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Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline

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Abstract

Background: Docosahexaenoic acid (DHA) plays an important role in neural function. Decreases in plasma DHA are associated with cognitive decline in healthy elderly adults and in patients with Alzheimer's disease. Higher DHA intake is inversely correlated with relative risk of Alzheimer's disease. The potential benefits of DHA supplementation in age-related cognitive decline (ARCD) have not been fully examined.

Objective: Determine effects of DHA administration on improving cognitive functions in healthy older adults with ARCD.

Methods: Randomized, double-blind, placebo-controlled, clinical study was conducted at 19 U.S. clinical sites. A total of 485 healthy subjects, aged $\geq$55 with Mini-Mental State Examination $>$26 and a Logical Memory (Wechsler Memory Scale III) baseline score $\geq$1 standard deviation below younger adults, were randomly assigned to 900 mg/d of DHA orally or matching placebo for 24 weeks. The primary outcome was the CANTAB Paired Associate Learning (PAL), a visuospatial learning and episodic memory test.

Results: Intention-to-treat analysis demonstrated significantly fewer PAL six pattern errors with DHA versus placebo at 24 weeks (difference score, -1.63 ± 0.76 [-3.1, -0.14, 95% CI], $P = .03$). DHA supplementation was also associated with improved immediate and delayed Verbal Recognition Memory scores ($P < .02$), but not working memory or executive function tests. Plasma DHA levels doubled and correlated with improved PAL scores ($P < .02$) in the DHA group. DHA was well tolerated with no reported treatment-related serious adverse events.

Conclusions: Twenty-four week supplementation with 900 mg/d DHA improved learning and memory function in ARCD and is a beneficial supplement that supports cognitive health with aging.

Trial Registration: Clinicaltrials.gov, Identifier: NCT0027813.

Keywords: cognition; memory; learning; aging; omega-3 fatty acid; docosahexaenoic acid; clinical trial; nutrition

Conflicts of Interest Disclosure: With the exception of Drs. Dror Rom, Andrew Blackwell, and Mary Stedman, the other authors are employed by Martek Biosciences Corporation. Dr. Rom and Dr. Blackwell were consultants to the study. The participating clinical investigators are listed as a group in the acknowledgement section of the manuscript.

Contributors: Blackwell, Nelson, Rom, Yurko-Mauro contributed to the study concept and design. Blackwell, McCarthy, Nelson, Rom, Yurko-Mauro and Stedman participated in study conduct and collection of the data. Rom had responsibility for the statistical analysis. Blackwell, Nelson, Rom, Ryan, Salem, Yurko-Mauro contributed to the interpretation of data. Blackwell, Ryan, Yurko-Mauro contributed to the drafting of the manuscript and McCarthy, Nelson, Rom, Ryan, Salem, Stedman and Yurko-Mauro participated in the revision of the manuscript. The corresponding author had full access to all data in the study after study unblinding, and had final responsibility for the writing of the report and the decision to submit for publication. The study was funded by Martek Biosciences Corporation.

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1. Introduction

A decline in memory and cognitive function is considered to be a normal consequence of aging. Memory loss is a prominent health concern, second only to heart disease for older individuals [1]. Prevalence estimates indicate that as many as 5.4 million older Americans (22.2%) have cognitive impairment without dementia. Approximately 12% of these elders will develop dementia annually [2]. Docosahexaenoic acid (DHA) is the principle long-chain polyunsaturated fatty acid (LCPUFA) in brain. Several epidemiological studies associate decreases in plasma DHA with cognitive decline in healthy elderly people [3,4] and in patients with Alzheimer's disease (AD) [5,6]. Populations with high dietary intake of DHA [7–9] and greater plasma DHA levels [4,6] have a lower risk of cognitive impairment or AD.

As an integral component of neural membrane phospholipid, DHA constitutes 30%–40% of LCPUFAs in grey matter cerebral cortex [10]. DHA is involved in multiple brain functions including cell membrane fluidity, receptor affinity, and modulation of signal transduction molecules [11]. In preclinical studies, DHA supplementation restored brain DHA levels and long-term potentiation [12], improved cerebral blood flow [13], and enhanced learning and memory tasks in aged animals [14]. DHA also reduced beta amyloid, plaque burden, and tau protein in transgenic AD models [15,16].

Clinical trials with LCPUFAs from fish oil (containing a mixture of eicosapentaenoic acid (EPA) and DHA) in healthy older adults or individuals with mild cognitive impairment (MCI) or AD have been conducted. No studies with DHA alone have investigated mild, age-associated cognitive changes (i.e., age-related cognitive decline, ARCD). A recent study of 302 elders with Mini-Mental State Examination (MMSE) scores >21, supplemented for 6 months with 400 or 1800 mg/d of DHA + EPA versus placebo, showed no significant changes on cognitive tests [17]. A small pilot study showed significant improvements in the Alzheimer Disease Assessment Scale cognitive subscale (ADAS-cog) in subjects with MCI but not in AD patients given 1.8 g/d DHA + EPA for 6 months versus placebo [18]. A trial of 204 mild to moderate AD patients showed no delay in rate of decline on ADAS-cog with DHA + EPA (2.3 g/d) administration [19]. However, in a sub-group of individuals with MMSE scores >27, there was a significant decrease in the MMSE rate of decline after 6 and 12 months supplementation. These results suggest that older adults with mild cognitive deficits may benefit the most from LCPUFAs.

We examined the potential benefits of 900 mg/d DHA on cognitive changes in individuals with ARCD in this double-blind, randomized, placebo-controlled, multi-center clinical trial, using the Cambridge Neuropsychological Test Automated Battery, CANTAB. The CANTAB cognitive battery is a validated, reliable neuropsychological battery [20], which consists of memory, learning, attention, problem-solving, and executive function tests [21]. Its measures of visuospatial associative learning demonstrate specificity and sensitivity in detecting isolated memory impairments in healthy older adults [22,23]. It was hypothesized that 24 weeks of DHA supplementation would improve cognitive function as assessed by the CANTAB Paired Associate Learning (PAL) test, a learning and episodic memory test. The PAL test was chosen as the primary endpoint because it uses mnemonic processes of the medial temporal lobe, a region where some of the earliest cognitive and neuronal dysfunctions are detected during aging and in pre-dementia conditions [24,25]. Collectively, these studies demonstrate PAL's sensitivity to early visuospatial learning and memory changes which may be affected by DHA.

2. Methods

2.1. Participants

A total of 485 male or female subjects, aged ≥55 years with a subjective memory complaint and who met criteria for ARCD were enrolled at 19 sites in the United States. The Diagnostics and Statistical Manual (DSM IV) defines ARCD as an ''objectively identified decline in cognitive functioning consequent to the aging process that is within normal limits given a person's age. Individuals may report problems remembering names or appointments or may experience difficulty solving complex problems.'' [26] A similar term, ''age-associated memory impairment'' has been used to describe specifically age-related memory loss [27]. We chose the DSM IV term ''age-related cognitive decline'' because of its acceptability as a standard, codified, diagnostic classification and broader definition encompassing cognitive function.

The Logical Memory sub-test of the Wechsler Memory Scale (WMS version III, 1997) was used to identify objectively individuals with a decline in cognitive function who had a baseline raw (immediate or delayed recall) score ≥1 standard deviation (SD) below the mean of younger adults (reference ages, 25–35 years). The cut-offs used for inclusion in the study were ≤28 for the Logical Memory immediate recall or ≤15 for the delayed recall score. Subjects were excluded if they had an MMSE score <26. To minimize the potential confounding effect of high DHA consumption before study entry, subjects who consumed >200 mg/d DHA in 2 months before randomization (determined by a DHA food frequency questionnaire [FFQ]) [28], or consumed omega-3 containing supplements, or used medications for AD, major antipsychotics, or anti-depressants were excluded from the study. A history or presence of major medical conditions (including a diagnosis of dementia or a Geriatric Depression score >5), and current or past alcohol or drug abuse also excluded subjects from eligibility. Institutional review board (New England IRB) approval was obtained, and all subjects provided written informed consent.

2.2. Procedures

The study consisted of a screening visit, followed 1 week later by a baseline visit, and three follow-up visits. Safety and

compliance measures were assessed at every visit. Efficacy assessments were obtained at baseline, week 12, and week 24. Eligible subjects were stratified by age (55–69; ≥70) and randomized 1:1 in blocks of four to active or placebo by site, using a centralized interactive voice randomization system (Fisher Clinical, FACTS services, Allentown, PA).

Subjects allocated to active treatment received 900 mg/d DHA, provided as (3) soft-gelatin capsules, each containing 300 mg DHA from algal triglyceride oil (DHA-S single cell oil from *Schizochytrium sp*, containing 40% DHA, ≤1% EPA, 15% docosapentaenoic acid [DPAn-6], plus antioxidants: 320 μg ascorbyl palmitate [8 IU ascorbic acid], 1.6 mg mixed tocopherols [7 IU d-α-tocopherol], and 2000 ppm rosemary extract). The 900 mg dose was chosen because cumulative cross-study dose response data demonstrated doubling in plasma DHA levels [29] and positive changes in cardiovascular lipid profiles [30]. DHA-S oil is a nutritional food ingredient that is Generally Recognized as Safe, GRAS Review Notification (GRN 137) [31], and manufactured under food Good Manufacturing Practice conditions. Placebo capsules were identical in size and appearance and consisted of 50% corn oil/50% soy oil, and the same antioxidant mixture. All capsules were orange-flavored and orange color to protect the study blind. Subjects were instructed to take capsules with food at the same time each day (e.g., 1 capsule/meal), starting at the baseline visit, and to not alter their normal diet during the study. The DHA FFQ was administered to assess ongoing dietary intake of LCPUFA and subject compliance. The primary measure of compliance was the week 24 change from baseline plasma phospholipid DHA level. A change greater than 1.5 wt% (based on historical dose response plasma DHA levels) was considered compliant for the DHA group. Capsule counts were conducted at each site visit and served as a secondary measure of compliance.

2.3. Outcome measures

The primary outcome was a week 24 change from baseline in the CANTAB PAL, a visuospatial learning and episodic memory test [22]. The test battery is computer based using a touchtone screen. A pre-baseline training session with CANTAB was conducted at screening to familiarize the subject with the computer battery and to minimize learning effects at subsequent test sessions. These data were not analyzed. The order of test sequence remained constant across all test sessions. Parallel versions of most tests were used at subsequent test sessions to minimize any potential ceiling effects. The following test variants were used: Pre-baseline Parallel version 1; Baseline = Parallel Version 2, Week 12 = Parallel Version 3, Week 24 = Parallel Version 4. All CANTAB data were collected electronically, processed, and validated by Cambridge Cognition Ltd. Final data sets were transferred to Prosoft Software, Inc. (Wayne, PA) for statistical analysis. The Wechsler Logical Memory test, used in screening, was not chosen as an outcome measure because it is not available in a computerized format and does not have parallel versions. These criteria are especially desirable in a large, multicenter clinical trial because they minimize potential bias and variability.

Secondary outcome measures included CANTAB Pattern Recognition Memory (PRM), a test of visual pattern recognition administered as a 2-choice forced discrimination series; CANTAB Verbal Recognition Memory (VRM), a test of immediate and delayed verbal memory; CANTAB Stockings of Cambridge (SOC), a test of executive function; and CANTAB Spatial Working Memory (SWM), a test of temporary spatial retention and search strategy. Other secondary measures included self-assessment tests of memory (Frequency of Forgetting-10 scale [32]) and Alzheimer's Disease Cooperative Study-Activities of Daily Living Prevention Instrument (ADCS-ADL PI scale) [33], MMSE [34], and the Geriatric Depression scale [35].

Safety assessments included adverse event monitoring, changes in vital signs, and physical examinations. Chemistry, hematology, and urinalysis tests were also conducted. Nonfasting blood samples were collected at baseline and at week 24. Plasma phospholipid fatty acids were analyzed as described previously [36]. APOE genotyping was not done.

2.4. Statistical analysis

The primary efficacy analysis was determined a priori and based on a linear univariate model of the change from baseline in the PAL 6 pattern error score at 24 weeks using treatment, site, age group (55–69 and ≥70 years), and education as factors, and the baseline PAL score as a covariate. The calculated effect size for the PAL was 0.19. The study was not designed to look at a rate of change in cognition over time. The primary efficacy analysis was tested in the intention-to-treat (ITT) population defined as all randomized subjects who received study treatment and had baseline evaluations. Planned per protocol analyses were also conducted. Levene's test examined homogeneity of variance across groups. All efficacy analyses used the ''Last Observation Carried Forward'' (LOCF) approach for handling missing data. Planned secondary efficacy analyses followed the method of the primary analysis. Safety analyses also used an analysis of covariance model or Fisher's Exact Test to assess treatment differences. A 2-fold increase in plasma phospholipid DHA levels was expected with 900 mg/d, based on previous dose response data [29].

A preplanned interim analysis (IA) for futility was conducted by an unblinded statistician not associated with data collection after 140 subjects completed the study. The objective of the early IA was to allow us to terminate the study if no efficacy was demonstrated. Preplanned conditional power calculations revealed a conditional power of 30% and indicated sample size adjustment based on imputed PAL error adjustments which overestimated total errors and increased variability. The planned interim look at a co-primary endpoint, PRM did not meet the conditional power threshold of 20%–30% and was thus specified as a secondary endpoint

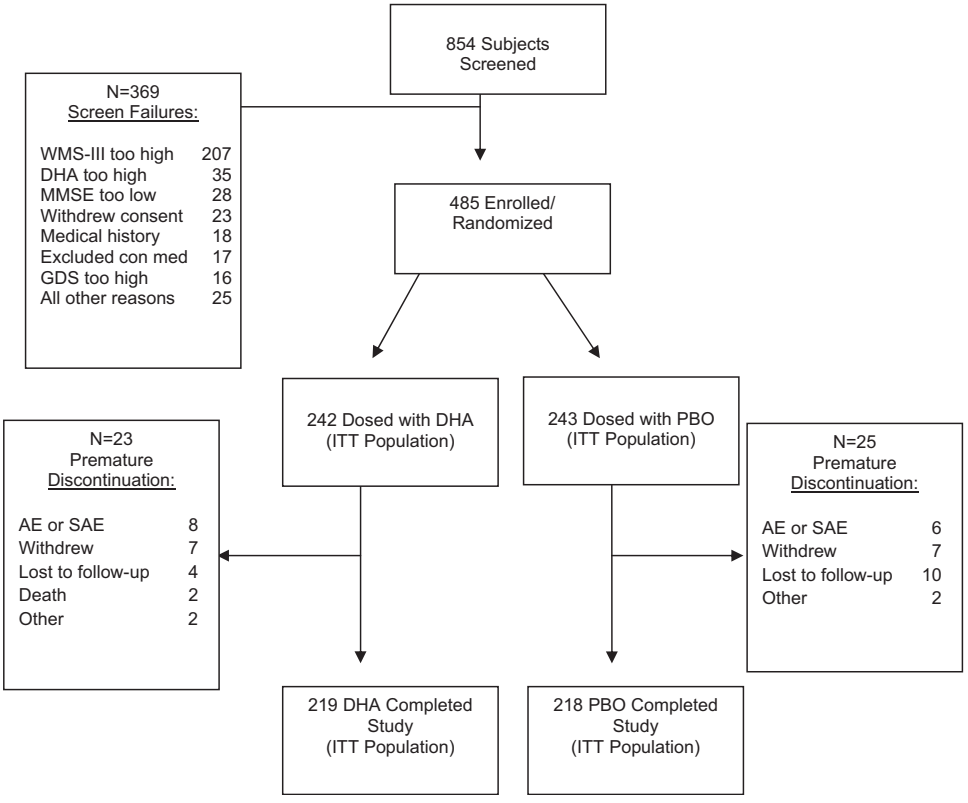

Fig. 1. Participant flow. Abbreviations: WMS, Wechsler Memory Scale; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale.

in the final analysis plan. For 80% (conditional) power, using the PAL 6 pattern error score (two-sided $P < .05$), a sample size of 325 subjects, post-IA was needed with 10% drop out rate calculated. All analyses were performed using SAS software, version 8.2.

3. Results

A total of 854 subjects were screened for the study, 369 subjects failed screening, and 485 subjects met study criteria and were randomized to DHA (242 subjects, ITT) or placebo (243 subjects) (Fig. 1). Of the subjects who failed screening, 56% did not meet the Logical Memory test criteria for ARCD. Only 9% of screen failures had elevated, disqualifying baseline DHA intake levels. All subjects received the correct treatment to which they were randomized. An overall study completion rate of 90% was achieved (219 subjects, DHA; 218 subjects, placebo). Baseline characteristics, including age, gender, race, education, and medication use (Table 1), showed no significant differences between groups.

3.1. Primary outcome

After 24 weeks, individuals in the DHA group had significantly fewer PAL 6 pattern errors (ITT difference score, -1.63 ± 0.76 (-3.1, -0.14, 95% CI), $P = .032$) compared with the placebo group (Table 2). There were no significant differences between groups on the PAL and other CANTAB tests at week 12 (data not shown). The per protocol ($P = .025$) analyses resulted in similar significant findings for PAL. The PAL data demonstrated homogeneity of variance across groups (Levene's test, $P = .58$), post hoc analysis of the univariate model without the cofactors age and education also showed a significant DHA response ($P = .029$), and an analysis using observed cases only displayed the same directional trend ($P = .067$).

The Logical Memory delayed recall score, as a covariate, was highly predictive of the change in PAL errors within the DHA group at week 24 ($r = -.36$, $P < .001$). Thus, worse baseline delayed recall scores were associated with greater improvement in PAL scores with DHA. Adjusting for the delayed recall score strengthened the DHA treatment effect (diff score, -1.73 ± 0.77, $P = .026$). Family history of dementia ($P = .054$) and concomitant statin medication use ($P = .049$, per protocol) were also predictive of the change in PAL within the DHA group. Other covariates (education, site, and age group) were not significant, although the older stratified group (age, ≥ 70) made more PAL errors than the younger group (55–69 years). The PAL treatment response was not analyzed by gender.

CANTAB has collected age-associated PAL normative cross-sectional data for individuals 55–93 years of age from several studies (*www.camcog.com*). Using these data as a frame of reference, at baseline, the PAL error performance data for the DHA group corresponded to a cognitive age of 72.6 years. After 24 weeks of supplementation, the

Table 1
Baseline characteristics

Parameter	DHA (n = 242)	Placebo (n = 243)	*P* value*
Gender (%)	44 M; 56 F	40 M; 60 F	.42
Age (±SD)	70 (9.3)	70 (8.7)	.98
Education years (±SD)	14.5 (2.5)	14.7 (2.6)	.80
Race (%)			.88
African-American	7	7	
Asian	.8	1.2	
White, non-Hispanic	85	83	
White, Hispanic	5	7	
Native American	1.2	0.4	
Baseline DHA intake mg/d (±SD)	103.5 (53.6)	104.7 (49.4)	nd
Alcohol consumption U/wk (±SD)	2.18 (3.4)	2.41 (3.7)	.37
Family history of dementia n (%)	83 (34)	93 (38)	.60
Logical memory, immediate recall mean (±SD)	25 (6.8)	25.1 (6.9)	.82
Logical memory, delayed recall mean (±SD)	11.3 (4.1)	11.2 (4.1)	.51
Statin use (%)	36	37	nd
Lipophilic statins	95	86	
Antihypertensive use (%)			nd
Diuretics	20	24	
Ace-inhibitors	15	14	
Ca^{++} channel blockers	9	9	
β-blockers	5	3	

Abbreviation: nd, not done.

*Based on an ANOVA model with effects treatment and pooled site for continuous parameters; or for categorical parameters, a Cochran-Mantel-Haenszel test for associations adjusting for pooled site.

cognitive age represented by the PAL scores was 65.6 years of age, indicating a 7 year improvement in PAL scores. In comparison, the PAL error performance scores for the placebo group at baseline corresponded to 70.6 years and 66.9 years at week 24, only a 3.6 year improvement. The CANTAB normative data provide, for illustrative purposes, a frame of reference comparing the magnitude of the changes in performance observed with DHA to the changes in performance that one could expect to see over time in the course of normal aging. When interpreting the positive cognitive effects associated with DHA, it should be kept in mind that the cited CANTAB norms are based on age-stratified, cross-sectional rather than longitudinal data.

3.2. Secondary outcomes

Additional CANTAB tests and other cognitive measures are also shown in Table 2. CANTAB VRM test showed significant week 24 change from baseline total immediate and delayed responses with DHA supplementation versus placebo. The baseline VRM delayed score was highly predictive and inversely associated with DHA treatment response ($r = -.52$, $P < .001$). At week 24, compared to baseline, PRM and SWM tests were not significantly different between groups. On CANTAB SOC, the placebo group demonstrated small, significant week 24 differences in the number of problems solved.

There were no significant differences in change from baseline scores on the MMSE, the Geriatric Depression scale (Table 2), or Frequency of Forgetting scale (mean change from baseline, DHA = 1.6 ± .5; placebo = 2.8 ± 0.5, $P < .12$), and the ADCS-ADL PI scale (mean change from baseline, DHA = −2 ± 0.3; placebo = −1.7 ± 0.3, $P < .59$). Dietary intake of omega-3 fatty acids, determined by the DHA FFQ, showed no differences between groups at any time point (mean baseline DHA intake = 104 mg/d vs. mean week 24 intake = 112 mg/d). This dietary intake corresponds with the average American DHA intake: 110 mg/d for males; 80 mg/d for females, aged 60–69 years [37]. As expected, plasma phospholipid DHA levels significantly increased by 3.2 wt% in the DHA group by week 24. Changes in plasma phospholipid fatty acids are shown in Table 3 and correspond to the known alterations in fatty acids with DHA supplementation [29]. Week 24 log plasma DHA levels were significantly correlated with the change from baseline PAL response ($r = -.11$, $P = .024$) (Fig. 2). Compliance, measured by plasma DHA levels, was >82% in the DHA group and >99% in the placebo group. As a secondary measure, capsule counts demonstrated >91% compliance.

There were no significant differences between groups on hematology and clinical chemistry measures, including hemoglobin/hematocrit, white blood cell count, total cholesterol, glucose, hs-crp, and liver transaminases. Alkaline phosphatase showed a nonclinically significant mean decrease of 3.6 IU from baseline with DHA versus placebo ($P < .001$), although both groups were in the normal range, 73 and 75 IU, respectively at week 24. There were no significant differences in systolic or diastolic blood pressure with DHA administration; however, a significant decrease in heart rate was detected in the DHA group at week 24 compared to baseline (−3.2 ± .59 bpm vs. −1 ± 0.61 bpm placebo, $P < .03$). The prevalence of cardiovascular disease in the study sample was 68%, consistent with the general population of this age group, although a slightly lower incidence of hypertension (43%) was found in our sample compared with 65–74-year-old individuals (67%) who were included in National Health and Nutrition Examination survey (NHANES) [38]. As reported in Table 1, 36% of the sample were taking statins, 50% were taking anti-hypertensive medications, and 41% took multivitamins or aspirin (37%). Except for statin use, tests for drug interactions of concomitant medications with DHA were not conducted.

The number of treatment-emergent adverse events were reported and the number of subjects reporting those events was similar across groups (45% DHA; 44.9% placebo). Twenty-one serious adverse events (SAEs) in 14 subjects (3%) were reported (13 SAEs/7 DHA subjects; 8 SAEs/7 placebo subjects). No SAEs were considered by investigators as treatment-related events. No significant difference in the incidence of treatment-emergent adverse events or SAEs was observed between groups (Tables 4 and 5).

Table 2
Cognitive and functional tests

Cognitive or functional measure	Baseline score, mean (SD)	Week 24 score, mean (SD)	Week 24 change from baseline, mean (SE)	Between group difference score (SE)*	*P* value†
CANTAB PAL (6 pattern stage errors)					
900 mg DHA (n = 241)	13.4 (11.6)	8.8 (9.9)	−4.5 (0.64)	−1.63 (0.76)	.032‡
Placebo (n = 242)	12.1 (10.9)	9.7 (10.4)	−2.4 (0.62)		
VRM free recall, total correct					
900 mg DHA	5.7 (1.9)	5.8 (2.1)	0.1 (0.13)	0.1 (0.23)	.791
Placebo	5.8 (1.9)	5.8 (2.1)	0 (0.13)		
VRM, immediate, total correct					
900 mg DHA	10.8 (1.5)	11.0 (1.4)	0.2 (0.11)	0.4 (0.17)	.018‡
Placebo	10.9 (1.5)	10.9 (1.4)	0.0 (0.11)		
VRM, delayed, total correct					
900 mg DHA	10.4 (1.8)	10.7 (1.5)	0.3 (0.11)	0.5 (0.18)	.012‡
Placebo	10.5 (1.8)	10.7 (1.8)	0.1 (0.11)		
PRM, delayed, number correct					
900 mg DHA	9.5 (1.6)	8.6 (2.0)	−0.9 (0.13)	−0.1 (0.16)	.573
Placebo	9.7 (1.5)	8.8 (1.8)	−0.9 (0.12)		
SOC, problems solved					
900 mg DHA	3.5 (1.2)	3.5 (1.3)	0.1 (0.09)	−0.23 (0.11)	.045‡
Placebo	3.5 (1.4)	3.7 (1.3)	0.2 (0.10)		
SWM, between errors					
900 mg DHA	20.3 (9.1)	20.5 (9.3)	0.2 (0.54)	1.8 (0.99)	.066
Placebo	20.3 (10.8)	19.3 (10.4)	−0.9 (0.61)		
MMSE					
900 mg DHA	28.3 (1.3)	28.0 (1.9)	−0.4 (0.12)	0 (0.15)	.866
Placebo	28.2 (1.3)	27.9 (1.9)	−0.3 (0.11)		
Geriatric depression					
900 mg DHA	1.3 (1.2)	1.4 (1.6)	0.1 (0.10)	0.1 (0.12)	.230
Placebo	1.3 (1.3)	1.3 (1.5)	0.0 (0.08)		

Abbreviations: PAL, Paired Associate Learning; VRM, Verbal Recognition Memory; PRM, Pattern Recognition Memory; SOC, Stockings of Cambridge; SWM, Spatial Working Memory; MMSE, Mini Mental State Examination.

*Model-adjusted difference score.

†For the ITT population, based on an ANCOVA model with effects treatment, pooled site, age group, education level, baseline parameter score, and treatment by pooled site interaction, if significant.

‡$P < .05$.

4. Discussion

This clinical study demonstrated that 900 mg/d of DHA supplementation improved episodic memory and learning in healthy, older adults with mild memory complaints. Over 24 weeks, compared with placebo, DHA supplementation produced a significant 2-fold reduction in the number of visuospatial learning and episodic memory errors on the CANTAB PAL 6 pattern test and significant increases in VRM. Cognitive changes were significantly correlated with week 24 log plasma DHA levels. The DHA effects are significant in that they represent an objective demonstration of improved memory in ARCD. Clinically, compared to age-associated normative CANTAB data as a point of reference, DHA supplementation yielded a 7-year improvement in PAL test performance versus a 3.6-year improvement with placebo. The placebo response was likely due to a small retest effect which is common with cognitive tests such as the PAL [39]. Mean PAL errors at baseline corresponded to a cognitive age of 72.6 years, and following 24 weeks of DHA supplementation, a cognitive age of 65.6 years. A 3.4 year *net* improvement in learning and memory function with DHA is likely beneficial to aging adults with mild memory complaints.

As described in reviews that consider various tools used for cognitive testing, the CANTAB PAL test appears to be a well-characterized episodic memory test that depends on mnemonic processes of the medial temporal lobe [22,40]. Studies have demonstrated that the PAL seems to discriminate well between healthy controls, mild cognitively impaired

Table 3
Plasma phospholipid fatty acids

Fatty acid	900 mg/d DHA (n = 209) change from baseline*	Placebo (n = 212) change from baseline*	*P* value†
DHA	3.2	−0.08	.001
ARA	−1.4	−0.12	.001
EPA	0.16	−0.06	.001
DPA n-6	0.38	−0.004	.001

Abbreviations: DHA, docosahexaenoic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPAn-6, docosapentaenoic acid.

*Weight % of total fatty acids.

†Based on ANCOVA model with effects treatment, pooled site, age group, education level, baseline parameter, concomitant statin use (if significant), and treatment by pooled site interaction, if significant.

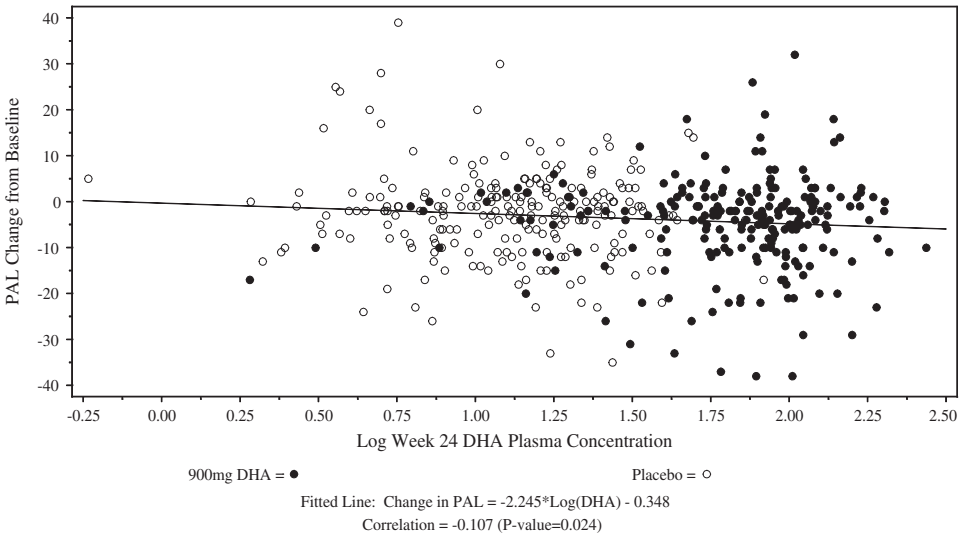

Fig. 2. Correlation of PAL change from baseline errors with plasma DHA levels. Scatterplot of the change from baseline in PAL errors (6 shapes) and the log week 24 DHA plasma levels (wt%) in ITT, LOCF population. Fitted linear regression line: change in PAL = −2.245*log (DHA)–0.348; $r = -.107$, $P = .024$. DHA = •, Placebo = O.

individuals, and AD patients [23,25,41]. The number of baseline PAL errors observed in our study are in line with previous trials that have also identified individuals with mild memory loss and illustrate mean scores that lie between those with normal cognition and MCI or ''questionable dementia'' [22]. The current results suggest that DHA supplementation may ameliorate early memory and learning deficits associated with cognitive aging [40].

The results also indicate that subjects in the DHA group who had lower baseline delayed recall Logical Memory scores showed greater improvement on the PAL. This finding supports the positive episodic memory effects resulting from DHA supplementation. A lower cut-off for the Logical Memory test was not established at entry. Thus, we cannot rule out the possibility that a few of these subjects with the lowest baseline scores would meet criteria for MCI. Within the DHA group, other cofactors, such as family history of dementia, and concomitant statin use were also associated with better performance on the PAL. This finding suggests that potential genetic and cardiovascular factors may influence the effects of DHA on cognition. Approximately 36% of the sample had a family history of dementia. APOE4 genotyping, as a risk factor for dementia, was not conducted and thus remains an interesting factor to further explore with DHA supplementation.

Cardiovascular disease, including hypertension, is considered by some to be a potential risk factor for cognitive disorders such as dementia [42]. Within our study population, 68% had a history of cardiovascular disease, 36% were taking statins, and 50% were on anti-hypertensives, suggesting comorbidity of cognitive and cardiovascular problems in aging individuals which may be ameliorated with additional DHA supplementation. It is noteworthy that there was a significant decrease in heart rate associated with DHA supplementation which may help reduce the risk of fatal cardiovascular events in this age group [43].

DHA supplementation did not produce changes in working memory (SWM) and executive function (SOC), cognitive

Table 4
Incidence of treatment-emergent adverse events

	DHA (n = 242)	Placebo (n = 243)	*P* value[†]
AEs by SOC*	n = 109	n = 109	
Number of subjects with AEs	n (%)	n (%)	
Infections/infestations	32 (13.2)	41 (16.9)	.310
Gastrointestinal disorders	30 (12.4)	41 (16.9)	.199
Musculoskeletal/connective tissue disorders	17 (7.0)	14 (5.8)	.584
Nervous system disorders	16 (6.6)	10 (4.1)	.234
Skin/subcutaneous tissue disorders	12 (5.0)	8 (3.3)	.373

*Adverse Events or Serious Adverse Events by MedDRA System Organ Class occurring in 5% or greater of subjects in either group.
[†]Fisher's Exact Test.

Table 5
Incidence of treatment-emergent serious adverse events

	DHA	Placebo
SAEs by SOC*	n = 7	n = 7
Number of subjects with SAEs[†,‡]	n	n
Infections/infestations	2	3
Musculoskeletal	2	0
Gastrointestinal	1	1
Nervous system	0	1

*Adverse Events or Serious Adverse Events by MedDRA System Organ Class occurring in 5% or greater of subjects in either group.
[†]Some subjects had multiple SAEs.
[‡]Two deaths unrelated to product: (1) congestive heart failure; (1) chronic obstructive pulmonary disease.

functions that are typically impaired in multidomain MCI and later stages of AD. Similar findings were also shown in a study of ''robust'' versus ''non-robust memory'' participants [22]. It is possible that significant changes in these other cognitive domains would be seen with more severe cognitive impairment or longer DHA supplementation, but this awaits confirmation. The MMSE and Geriatric Depression scale were unchanged in both groups over the 24-week period. Self-assessment tests of memory and daily living skills showed trends of improvement over time but no differential effects with DHA. This is likely due to the mild cognitive deficits of the study sample that typically show no functional activity impairment. The 900 mg/d dose of DHA doubled plasma DHA levels as expected and was well tolerated with good compliance.

DHA plays an essential role in neuronal development and in multiple brain functions. Previous clinical studies with LCPUFAs have demonstrated small but significant benefits in patients with MCI or mild AD. However, no benefits were demonstrated in a recent study of cognitively healthy elders [17]. In this study, the mean MMSE score was 28, yet the range of impairment was wide (MMSE scores, 23–30) which likely contributed to greater variability in cognitive responses among treatment groups. Recruited subjects had an average LCPUFA intake of ~300 mg/d, higher than the average U.S. intake (~100 mg/d) [37]. Thus, higher baseline intake status may have reduced the ability to identify cognitive improvements. This study also found significant ceiling effects with the cognitive tests administered, making it difficult to detect an omega-3 benefit. Differences in study design may have accounted for our dissimilar positive results.

Our results are the first to clinically confirm that DHA significantly improves episodic memory and learning functions in healthy adults with ARCD. The magnitude of the improvement in episodic memory may appear to be moderate. However, considering the duration of treatment (24 weeks) and the fact that healthy older adults with mild memory loss were considered the findings reported herein are important. Some studies have also shown that changes in episodic memory can be determined by the PAL test, and such changes are predictive of pre-clinical AD [39,40]. The positive findings here indicate that 900 mg/d of DHA may serve as a nutritional neuroprotective agent in improving some very early cognitive deficits. Such cognitive changes likely occur as a consequence of normal aging or may be observed before a diagnosis of MCI or mild AD. The present study was not designed to assess long-term effects of DHA on cognitive decline rates or conversion rates to MCI or mild AD. On the basis of epidemiological and clinical data to date, DHA is potentially beneficial for prevention of cognitive decline but will need confirmation with long-term prevention trials (*www.clinicaltrials.gov*). Our study results demonstrate that DHA is well tolerated and may have a significant positive effect on gradual memory loss, which is a major health concern of older individuals.

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