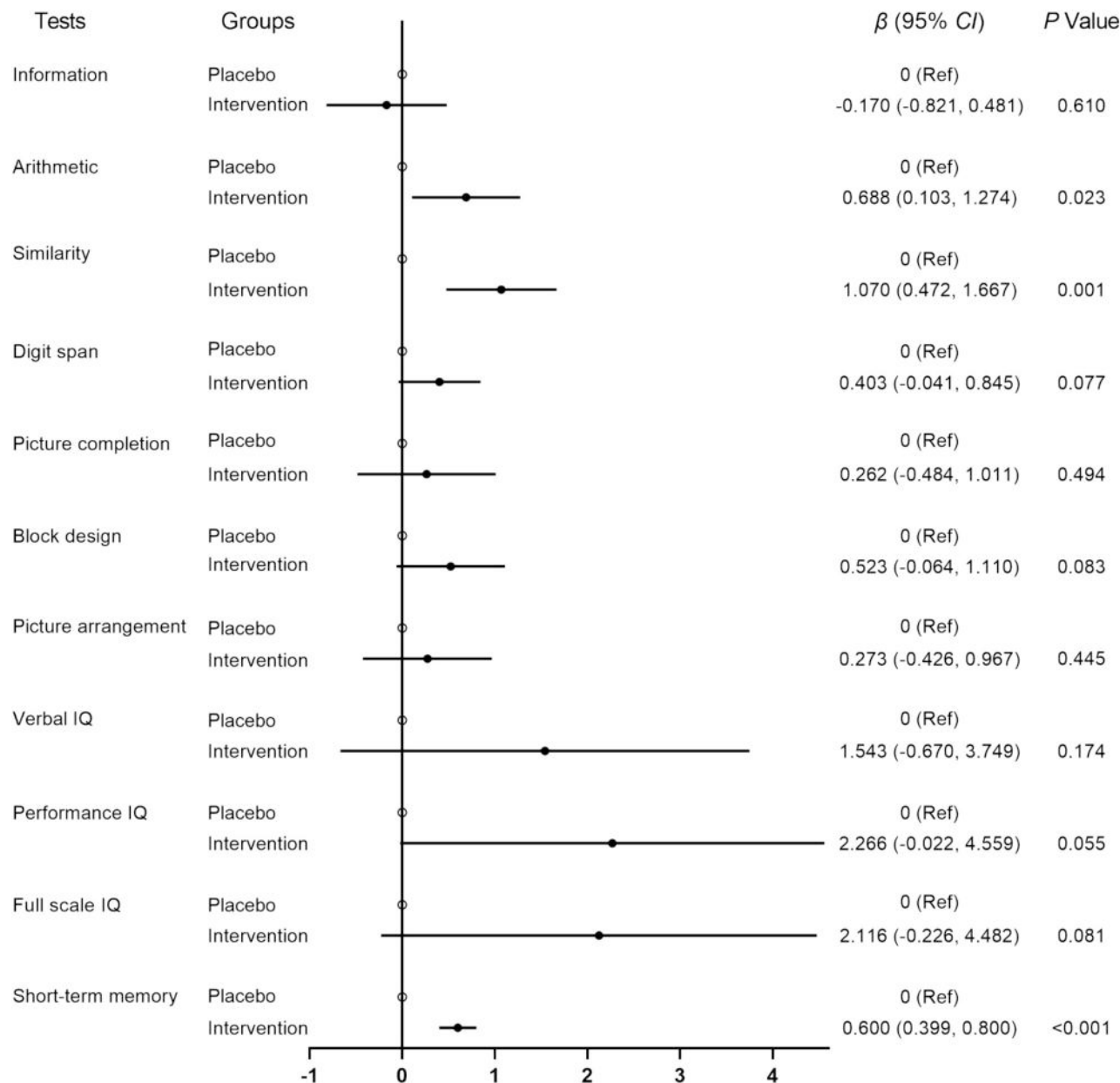


Fig. 2. Longitudinal analysis of intervention on cognitive function between two groups. Model adjusted for social demographics (age, sex, educational duration, living alone, BMI), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities), medical history (diabetes, hypertension, cardiovascular disease), nutritional supplements, MMSE score, ADL score, SAS score, SDS score, *APOE* ϵ 4 and *MTHFR* C677T genotype. IQ, Intelligence Quotient.

shown in Table S7 of supplement 2.
No significant difference of serum folate and homocysteine between two groups was found (Tables S2 and S7 of supplement 2).

3.4. Causal mediation analysis

Based on the results of intervention on cognitive function, serum n-3 PUFAs and neurotransmitters, we further analyzed the relationship between serum n-3 PUFAs, neurotransmitters and cognitive function as shown in Table S3 of supplement 2. Furthermore, we did causal mediation analysis to explore the potential mechanism. Table 3 shows that intervention may improve short-term memory by increasing serum ALA levels (average causal mediation effect = 0.132, 95 % CI, 0.053–0.225) with 19.7 % mediation proportion, but not via serum DHA levels (average causal mediation effect = -0.042, 95 % CI, -0.102–0.0003). In PP analysis, same results were found, intervention may improve short-

term memory by increasing serum ALA levels (average causal mediation effect = 0.086, 95 % CI, 0.022–0.167) with 11.6 % mediation proportion (Tables S9 and S10 of supplement 2).

3.5. Compliance and adverse events

Of the 177 participants who completed the trial, 7 participants discontinued in the placebo group of 7.4 % drop-out rate and 8 in the intervention group of 8.4 % drop-out rate, for an average drop-out rate <10 % over both groups. Besides, there were no serious adverse events in either group (Tables S4 and S11 of supplement 2).

4. Discussion

To our knowledge, this randomized clinical trial (RCT) is the first to discuss that a phosphatidylserine containing supplement improved

Table 2

Longitudinal analysis of intervention on serum n-3 PUFAs and neurotransmitters between two groups.

Indicators	Group	Model 1 β (95 % CI)	P value	Model 2 β (95 % CI)	P value
ALA	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	1.504 (0.657, 2.350)	0.0006	1.620 (0.967, 2.265)	<0.0001
DHA	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	2.969 (1.021, 4.917)	0.0032	2.797 (1.075, 4.532)	0.0018
EPA	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	1.320 (−0.035, 2.674)	0.0578	1.472 (0.296, 2.643)	0.0152
Acetylcholine	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	0.438 (0.410, 0.467)	<0.0001	0.441 (0.415, 0.468)	<0.0001
GABA	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	0.008 (−0.001, 0.016)	0.0681	0.009 (0.001, 0.016)	0.0287
Dopamine	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	−0.002 (−0.007, 0.003)	0.4815	−0.001 (−0.006, 0.004)	0.6503
5-HT	Placebo group (n = 95)	0 (Ref)		0 (Ref)	
	Intervention group (n = 95)	0.131 (0.031, 0.230)	0.0108	0.160 (0.081, 0.238)	<0.0001

Model 1 is univariate model. Model 2 adjusted for social demographics (age, sex, educational duration, living alone, BMI), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities), medical history (diabetes, hypertension, cardiovascular disease), nutritional supplements, MMSE score, ADL score, SAS score, SDS score, *APOE* ϵ 4 and *MTHFR* C677T genotype. β indicates the expected change in the dependent variable when the independent variable increases by one unit. PUFAs, polyunsaturated fatty acids; ALA, α -Linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GABA, γ -aminobutyric acid; 5-HT, 5-hydroxytryptamine.

cognitive function for older adults with MCI, although here the amount of Ginkgo extract constituents were far too low to be effective in any way. Thus, this trial only tests the therapeutic potential of the other components present in the supplement. Compared with the placebo group, 3 of the many cognitive scores of the intervention group were significantly higher at 12 months, and serum n-3 PUFAs and neurotransmitters were significantly increased as well. In addition, increased plasma A β 40, A β 42 and A β 42/A β 40 in the placebo group suggested that the supplement might delay A β deposition. Moreover, the improvement of short-term memory would partially be mediated by serum ALA.

Previously, n-3 PUFAs supplements have been reported positive cognitive outcomes of people with cognitive impairment (Johnson and Schaefer, 2006; Hooper et al., 2018; Andriambelo et al., 2023) and improved memory function in the hippocampus (Yamada et al., 2014; Nasehi et al., 2018). But most studies focused on DHA and EPA instead

of ALA. ALA is a precursor for the synthesis of long-chain n-3 PUFAs, it could be converted to EPA and DHA (Burdge and Calder, 2005). Studies indicated that ALA can pleiotropically activate transcriptional and translational programs in the brain to promote neuronal survival and improve cognitive function, but the optimal dose to exert these effects in humans requires further research (Pan et al., 2015; Piermartiri et al., 2015). A RCT of sixty healthy older adults with no cognitive impairment aged 65 to 80 years of 3.7 g/day of flaxseed oil containing 2.2 g ALA showed improved cognitive functions, specifically verbal fluency (Ogawa et al., 2023). In the present study, our results showed the improvement of logical reasoning, calculation, problem-solving skills, attention, abstract generalization and short-term memory after 12 months intervention. Besides, we found increased levels of serum ALA, DHA, EPA. In addition, we also found an improvement of short-term memory after 12 months intervention with ALA as a potential mediator. We hypothesize that this effect may be related to a decrease in relevant markers and the regulation of protein expression.

However, the combination of phosphatidylserine and *Ginkgo biloba* extract for cognitive effects remained controversial (Kennedy et al., 2007; Jackson et al., 2016). Interestingly, a supplement of PS, *Ginkgo biloba* leaves, vitamin E and pyridoxine could significantly improve memory in aged beagles (Araujo et al., 2008). Similarly, a RCT with multivitamin supplement of DHA, EPA, *Ginkgo biloba* extracts, PS, α -tocopherol, folic acid, and vitamin B₁₂ of postmenopausal women, results showed a significant improvement on cognition (Strike et al., 2016). Oral PS supplement was reported to improve cognitive function (Delwaide et al., 1986), and its combination of n-3 fatty acids showed favorable effects on memory in subjects with subjective memory disorders older adults (Richter et al., 2010; Vakhapova et al., 2010). Besides, intravenous injection of purified bovine brain PS for 8 days attenuated the reduction of acetylcholine release from the parietal cortex of aged rats, which may be caused by providing more choline for acetylcholine synthesis (Kim et al., 2014). Similarly, *Ginkgo biloba* extracts may also have indirect actions upon the prevention of serotonin receptor reduction, enhance the function of acetylcholine neurons and the release of GABA (Gold et al., 2002). Nevertheless, serum neurotransmitters were not statistically associated with cognitive function, and were not possible mediators of the intervention's improvement in cognitive function although increased levels of serum acetylcholine, GABA and 5-HT were found. This could be possibly caused by different transportation of circulating nutrients (substrates for synthesizing neurotransmitters) through blood-brain barrier (Pardridge, 1981). We believed that brain neurotransmitters could explain the results better than serum transmitters although clinical data was unavailable.

In addition, we didn't find statistical significance of serum folate and homocysteine between the two groups after intervention, which suggested that the dosage of Vitamin B₁, Vitamin B₆ and folic acid in the supplements do not affect the effect of the main functional components (ALA, PS and Ginkgo total flavonoids) on cognitive function. Although the sample size of this trial meets certain statistical efficacy, it's still lower than many other MCI RCTs. Besides, the insignificant results in several subtests could also suggest that our findings may have some limitations.

4.1. Strengths and limitations

This study has several strengths. First, our strict and standardized supervision had a high adherence rate and a low drop-out rate. Second, we adjusted possible confounding factors, which could reduce confounding bias to some extent. Third, we performed ITT and PP analyses, and all the analyses of the main results presented similar effects. Moreover, we used plasma A β 40, A β 42 and A β 42/A β 40 to objectively observe the cognitive changes besides the scale. However, there are limitations of this study that need to be pointed out. First, the study population was recruited from single center, which would limit the extent of extrapolation of these results. Second, we did not conduct a

Table 3
Mediating effects of serum n-3 PUFAs on relationship of supplements and cognitive function.

	β (95 % CI)	P value	Mediated proportion
Intervention → ALA → Short-term memory ^a			0.197 (0.090, 0.330)
ACME	0.132 (0.053, 0.225)	<0.0001	
ADE	0.530 (0.356, 0.709)	<0.0001	
Total effect	0.662 (0.474, 0.846)	<0.0001	
Intervention → DHA → Short-term memory ^b			−0.060 (−0.169, 0.0004)
ACME	−0.042 (−0.102, 0.0003)	0.054	
ADE	0.697 (0.505, 0.889)	<0.0001	
Total effect	0.655 (0.467, 0.838)	<0.0001	

β represents the strength of the mediating variable in the relationship between the independent and dependent variables. PUFAs, polyunsaturated fatty acids; ACME, average causal mediation effect; ADE, average direct effect. ALA, α -Linolenic acid; DHA, docosaheaxaenoic acid.

^a Model adjusted for social demographics (age, sex, educational duration, living alone, BMI), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities), medical history (diabetes, hypertension, cardiovascular disease), nutritional supplements, MMSE score, ADL score, SAS score, SDS score, *APOE* ϵ 4, *MTHFR* C677T genotype, baseline serum ALA and short-term memory scores.

^b Model adjusted for social demographics (age, sex, educational duration, living alone, BMI), lifestyles (smoking status, drinking alcohol, exercise, participation in leisure activities), medical history (diabetes, hypertension, cardiovascular disease), nutritional supplements, MMSE score, ADL score, SAS score, SDS score, *APOE* ϵ 4, *MTHFR* C677T genotype, baseline serum DHA and short-term memory scores.

dietary assessment due to implementability, although we adjusted for nutritional supplements included calcium, folate, Vitamin B and multi-vitamin, certain foods, such as nuts, seeds, vegetable oils could also introduce bias to the results. Third, despite adjusting for known potential factors, the possibility of unmeasured confounders remains. Finally, longer follow-up duration is needed since cognitive decline was a slow process.

5. Conclusions

In this trial of MCI patients, a phosphatidylserine containing supplement could improve their computational ability, logical reasoning ability, attention and short-term memory. The supplement could also increase serum ALA, DHA, EPA, acetylcholine, GABA, 5-HT levels. One potential mechanism of improving short-term memory was possibly via increasing serum ALA levels.

CRediT authorship contribution statement

Huilian Duan: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Ning Xu:** Investigation. **Tong Yang:** Investigation. **Moyan Wang:** Resources. **Chunlai Zhang:** Resources. **Jiangang Zhao:** Resources. **Zhenshu Li:** Methodology. **Yongjie Chen:** Methodology. **Jing Yan:** Methodology. **Meilin Zhang:** Methodology. **Wen Li:** Methodology. **Zhongbao Yue:** Project administration. **Fei Ma:** Methodology. **Ruikun He:** Supervision, Project administration, Funding acquisition, Conceptualization. **Guowei Huang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

Data availability statement

The raw data supporting the conclusions of this article will be available upon request from the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jad.2024.09.131>.

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