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Impact of Omega-3 Fatty Acid Supplementation on Memory Functions in Healthy Older Adults

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Abstract. As the process of Alzheimer's disease (AD) begins years before disease onset, searching for prevention strategies is of major medical and economic importance. Nutritional supplementation with long-chain polyunsaturated omega-3 fatty acids (LC-n3-FA) may exert beneficial effects on brain structure and function. However, experimental evidence in older adults without clinical dementia is inconsistent, possibly due to low sensitivity of previously employed test batteries for detecting subtle improvements in cognition in healthy individuals. Here we used LOCATO, recently described as a robust and sensitive tool for assessing object-location memory (OLM) in older adults, to evaluate the impact of LC-n3-FA supplementation on learning and memory formation. In a double-blind placebo-controlled proof-of-concept study, 44 (20 female) cognitively healthy individuals aged 50–75 years received either LC-n3-FA (2,200 mg/day, $n = 22$) or placebo ($n = 22$) for 26 weeks. Before and after intervention, memory performance in the OLM-task (primary) was tested. As secondary outcome parameters, performance in Rey Auditory Verbal Learning Test (AVLT), dietary habits, omega-3-index, and other blood-derived parameters were assessed. Omega-3 index increased significantly in the LC-n3-FA group compared with the placebo group. Moreover, recall of object locations was significantly better after LC-n3-FA supplementation compared with placebo. Performance in the AVLT was not significantly affected by LC-n3-FA. This double-blind placebo-controlled proof-of-concept study provides further experimental evidence that LC-n3-FA exert positive effects on memory functions in healthy older adults. Our findings suggest novel strategies to maintain cognitive functions into old age.

Keywords: Cognitive aging, dietary prevention, docosahexaenoic acid, eicosapentaenoic acid, fish oil

INTRODUCTION

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease [1]. As the process of AD begins years, if not decades, before the diagnosis of clinical dementia [2], efforts have been directed to search for early preventative strategies to

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lower AD risk [3, 4]. However, long-term preventive treatments in healthy older adults must be well tolerated, easy-to-implement, low-cost, and safe for long-term use. Nutritional intervention via dietary supplements is well suited to meet these requirements. Specifically, the beneficial role of long-chain omega-3 polyunsaturated fatty acids (LC-n3-FA), primarily eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) derived from fish oil, on brain function is the focus of intense current research [5, 6].

The healthy brain is rich in FA, and DHA is the most abundant LC-n3-FA in the brain [7]. Diverse mechanisms underlying cognitive benefits of LC-n3-FA administration have been suggested and described in detail elsewhere (e.g., [4–6, 8]). In short, mechanisms comprising neuroprotective actions such as neurogenesis, neural membrane plasticity, synaptogenesis, anti-inflammatory, antithrombotic, and vasodilatory effects, as well as regulations in transport and uptake of glucose may impact cognitive performance via improved signal transduction, neurotransmission, increased cell survival, and enhanced cerebral blood flow [9–11].

Among age-related cognitive decline, deterioration in formation of novel memories represents one of the most frequent features, probably due to altered functional properties of hippocampal neuronal networks such as decreased synaptic transmission and neuronal excitability [12]. Moreover, *in-vitro* data from animal studies suggested that DHA deficiency mostly affects cortical and hippocampal areas [4]. Thus, the hippocampus emerges as a structure specifically vulnerable to age-related changes. Hence, availability of DHA and other LC-n3-FA may play a crucial role in maintenance of hippocampal-dependent cognitive functioning, as suggested by interventional studies in animals [13, 14] and humans [15].

However, evidence from randomized controlled trials in healthy older adults is limited and results are somewhat inconsistent [5, 16–19]. While some studies did not substantiate a positive effect of LC-n3-FA on any cognitive domain (e.g., [16, 20–22]), others reported improvements in some cognitive functions, mostly memory, but also executive functions after long-term supplementation with LC-n3-FA compared to placebo (e.g., [3, 17, 23–25]).

Of note, cognitive assessments primarily conceptualized for clinical use in adults with manifest or incipient cognitive impairment may not always be sensitive to detect small changes in high-functioning healthy older adults and may have masked beneficial effects in some trials [5, 23, 26]. These limitations might be addressed by implementing more sensitive assessments of learning and memory formation. Recently, we have developed a task called LOCATO [27]. Here, we demonstrated that LOCATO is not only sensitive to detect small intervention-related changes [28], but is also a robust and ecologically meaningful assessment of object-location-memory (OLM) in older adults with and without mild cognitive impairment. Moreover, we illustrated that retesting after six months revealed only small practice effects in learning and recall. In brief, pictures of real-life buildings have to be associated with positions on a two-dimensional street map by repetitions of "correct" object-location pairings over the course of five training blocks, followed by a recall task. OLM is thus of high functional relevance in everyday life [29], and known to largely depend on proper hippocampal functioning [30–33]. Therefore, in a double-blind placebo-controlled proof-of-concept study, we investigated whether daily supplementation with LC-n3-FA versus control intervention, for a period of 26 weeks, would beneficially influence recall in an OLM-task in healthy older adults. Moreover, for comparison with previous studies, we assessed recall and recognition performance in an episodic memory task taken from a standard neuropsychological test battery.

MATERIALS AND METHODS

Subjects

Data reported here were assessed as add-on during a double-blind placebo-controlled randomized dietary interventional trial (NCT00996229) conducted between 2010 and 2013 at the Department of Neurology at the Charité Berlin, Germany. Here, healthy older adults aged between 50 and 80 years were recruited via advertisements from Berlin, Germany. They were pre-screened by phone by trained research personnel to clarify major exclusion criteria such as diabetes mellitus type 2, intake of antidepressants, daily consumption of >50 g of alcohol, >10 cigarettes, or >6 cups of coffee, habitual intake of fish oil supplements before starting the trial, and not-fluent German speaking.

Eligible participants underwent a medical screening before baseline testing including assessment of global cognitive functioning (Mini-Mental State Examination, MMSE; [34]), structural magnetic

resonance imaging (MRI) of the brain and anthropometry to exclude subjects with a history of severe untreated medical, neurological, and psychiatric diseases, Body Mass Index (BMI) <25 or >30 kg/m^2, and signs of dementia (MMSE of minimal <26 points). Psychiatric comorbidity was monitored by Beck's Depression Inventory (BDI, [35]) and Spielberger's State-Trait anxiety inventory [36]. Before (baseline) and after an intervention period (26 weeks), learning and recall scores in a computerized visuospatial memory task called LOCATO ([27]; details see below) were assessed as well as standard neuropsychological tests, blood parameters, ApoE genotype, vascular markers, and anthropometry.

The analysis was based on a subsample of a previously reported study [23] and was restricted to subjects that successfully participated in LOCATO at baseline and follow-up testing. As can be seen in Fig. 1, 80 subjects were initially enrolled in a double-blind placebo-controlled parallel-group study and were allocated (1:1 ratio) by block randomization (blocks of 10; computer generated random number list prepared by an investigator with no clinical involvement in the trial) to intervention or placebo group. Twelve subjects were drop-outs, another three failed to follow dietary instruction, and six subjects were excluded due missing LOCATO data, but this group ($n = 21$) did not differ with regard to age, gender, or education from the remaining subjects ($n = 59$, all $p's > 0.57$). For the present analysis, participants had to successfully perform LOCATO at baseline and follow up visit with a reliable number of responses in the computer-based memory task (inclusion criteria: number of nonresponses is less than mean + 1SEM (equals less than 7%)), leaving 49 subjects. Out of these, 22 subjects were randomly assigned to LC-n3-FA supplementation (LC-n3-FA-group) during trial randomization procedure. To derive groups of identical sample sizes for the present analysis, and to minimize the influence of factors known to modulate memory performance, the placebo group ($n = 27$) was matched to the LC-n3-FA-group according to the following criteria: gender, age, years of education, and order of LOCATO parallel versions (A,B) for repeated testing, leaving 44 subjects (20 female) for the final analysis (mean age of 62 ± 6 years, mean duration of education of 16 ± 3 years; see also Table 1). Results on standard neuropsychological tests are presented in Supplementary Table 1. All subjects gave written informed consent prior to the study and received a small reimbursement. Each part of the study was approved by the Ethics Committee of the Charité University Hospital Berlin, Germany, and was conducted in accordance with the declaration of Helsinki.

Dietary intervention

The LC-n3-FA group received fish oil capsules for 26 weeks (4 capsules daily). The daily dose of four capsules contained 2,200 mg of LC-n3-FA (1,320 mg EPA + 880 mg DHA; given as $4 \times 1{,}000$ mg fish oil and 15 mg vitamin E). Placebo group received a daily dose of four capsules each filled with 1,015 mg of sunflower oil. All capsules were identical in shape and color and were provided by Via Vitamine, Oberhausen, Germany. Subjects and investigators were blinded to the treatment group. Compliance was monitored by capsule count after 12 and 26 weeks. Moreover, subjects were instructed not to change their dietary habits, for instance frequency of fish consumption, throughout the intervention. At the end of the study, a questionnaire was administered that evaluated changes in dietary habits, regular intake of capsules, and side effects.

Visuospatial object-location-memory task (LOCATO)

For LOCATO, 15 pictures of real-life buildings were associated with different positions (location) on a two-dimensional street map. Subjects had to learn the correct object-location pairing over the course of five training blocks followed by a recall task. In detail, each block comprised 60 trials presenting 2×15 correct object-location pairings as well as 30 incorrect object-location pairings in randomized order (total of 300 training trials across five blocks). Over the course of five training blocks the "correct" pairing position of a building occurred 10 times more frequently compared with "incorrect" positions (shown only once, respectively). Each trial comprised a picture of a schematized street map with one building presented for a duration of 3000 ms and an inter-stimulus interval of 1000 ms. Within these time frame subjects had to indicate by button press on a response pad as accurate as possible if, in their opinion, object and position matched, or did not match (button "correct" or "incorrect"). Correct/incorrect responses were recorded and no online feedback on performance was provided. Acquisition performance were tested by accuracy on a given block in terms of percent correct (PC) compiled on the basis of hits and correct rejections within a training block. Reaction times were addition-

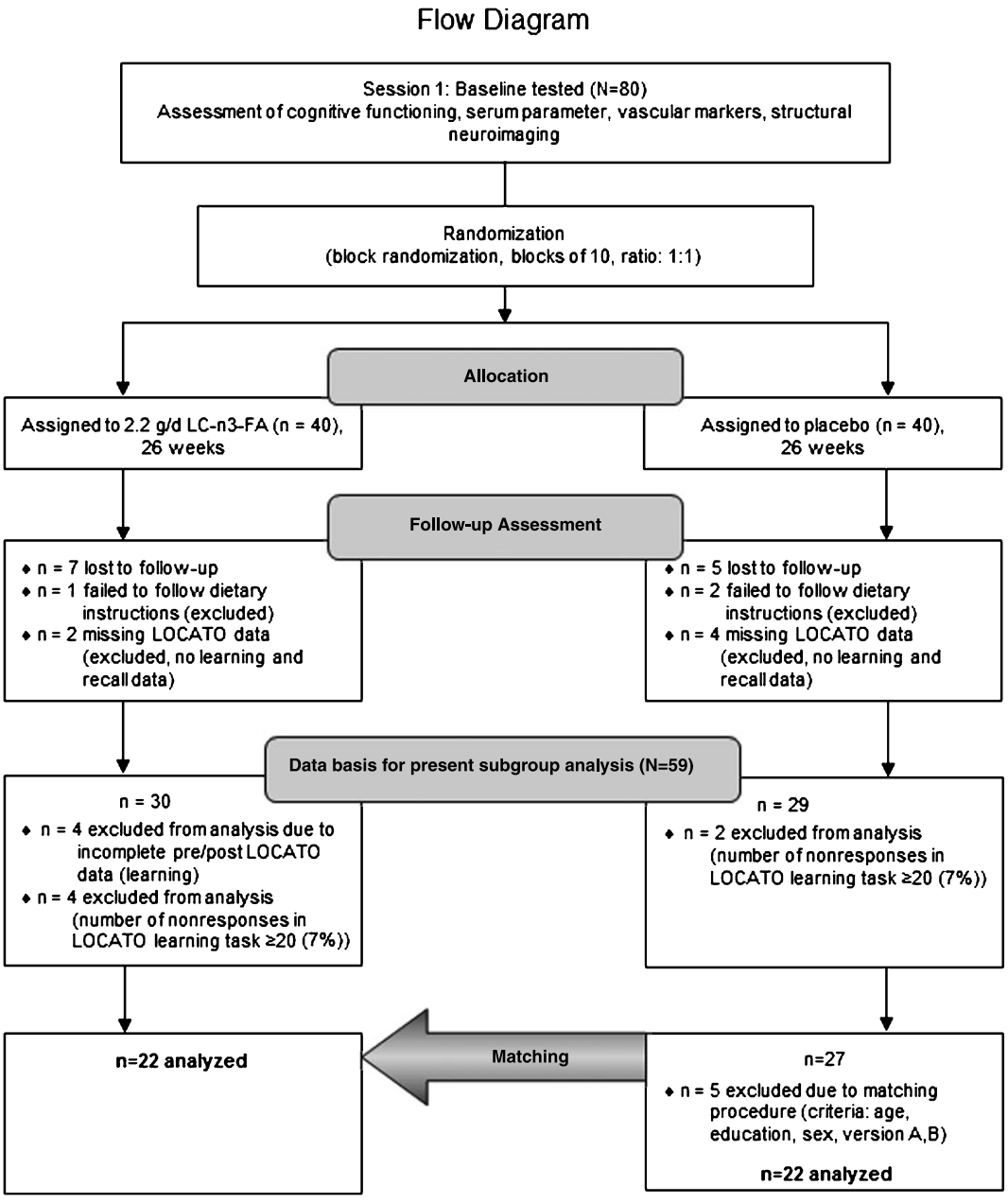

Fig. 1. Flow chart of the study. Of 80 baseline tested subjects (including assessment of cognitive functioning, serum parameter, vascular markers, structural neuroimaging), 40 subjects were randomized by block randomization (blocks of 10, ratio 1:1) either to intervention arm receiving LC-n3-FA supplementation or placebo arm. Twelve subjects were drop-outs, while another three failed to follow dietary instructions. Out of these 65 subjects, six subjects had to be excluded for the present proof-of-principle study due to missing LOCATO data, another four subjects were excluded due to incomplete LOCATO testing at baseline or follow-up session, and six subjects did not met criteria for a reliable performance analysis (number of nonresponses $\geq$20 (7%) in LOCATO learning task), leaving 22 subjects receiving LC-n3-FA and 27 subjects receiving placebo. Placebo group were then matched to LC-n3-FA group according to age, education, gender, and parallel LOCATO versions to derive groups of identical sample size.

ally analyzed. Immediately after the training blocks, memory was tested with a cued recall test (CR) as the primary outcome of this study. Therefore, three possible locations for a particular building were shown on the map and the subject had to choose the building's "correct" position (3-alternative forced choice

Table 1
Baseline characteristics for LC-n3-FA and placebo group

	LC-n3-FA	Placebo	*p*-value
n (women)	22 (10)	22 (10)	
Age (years)	63 ± 6	61 ± 6	0.30
Education	University degree	University degree	0.20[1]
Modus of educational level (*n*)	(55%)	(59%)	
number of years	16 ± 3	17 ± 2	0.07
ApoE genotype (ε4 allele carriers; non-carriers)	13.6 %; 86.4%	22.7%; 77.3%	0.53[1]
Mini-Mental State Examination	29 ± 1.2	29.3 ± 0.7	0.79[2]
Beck's Depression Index	4.7 ± 4.3	5.7 ± 4.9	0.43[2]
State-Trait-Anxiety-Inventory	32.1 ± 7.2	34.8 ± 9.7	0.29

Data are given as mean ± SD and *p*-values are presented of unpaired *t*-tests unless otherwise mentioned. [1] χ^2-test, [2]Mann-Whitney U-test.

task). Responses (accuracy assessed as percentage of correctly selected positions) were measured, no time limit for subject's response was set, and no online feedback was provided. Two parallel versions of LOCATO were used, each with a different set of 15 buildings, and with the street map rotated for 180° for version B, in balanced order across subjects. Psychometric properties are not available so far, but usefulness of this version of LOCATO in repeated assessing of associative visuospatial memory has been previously tested [27].

Standard neuropsychological tests

To compare this study to previous trials memory was also assessed by the German version of the Rey Auditory Verbal Learning Test (AVLT, [37]), implemented in most standard neuropsychological test batteries. Subjects had to learn a list of 15 concrete nouns within five immediate recall trials, followed by a 30-min delayed recall and recognition test. The sum of words immediately remembered across the five learning trials represents a score for learning ability. Delayed recall was defined by number of correctly remembered words after 30 min and a recognition score was determined by the number of correctly recognized words minus false positives out of a subsequent list of 45 words (15 learned words intermixed with 30 distractor words). Parallel versions were used to minimize test-retest effects. In addition, the affective state at the time of the testing was assessed with the Positive and Negative Affect Schedule (PANAS, [38]).

Omega-3-index

For evaluating supplementation-induced changes of LC-n3-FA, omega-3-index values [39] were determined at baseline and after 26 weeks. Blood samples were taken from subjects, immediately centrifuged, and the erythrocyte fraction was stored at –80°C until assayed. The omega-3-index was defined as the percentage of EPA (C20:5n3) + DHA (C22:6n3) of total fatty acid areas in erythrocytes. Analyses were done on coded samples using a gas chromatograph (HP 5890 Series II with Autosampler) by Lipidomix Laboratory, Berlin, Germany. Two samples had to be excluded from baseline assessment due to technical problems.

Serum based parameters

Fasting blood samples were collected from all subjects to assess lipid profile (total cholesterol, high- and low-density-lipoprotein cholesterin (HDL, LDL), triacylglycerides), markers of inflammation (tumor necrosis factor (TNF)-α, interleukin-6, high-sensitive C-reactive protein (hsCSP), glucose metabolism (fasting glucose, glycated hemoglobin A1c (HbA1c), insulin), leptin, brain derived neurotrophic factor (BDNF), and vitamin B12. All parameters were analyzed by the institute of medical diagnostic (IMD) Laboratory, Berlin, Germany. Analyses were done by IMD-staff on coded samples blinded to intervention.

Apolipoprotein E (ApoE,) genotyping, cardiovascular, and anthropometric data

To assess individual ApoE status, DNA was extracted from whole blood using a blood mini-kit (Qiagen, Hilden, Germany). Genotyping on coded samples was performed by the lab of Prof. Dr. Dan Rujecscu, University Halle, Germany (procedure is described in more detail elsewhere; [40]). Systolic and diastolic blood pressure was measured by a semi-

automatic device. Anthropometry incorporated the assessment of weight, height, and body fat (percentage, measured by bioelectrical impedance analysis). Subject's physical activity and other lifestyle habits were estimated by using the Freiburger physical activity questionnaire [41] implemented in a questionnaire on lifestyle habits [42].

Data aggregation and statistical analysis

For OLM, learning (accuracy and reaction times) and recall performance (primary outcome) in LOCATO were determined. Learning performance for each training block was described as Percent Correct (PC) and was composed as follows: PC = (number of hits + number of correct rejections)*100)/total number of buildings presented within a training block. Strong response tendencies (to respond with "no" or "yes" to every stimulus) were monitored by taking into account all "yes" responses (comprising hits and false alarms) in relation to number of presented pictures. Mean reaction times (RT) to correct responses were determined by averaging the individual medians for hits and correct rejections per training block. Recall scores in the cued recall task were expressed as percentage of correctly selected positions. Learning and recall scores were analyzed using a repeated measurement analysis of variance ($ANOVA_{RM}$), respectively, with GROUP (LC-n3-FA versus placebo) as between- and TIME (baseline, follow-up) as within-subject factor. For analyzing learning performance the within-subject factor BLOCK (block 1–5) was added to consider learning curve as well.

As exploratory analyses, we performed separate GROUP × TIME $ANOVA_{RM}$ on other standard neuropsychological outcomes (AVLT, mood), blood-based biomarkers, questionnaires, omega-3-index, and anthropometric data to detect differential changes over time by significant GROUP × TIME interactions. Demographic characteristics at baseline were compared by independent *t*-tests or in case of insufficient normal distribution of the data (|skewness| <1) by Mann-Whitney-U-tests. Differences in potential modulators (ApoE, global cognitive functioning, depression, and anxiety) and compliance (dietary changes, capsule intake) between groups were analyzed by chi-square-tests (ApoE) and *t*-tests or Mann-Whitney-U-tests, respectively. To statistically control violations in compliance these scores were incorporated as covariates in ANCOVAs if necessary. Bivariate correlations to evaluate associations between changes in serum parameters and cognitive functions were assessed using Spearman correlations.

A two-sided significance level $\alpha = 0.05$ was used in all analyses. Reported effect size is partial η^2 (subsequently shortened to η^2). All statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences for Windows, SPSS Inc. USA). Because of the small sample size and proof-of-concept nature of the study no adjustment for multiple testing was applied. No imputation on missing data was done.

RESULTS

As shown in Table 1 the groups were comparable with regard to age, educational level, and other memory modulating factors (confounders) such as ApoE genotype, global cognitive functioning, depression, or anxiety.

Compliance and side-effects

Capsule count indicated high compliance with capsule intake (number of missed capsules/month below 5%). These scores did not significantly differ among groups ($\chi^2(2) = 5.24$, $p = 0.07$), but subjects of the placebo group more frequently reported a change in dietary habits during intervention ($\chi^2(1) = 5.5$, $p = 0.019$; LC-n3-FA group: $n = 1$ versus placebo group: $n = 7$).

No severe adverse events were reported over 26 weeks. However, adverse events included gastrointestinal symptoms during the first days, e.g., flatulence, digestions, diarrhea, and burps ($n = 7$), headache over several days ($n = 1$), and skin irritations for a few days ($n = 1$).

Object-location-learning- and memory

For cued recall a significant interaction effect emerged (GROUP × TIME: $F(1,42) = 4.11$, $p = 0.049$, $\eta^2 = 0.09$, see also Table 2) pointing to better retrieval of correct object-location-associations after LC-n3-FA compared to placebo treatment (mean improvement in %: 13.2 versus 3.5; see also Fig. 2A). Performance at baseline was comparable between groups (LC-n3-FA: 65.6% ± 13.8, placebo: 69.6% ± 15.6; $t_{(42)} < 1$). Further control analyses (ANCOVAs) were conducted to adjust for "change in dietary habits" and additionally for "ApoE-ε4 allele carrier status" since the presence or absence of ApoE-ε4 allele appears to be an

Table 2
Results of $ANOVA_{RM}$ (degree of freedoms, F and *p*-values, effect size partial η^2, 95% CI) for cued recall (GROUP × TIME) and learning accuracy (GROUP × TIME × BLOCK) and main (highest order interaction) results of adjusted Models (ANCOVA). Note that for GROUP × TIME × BLOCK $ANOVA_{RM}$ on all yes responses (hits and false alarms) no strong response tendencies (all $p's \geq 0.15$ for all main and interaction effects with factor GROUP) were found for the LC-n3-FA group (pre = 0.5 ± 0.1, post = 0.5 ± 0.2) and for the placebo group (pre = 0.5 ± 0.1, post = 0.5 ± 0.1)

Source of variance	df	F	*p*	η^2	Mean (in %) ± SD [95% CI]			
Cued recall					LC-n3-FA		Placebo	
					pre	post	pre	post
GROUP × TIME	1,42	4.11	**0.049**	0.09	65.6 ± 2.9 [59.3, 71.4]	78.8 ± 2.4 [73.2, 84.3]	69.5 ± 3.3 [63.2, 76.0]	73.0 ± 3.1 [67.5, 78.6]
Adjusted for "*change in dietary habits*" (covariate)	*1,40*	*9.25*	***0.004***	*0.18*				
Adjusted for "*change in dietary habits, ApoE- ε4 allele carrier status*" (covariates)	*1,39*	*8.01*	***0.007***	*0.17*				
GROUP	1,42	<1						
TIME	1,42	12.13	**0.001**	0.23				
Learning (accuracy)					LC-n3-FA		Placebo	
					pre[1]	post[1]	pre[1]	post[1]
GROUP × TIME × BLOCK	4,168	1.10	0.36	0.03	75 ± 2.1 [70.8, 79.2]	78.3 ± 2 [74.2, 82.3]	72.8 ± 2.1 [68.5, 76.9]	78.9 ± 2 [74.9, 83]
Adjusted for "*change in dietary habits*" (covariate)	*4,164*	*<1*						
Adjusted for "*change in dietary habits, ApoE- ε4 allele carrier status*" (covariates)	*4,156*	*<1*						
GROUP × TIME	1,42	1.54	0.22	0.04				
GROUP × BLOCK	4,168	<1						
TIME × BLOCK	4,168	5.64	**<0.001**	0.12				
GROUP	1,42	<1						
TIME	1,42	14.73	**<0.001**	0.26				
BLOCK	4,168	200.5	**<0.001**	0.83				

Only if F-values ≥1 results of $ANOVA_{RM}$ and ANCOVA are reported. Significant effects ($p \leq 0.05$) are indicated by bolding the *p*-value. Main results of ANCOVAs are printed in italic. 1) Mean performance and SD of the fifth learning block.

important modifier of the relationship between LC-n3-FA and cognitive outcome [43]. As can be seen in Table 2 the interaction effect GROUP × TIME remained in the ANCOVAs indicating enhanced recall of correct-object-associations after LC-n3-FA treatment also after controlling for these variables. Analysis of learning performance (details are represented in Table 2) revealed no significant interaction effects of the GROUP × TIME × BLOCK $ANOVA_{RM}$ suggesting similar linear learning slopes across blocks at baseline and follow-up testing. Further, a general training effect over time was observed as indicated by a significant effect of TIME × BLOCK pointing to better learning performance at follow-up (78.6% ± 9.4) compared to baseline testing (73.9% ± 9.7) independent of intervention. Similarly, reaction time data indicated linear learning at both sessions (pre and post) and showed decreased reaction times across blocks independent of time and intervention (main effect BLOCK: $F(4,168) = 70.75$, $p < 0.001$, $\eta^2 = 0.63$). However, a general improvement in reaction time over time from pre to post testing was not observed (main and interaction effects with factor TIME all $p's > 0.11$). Correlational analysis revealed no significant linear relationship between changes in omega-3-index and recall or learning scores (all $r's < 0.28$ and $p's > 0.20$).

Changes in Omega-3-index

An increase of omega-3-index, i.e., higher proportions of EPA and DHA in membranes of erythrocytes of peripheral blood [44], was observed after 26 weeks of supplementation with LC-n3-FA compared to placebo ($ANOVA_{RM}$: GROUP × TIME: $F(1,40) = 12.39$, $p = 0.001$, $\eta^2 = 0.24$; see Fig. 2B). Notably, an increase was observed in both EPA and

DHA (see also Table 3) over time. Significant group differences remained for omega-3-index and proportion of EPA after correction for "capsule count" and "change score in dietary habits" in the corresponding ANCOVAs (GROUP × TIME: all p'$s \leq 0.007$), but attenuated the effect for DHA (GROUP × TIME: $F(1,38) = 2.47$, $p = 0.12$, $\eta^2 = 0.06$).

In sum, supplementation of LC-n3-FA led to an increase in omega-3-index in the LC-n3-FA group, which was not seen in the placebo group. Moreover, on average supplementation of LC-n3-FA resulted in significantly improved recall of correct object-location-associations for LC-n3-FA compared to placebo, without significantly affecting learning and reaction time.

Standard neuropsychological tests

$ANOVA_{RM}$ testing for intervention-induced differences in verbal learning and memory scores (delayed recall and recognition) yielded no significant GROUP × TIME effects in any of the tests (all p'$s > 0.33$). No differences were found in affective positive or negative state at pre and post testing between groups (all p'$s > 0.69$; data are presented in Supplementary Table 1).

Anthropometric, cardiovascular, and biomarker

No substantial differences between groups were observed in weight, body mass index, body fat, mean physical activity or blood pressure (GROUP × TIME: all p'$s \geq 0.07$, see also Table 3) after 26 weeks of LC-n3-FA supplementation compared to placebo. Likewise, in other blood biomarkers (lipid profile, triacylglycerides, inflammatory- and glucose metabolism marker, leptin, vitamin B12, BDNF) no significant changes were noted between groups (all p'$s > 0.15$).

DISCUSSION

The present study demonstrated that performance in cued recall in a previously developed OLM task was sensitive in detecting beneficial effects of LC-n3-FA supplementation, after controlling for possible confounders. Conversely, differential effects of LC-n3-FA supplementation could not be detected on recall or recognition performance from a standard neuropsychological test battery (AVLT).

Our findings extend previous studies reporting beneficial effects of LC-n3-FA supplementation on cognition (e.g., [17, 23, 45]) by demonstrating improved OLM. Indeed, in randomized controlled trials benefits on memory functions, as commonly measured by standard neuropsychological tests [15] such as the AVLT or similar word-list learning and recall paradigms [46, 47], have been shown in older adults with subjective memory complaints [48] or mild cognitive impairment [3, 25, 49, 50], but not in healthy older adults [20–22, 45], which is also in line with our results. Differences between studies in older adults with objective deficits in memory performance [3, 25, 49, 50] versus healthy adults might be due to ceiling effects in neuropsychological assessments of healthy subjects [18] but not in patients with memory deficits [51]. Moreover, differences in dosage and compositions of EPA and DHA (e.g., [20]) as well as intervention length (e.g., [21]) may account for differential findings between healthy individuals and patients with subjective memory complaints. However, given the significant improvements in cued recall in a sensitive test of OLM, but lack of effect on a standard memory test, it could be hypothesized that some results of previous trials in healthy older adults were biased by insensitive outcomes measures. Thus, task sensitivity might be an important issue when including cognitively high-functioning older adults. This was also supported by other studies, e.g., Nilsson et al. [17] who conducted an interventional study of daily consumption of fish oil capsules over a period of five weeks in healthy middle aged to older adults. Here, the authors found the largest performance differences in the most demanding parts of a working memory task. In sum, cued recall task of LOCATO constitutes an ideal scenario for monitoring intervention induced cognitive changes over time in a cohort of cognitively intact older adults.

Improvements in memory functions seen by the intervention with LC-n3-FA supplements may result from beneficial effects on nerve cell membranes, synaptogenesis, and improved synaptic transmission [9, 52] that in turn modulate expression and function of glutamate receptors [10] implicated in learning and memory. It has been shown that dietary administration of DHA mediates synaptic fluidity and enhanced neurogenesis in subgranular zone of rats [53], increased neurogenesis within the hippocampus of adult fat-1-transgenic mice with high endogenous DHA levels compared to wild type-mice [54], and that LC-n3-FA promoted long-term potentiation in rats [55]. DHA was also reported to increase hippocampal BDNF in rats [56] and higher BDNF levels have been linked to larger hippocampal volume [57]

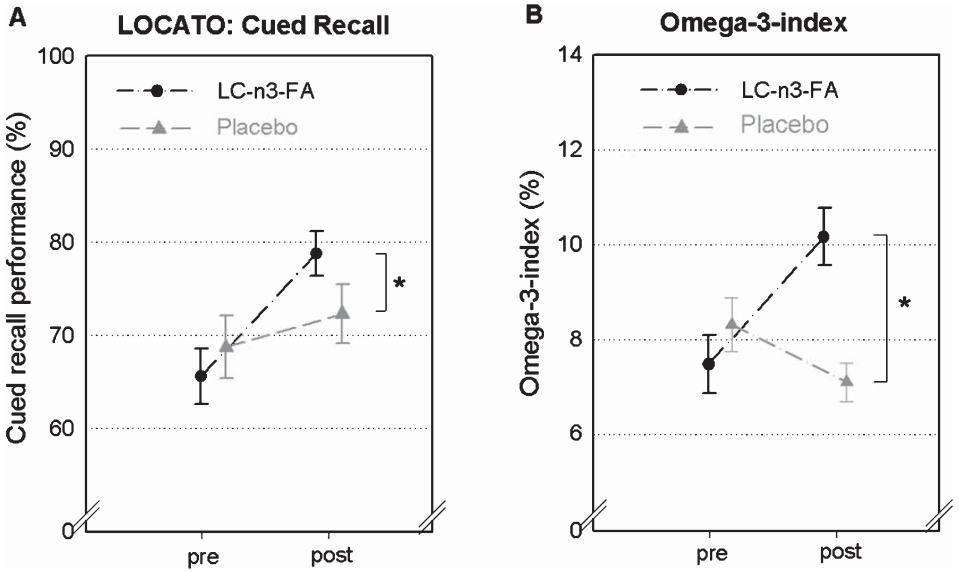

Fig. 2. A) Cued recall performance (mean ± standard error of the mean of percentage of correctly selected positions in a 3-alternative forced choice task) in LOCATO before (pre) and after (post) 26 weeks of supplementary LC-n3-FA or placebo capsules intake. Subjects of LC-n3-FA group significantly improved recall performance after treatment (circles, black) compared to placebo (triangles, gray). B) Omega-3-index (mean ± standard error of the mean of relative content of EPA and DHA in erythrocyte membranes) before (pre) and after (post) 26 weeks of supplementation with LC-n3-FA. Omega-3-index significantly increased in the LC-n3-FA group ($p = 0.013$, paired t-test; circles, black) and decreased in the placebo group ($p = 0.032$, paired t-test; triangles, gray).

and superior memory [58] in humans. However, in our study both groups showed an increase in BDNF concentration at post-intervention measurement, which may be due to changes in general lifestyle habits such as diet or exercise. Moreover, peripheral BDNF levels may not adequately reflect central BDNF concentrations. Taken together, although the specific mechanisms relevant to cognitive benefit in humans remain difficult to assess *in-vivo*, previous findings point to modulations of neurotransmission pathways [9, 10] relevant for synaptic consolidation processes.

Consolidation can be tested on a behavioral level via recall tests. In our study, 26 weeks of supplementation with LC-n3-FA significantly affected cued recall. Interestingly, these differential findings for recall were seen without significant differences in learning rate between the groups, possibly due to the underlying cognitive processes required for acquisition and cued recall, respectively. Within LOCATO, learning is supported in part by implicit processes such as statistical learning (frequency of correct object-locations pairs accumulates over time), which might be less susceptible for LC-n3-FA effects in contrast to more hippocampal-dependent tasks such as cued recall. Moreover, experience-based task-learning strategies might have facilitated learning performance [27] independent of LC-n3-FA effects and thus may have contributed to our results.

No significant correlation between changes in memory performance and omega-3-index were observed, suggesting that memory benefits were not associated in a simple linear fashion with changes in omega-3-index. In addition, the association might have been further masked by restrained responsiveness to supplementation [5] due to high baseline levels. However, a relative improvement in omega-3-index was observed after LC-n3-FA supplementation in the LC-n3-FA, but not the placebo group, suggesting that the intervention was successful in increasing DHA and EPA in cell membranes. Moreover, a trend for an inverse relationship between changes in triacylglyceride level and memory improvement was noted for the LC-n3-FA group ($r = –0.39$, $p = 0.073$) but not for the placebo group ($r = 0.27$, $p = 0.245$). These results are in line with Nilsson et al. [17] who also found a trend for an association between lower cognitive performance and higher triacylglyceride after five weeks of intervention with LC-n3-FA. In sum, memory may have also benefitted from omega-3 supplementation via its triacylglyceride lowering [59] and thus possibly cerebrovascular protective effect.

Table 3
Omega-3-index, anthropometric and vascular measures, and fasting blood parameters before and after intervention period for LC-n3-FA group and placebo group

Parameter	LC-n3-FA		Placebo		
	pre	post	pre	post	*p*-value
Omega-3-index (%)*	7.8 ± 2.6	10.2 ± 2.9	8.6 ± 2.3	7.1 ± 1.8	0.001
EPA (%)*	1.8 ± 0.7	3.2 ± 0.9	2.0 ± 0.6	1.5 ± 0.6	**<0.001**
DHA (%)*	6.0 ± 2.0	6.9 ± 2.2	6.6 ± 2.0	5.7 ± 1.4	**0.05**
Weight (kg)	84.9 ± 9.3	85.54 ± 9.6	81.0 ± 8.7	80.5 ± 8.4	0.07
BMI (kg/m^2)	27.7 ± 1.8	27.8 ± 1.9	27.1 ± 1.5	27.0 ± 1.4	0.10
Body fat (%)	31.4 ± 6.5	31.4 ± 6.8	28.4 ± 9.2	30.2 ± 10.0	0.09
Mean physical activity* (kcal/week)	5930 ± 5795	4041 ± 2945	5567 ± 5219	6008 ± 4731	0.16
Vascular markers					
Systolic BP (mmHg)	143.3 ± 17.5	142.8 ± 16.9	144.1 ± 16.7	143.2 ± 14.7	0.95
Diastolic BP (mmHg)	89.1 ± 6.1	84.0 ± 10.2	90.14 ± 9.0	87.3 ± 7.7	0.38
Lipid profile					
HDL (mg/dl)	65.1 ± 13.2	69.3 ± 15.2	56.8 ± 11.0	58.2 ± 11.4	0.19
LDL (mg/dl)	127.3 ± 21.2	133.3 ± 20.8	137.5 ± 32.5	136.7 ± 34.1	0.21
HDL to LDL-ratio	2.0 ± 0.5	2.0 ± 0.5	2.5 ± 0.7	2.4 ± 0.7	0.54
Total cholesterol (mg/dL)	215.9 ± 30.0	218.2 ± 28.4	216.0 ± 39.7	216.1 ± 39.7	0.73
Triacylglycerides (mg/dL)	98.1 ± 37.1	84.6 ± 28.1	105.0 ± 40.6	107.8 ± 48.2	0.14
Inflammatory markers					
Interleukin-6 (pg/mL)*	3.7 ± 4.0	2.1 ± 0.5	4.4 ± 6.3	2.5 ± 1.0	0.87
TNF-α (pg/m)*	11.0 ± 2.6	9.3 ± 1.4	13.9 ± 8.4	10.0 ± 2.0	0.24
hsCRP (pg/m)	1.9 ± 2.3	2.0 ± 3.6	3.1 ± 4.0	1.8 ± 2.1	0.29
Markers of glucose metabolism					
Insulin (μU/mL)	8.8 ± 4.5	8.7 ± 3.7	8.2 ± 3.8	8.3 ± 2.8	0.82
Glucose (mg/dL)	89.6 ± 8.0	93.1 ± 7.9	91.2 ± 9.1	91.8 ± 9.1	0.24
HbA1c (%)	5.8 ± 0.2	5.8 ± 0.3	5.8 ± 0.2	5.9 ± 0.3	0.56
Others					
Leptin (ng/mL)	15.7 ± 13.8	12.1 ± 12.2	16.4 ± 13.1	12.4 ± 13.3	0.90
Vitamin B12 (pmol/L)	419.0 ± 115.8	419.9 ± 101.1	411.4 ± 106.1	405.6 ± 105.9	0.76
BDNF (pg/mL)	4000.2 ± 630.2	4266.3 ± 869.5	4082.2 ± 854.7	4315.5 ± 1029.2	0.92

Data are given as mean ± SD. *p*-values are depicted for interaction effects of GROUP × TIME ANOVArm. Significant effects are indicated by bolding the *p*-value. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BMI, Body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TNF-α, tumor necrosis factor-alpha; hsCRP, high-sensitive C-reactive protein; HbA1c, glycated hemoglobin A1c; BDNF, brain-derived neurotrophic factor. $N = 44$ unless otherwise mentioned. *Reduced sample size due to missing data because of technical problems in assessing omega-3-index (inclusive EPA and DHA; placebo: $n = 20$), missing values in a questionnaire of mean physical activity (LC-n3-FA: $n = 19$; placebo: $n = 21$), and in non-fasting blood samples in placebo group ($n = 20$).

Limitations

Several limitations must be considered when interpreting our findings. First, this proof-of-concept study used a recently developed OLM-task (LOCATO), which has not been tested for psychometric properties so far. However, usefulness of LOCATO in repeated assessing of associative visuospatial memory in older adults has been demonstrated [27]. Second, this proof-of-concept study does not meet all criteria of a randomized controlled trial. However, it closely complies with most of the CONSORT guidelines, particularly with methodological criteria including the double-blind placebo-controlled study design. Thus, we believe that the present study provides additional evidence of beneficial effects of LC-n3-FA supplementation on memory in healthy older adults. Third, statistical power was limited due to small sample size, and external validity may be diminished because the applied exclusion criteria favored selection of extremely healthy older adults. However, groups were well matched with regard to memory confounders (age, gender, education, mood) and controlled for a potentially important genetic modifier (ApoE-ε4 allele carriers) of the association between LC-n3-FA and memory (e.g., [43]). Thus, it seems unlikely that these factors have substantially biased LC-n3-FA induced effects in this study. Fourth, eight subjects reported that they changed their lifestyle

habits during the intervention period. However, we included this information in our statistical models and found no significant modulation of the main outcome.

Conclusion and outlook

Taken together, results of this double-blind placebo-controlled proof-of-concept study provide experimental evidence that LC-n3-FA exert positive effects on memory functions in healthy older adults. However, the clinical relevance of the statistical significant treatment effect remains to be evaluated with appropriate scales and questionnaires that also assess the impact of LC-n3-FA supplementation on activities of daily life in cognitively impaired populations. Given that nutritional interventions are a cost-effective, well-tolerated, and safe approach that can thus be employed years or even decades before onset of pathological aging, our results support further large, long-term randomized controlled trials with sensitive outcome measures to determine the impact of LC-n3-FA on cognitive functions in healthy populations.

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SUPPLEMENTARY MATERIAL

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