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Long-Chain Omega-3 Fatty Acids Improve Brain Function and Structure in Older Adults

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Higher intake of seafish or oil rich in long-chain omega-3 polyunsaturated fatty acids (LC-n3-FA) may be beneficial for the aging brain. We tested in a prospective interventional design whether high levels of supplementary LC-n3-FA would improve cognition, and addressed potential mechanisms underlying the effects. Sixty-five healthy subjects (50–75 years, 30 females) successfully completed 26 weeks of either fish oil (2.2 g/day LC-n3-FA) or placebo intake. Before and after the intervention period, cognitive performance, structural neuroimaging, vascular markers, and blood parameters were assayed. We found a significant increase in executive functions after LC-n3-FA compared with placebo ($P = 0.023$). In parallel, LC-n3-FA exerted beneficial effects on white matter microstructural integrity and gray matter volume in frontal, temporal, parietal, and limbic areas primarily of the left hemisphere, and on carotid intima media thickness and diastolic blood pressure. Improvements in executive functions correlated positively with changes in omega-3-index and peripheral brain-derived neurotrophic factor, and negatively with changes in peripheral fasting insulin. This double-blind randomized interventional study provides first-time evidence that LC-n3-FA exert positive effects on brain functions in healthy older adults, and elucidates underlying mechanisms. Our findings suggest novel strategies to maintain cognitive functions into old age.

Keywords: cognitive aging, diffusion tensor imaging, executive functions, intima media thickness, voxel-based morphometry

Introduction

Due to the constant growth of the elderly population worldwide, the incidence of Alzheimer's disease (AD) increase exponentially (Plassman et al. 2011). As the process of AD begins years, if not decades, before the diagnosis of clinical dementia (Morris 2005), searching for new prevention strategies is of major economic and medical importance (Lowin et al. 2001).

Long-chain omega-3 polyunsaturated fatty acids (LC-n3-FA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may exert beneficial effects on the aging brain (Gomez-Pinilla 2008; Fotuhi et al. 2009; Hooijmans et al. 2012). For example, animal experiments showed that LC-n3-FA supplementation up-regulates synaptic membrane proteins implicated in synaptic plasticity (Cansev and Wurtman 2007) and improves executive functions and learning abilities (Hooijmans et al. 2012). Human studies showed that higher LC-n3-FA consumption correlates with better cognitive functioning (Kalmijn et al. 2004), a reduced risk for dementia (Barberger-Gateau et al. 2007), lower β-amyloid 42 (Aβ 42) plasma levels (Gu et al. 2012), higher total brain (Tan et al. 2012) and hippocampal volume (Conklin et al. 2007), and with reduced white matter hyperintensities (Conklin et al. 2007). Others reported inverse associations between DHA concentrations and carotid intima media thickness (CIMT), a surrogate marker of atherosclerosis (Sekikawa et al. 2011).

However, interventional studies of supplementary LC-n3-FA showed contradictory results. Some studies reported improved cognition after 6 months of LC-n3-FA, compared with placebo in healthy elderly with subjective memory complaints (Yurko-Mauro et al. 2010) and in small groups of patients with mild cognitive impairment (MCI; Chiu et al. 2008) or very mild AD (Freund-Levi et al. 2006). In contrast, others did not report significant effects in healthy elderly (van de Rest et al. 2008) and in AD patients (Quinn et al. 2010). In summary, the impact of LC-n3-FA supplementation on cognitive functions in humans is still a matter of debate, and underlying mechanisms on the systemic and neuronal level in humans remain unclear. We therefore assessed the effects of supplementary LC-n3-FA (2200 mg/day) over 26 weeks on cognitive performance in a cohort of healthy older adults, using composite scores of memory and executive functions (Chiu et al. 2008; van de Rest et al. 2008) in a double-blind randomized interventional study. Moreover, white matter integrity, gray matter (GM) volume, CIMT, and peripheral parameters (omega-3 index, glucose/insulin metabolism, inflammatory markers, and neurotrophins) were assessed to examine potential mechanisms.

Materials and Methods

Subjects

Participants were recruited via advertisements in Berlin, Germany. Exclusion criteria were severe disease including diabetes mellitus type 2, neurological disorders, psychiatric medication, a mini-mental state examination (MMSE; Folstein et al. 1975) <26 points, a body mass index (BMI) <25 kg/m^2 or >30 kg/m^2, intake of acetylsalicylic acid, daily consumption of >50 g alcohol, >10 cigarettes, or >6 cups of coffee, non-fluent German, and left-handedness. Subjects did not take dietary supplements containing fish oil before starting the trial.

Subjects underwent a medical examination before baseline testings, which included neuropsychological testing, structural magnetic resonance imaging (MRI) of the brain, and assessment of vascular markers, blood parameters, and anthropometry (Session 1; see Fig. 1). Psychiatric comorbidity was additionally monitored using the Beck's Depression Inventory (BDI; Kuhner et al. 2007) and Spielberger's State-Trait Angst Inventar (STAI 1 and 2; Laux et al. 1981). Participants were randomized into 3 groups: (1) LC-n3-FA ($n = 40$), (2) placebo ($n = 40$), and (3) into a separate study testing cognitive effects of caloric restriction (reported separately, $n = 41$). The LC-n3-FA group received fish oil capsules for 26 weeks (4 capsules daily) comprising

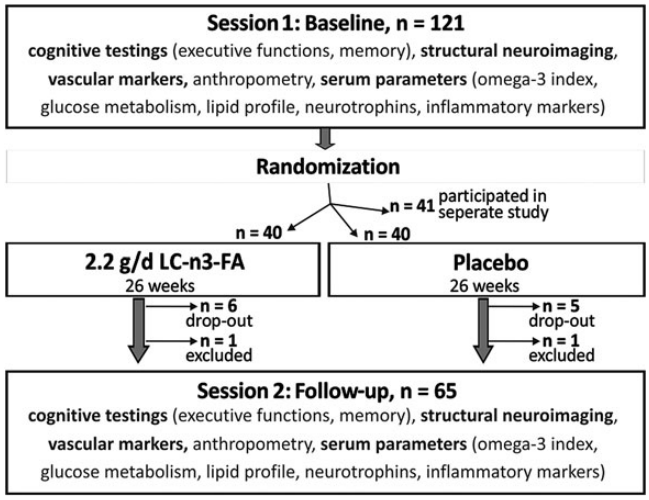

Figure 1. Flow chart of the study. Of 743 volunteers who were screened for inclusion and exclusion criteria, 121 subjects completed baseline sessions including cognitive testings, structural neuroimaging and assessment of vascular markers, and fasting blood parameters. Subjects were randomly allocated either to the intervention arm receiving supplementary LC-n3-FA ($n = 40$), or to the placebo arm ($n = 40$), or to a separate study ($n = 41$). Twelve subjects were drop-outs, another 3 failed to follow dietary instructions, leaving 65 healthy older adults for analysis who successfully completed 26 weeks of either LC-n3-FA ($n = 32$) or placebo intake ($n = 33$). All measurements were repeated at follow-up sessions.

Table 1
Baseline characteristics

	LC-n3-FA	Placebo	*P*-value
N (*n* women)	32 (15)	33 (15)	—
Age [years]	65 ± 6.3 (51–75)	62.9 ± 6.8 (50–75)	0.77[a]
Education [degree] (range: 0 = no education–5 = university degree)	4 ± 1.2 (1–5)	4.3 ± 1.2 (1–5)	0.21[c]
Mini-mental state examination (MMSE) [score]	29.1 ± 1.2 (26–30)	29.4 ± 0.8 (27–30)	0.52[b]
Beck's Depression Index [score]	6.3 ± 7.6 (0–32)	6.9 ± 5.2 (0–18)	0.27[a]
State-Trait Anxiety Inventory-X1 [score]	34.4 ± 9.5 (20–66)	34.8 ± 8.3 (20–57)	0.44[a]
Right-handedness [%]	80.2 ± 15.9 (50–100)	82.0 ± 16.8 (40–100)	0.57[b]

Note: Data expressed as mean ± SD and range (minimum–maximum). Handedness scores were determined according to the Edinburgh Handedness Inventory.
[a]Unpaired *t*-test.
[b]Mann–Whitney *U*-test.
[c]χ^2 test.

2200 mg LC-n3-FA (1320 mg EPA + 880 mg DHA, given as 1000 mg fish oil and 15 mg vitamin E). Subjects of the control group received placebo capsules (sunflower oil). All capsules were provided by Via Vitamine, Oberhausen, Germany, identical in shape and color. Twelve subjects were drop-outs (MRI pathologies, $n = 6$; withdrawal: nausea, $n = 2$; and time constraints, $n = 4$). Three subjects failed to follow dietary instruction (i.e. self-reported misses of capsule intake >5 times/week), leaving 65 subjects for analysis who successfully completed this double-blind interventional trial (mean age: 63.9 years ± 6.6 SD, range from 50 to 75 years, 30 females, mean BMI: 27.6 kg/m^2 ± 1.7 SD, with 10–22 years of formal education, mean 16.3 years ± 3 SD). Those subjects that were included into the analysis did not differ with regard to age, sex, or education from those excluded (all $P > 0.77$), or those of the caloric restriction study (all $P > 0.12$), or drop-outs (all $P > 0.56$). Baseline characteristics did not differ between groups (Table 1). After 26 weeks of intervention/control period, all baseline measurements were repeated (Session 2; see Fig. 1).

The study was conducted at the Department of Neurology at the Charité University Hospital of Berlin, Germany. All subjects provided written informed consent and received reimbursement. The research protocol was in accordance with the Declaration of Helsinki and approved by the local Ethics Committee.

Compliance

Compliance was monitored by an evaluation questionnaire at the end of the study and by capsule counts after 12 and 26 weeks. In addition, the omega-3 index (von Schacky and Harris 2007) served as a measure of LC-n3-FA intake, and subjects had to fill out detailed nutrition protocols over periods of 7 days at baseline, after 12 weeks, and again after 26 weeks, to monitor dietary intake of fatty acids. They were instructed not to change dietary habits, for example, monthly fish consumption, throughout the intervention.

Omega-3 Index

Erythrocyte membrane fatty acid compositions were assessed at baseline and after 26 weeks. Blood samples were collected and immediately centrifuged, and the erythrocyte fraction was stored at −80 °C until assayed. Two samples of the control group had to be excluded due to technical problems. The omega-3 index (von Schacky and Harris 2007) was defined as the percentage of EPA (C20:5n-3) + DHA (C22:6n-3) of total fatty acid areas, determined using a gas chromatograph (HP 5890 Series II with Autosampler). Analyses were performed by Lipidomix Laboratory, Berlin, Germany.

Neuropsychological Testing

Neuropsychological testing comprised verbal fluency, trail making test (TMT) part A and B, Stroop Color-Word test, auditory verbal learning task (AVLT), and forward and backward digit spans (Lezak 2004; van de Rest et al. 2008). In the AVLT (Lezak 2004), participants had to remember and recall a list of 15 words within 5 immediate recall trials, followed by a 30-min delayed recall and recognition trial. Memory consolidation was defined as the number of correct words recalled after the fifth trial subtracted by those correctly recalled after the 30-min delay. Parallel versions were used to avoid test–retest effects. Test scores were z-transformed and averaged to create composite scores for executive functions, memory performance (primary outcomes), sensorimotor speed, and attention, according to van de Rest et al. (2008). Composite scores were defined as follows: executive functions = [z phonemic fluency + z semantic fluency −z TMT (part B − part A)/part A −z STROOP (part 3 − (part 1 + part 2))/2]/4; memory = (z AVLT learning + z AVLT delayed recall + z AVLT recognition + z digit span backward)/4; sensorimotor speed = (−z TMT part A − z STROOP part A − z STROOP part B)/3; attention = z digit span forward. Mood during testing was assessed by the positive and negative affect schedule (PANAS; Krohne et al. 1996; 2 subjects could not be evaluated due to missing values).

Magnetic Resonance Imaging

MRI was performed on a Siemens Trio system operating at 3 T using a 12-channel head coil. Each subject underwent a 3-dimensional scanning protocol using diffusion-weighted images using a spin-echo planar imaging sequence (time to repeat, TR = 7500 ms, time to echo, TE = 86 ms, 61 axial slices, voxel size of 2.3 × 2.3 × 2.3 mm^3; 64 directions with a *b*-value of 1000 s/mm^2 and one *b*0). In addition, high-resolution T_1-weighted magnetization prepared rapid gradient-echo images (TR = 1900 ms, TE = 2.52 ms, 192 sagittal slices, voxel size of 1.0 × 1.0 × 1.0 mm^3, flip angle = 9°) were acquired. Image preprocessings and analyses were done using the software packages FSL (www.fmrib.ox.ac.uk/fsl) and freesurfer (http://surfer.nmr.mgh.harvard.edu/).

For voxel-wise analysis of changes in regional white matter microstructure, we used a customized longitudinal version of tract-based spatial statistics (TBSS; Smith et al. 2006). Nine subjects had to be excluded due to missing scans, technical problems, or registration errors, leaving 56 for analysis. For voxel-wise analysis of changes in GM volume, we used a customized longitudinal version of voxel-based morphometry (VBM; Good et al. 2001), implemented in FSL (Douaud

et al. 2007). Two subjects had to be excluded due to missing scans, leaving 63 for analysis. For details of TBSS and VBM procedures, see Supplementary Information.

Carotid Intima Media Thickness

All subjects underwent B-mode duplex ultrasound of the distal right and left common carotid artery. Recordings were obtained with the subject resting in a supine position with the head turned to the contralateral side of the respective artery. CIMT was assessed according to the Mannheim Intima Media Thickness Consensus (Touboul et al. 2007). CIMT of the far vessel wall was semi-automatically measured with the ultrasound transducer positioned 1 cm proximal to the carotid bulb using a commercially available standardized real-time measurement method (Esaote Mylab25Gold, Cologne, Germany). CIMT was defined as the distance between the characteristic echoes of the lumen-intima interface and the media-adventitia interface. Mean values (measured in μm) were created by performing 3 CIMT measurements of each side.

Blood Parameters and Anthropometric Data

Fasting serum levels were collected to assess levels of triacylglycerides, total cholesterol, high-to-low density lipoprotein (HDL-to-LDL) ratio, insulin, glucose, glycated hemoglobin A1c (HbA1c), brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1, high-sensitive C-reactive protein, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). All parameters were analyzed by IMD Laboratory, Berlin, Germany. Anthropometry included weight, height, and body fat (percentage, measured using bioelectrical impedance analysis). Subjects also reported their physical activity and other lifestyle habits using the Freiburger physical activity questionnaire (Frey et al. 1999) implemented in a questionnaire on lifestyle habits (Floel et al. 2008).

Statistical Analysis

Analyses were done using SPSS 19 (PASW, SPSS, IBM). To detect significant effects of LC-n3-FA supplementation on executive functions and memory performance, we used repeated-measures analysis of variance ($ANOVA_{RM}$) with dependent variable "executive functions" or "memory," a repeated factor "time" (baseline vs. follow-up), and a between-subject factor "group" (LC-n3-FA vs. placebo). Analysis was conducted without prespecified statistical analysis plan prior to the study. Correction for multiple comparisons was done using the Bonferroni method; significance was set to $P < 0.05/2$. Student's *t*-tests were run for post hoc comparisons, if appropriate.

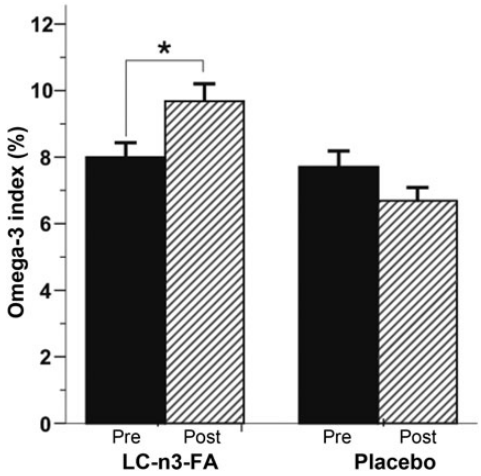

Figure 2. Proportion of LC-n3-FA in erythrocyte membranes (omega-3 index) at baseline (pre, black bars) and after intervention/control period (post, striped bars). Note that subjects of the LC-n3-FA supplementation group had a significantly higher omega-3 index after the intervention compared with placebo ($ANOVA_{RM}$, $P = 0.002$). Error bars indicate standard error. *$P < 0.05$ according to post hoc *t*-test.

As exploratory analyses, we conducted $ANOVA_{RM}$ for other neuropsychological outcome parameters and omega-3 index to detect significant group by time interactions. Additionally, we focused on a subgroup of subjects that adhered best to the intervention, defined as a change in omega-3 index of at least or at max 1 confidence interval of the group's mean, $n = 36$ (cutoff values: LC-n3-FA group, minimum +0.35%, $n = 18$; placebo, maximum −0.04%, $n = 18$). Demographic characteristics at baseline were compared between groups using independent *t*-tests, Mann–Whitney *U*-tests, or χ^2 tests. Changes over time in vascular parameters, anthropometric measures, self-reported physical activity, mood, and serum parameters were evaluated using paired *t*-tests or nonparametric tests, as appropriate. Associations between changes in cognitive functions and serum parameters were assessed using Spearman's correlations. Levels of significance were set at $P < 0.05$.

Longitudinal Voxel-Wise Statistics of White Matter Microstructure and Gray Matter Volume

After preprocessing, fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and GM volume datasets were voxelwise tested for the statistical inference of group by time interactions using permutation-based methods, accounting for nonlinearity of structural images. Subject-specific differences in cortical FA, MD, RD, and GM between timepoints 1 and 2 were calculated and voxelwise compared between groups (LC-n3-FA vs. placebo) using the "randomise" tool implemented in FSL. Correction for multiple comparisons was done using threshold-free cluster enhancement (TFCE). A minimal cluster size of 5 voxels was defined. Significance was set at $P < 0.001$.

Results

Changes in Omega-3 Index

After the intervention, subjects of the LC-n3-FA group displayed significantly higher proportions of DHA and EPA measured in the membranes of erythrocytes of peripheral blood (omega-3 index; von Schacky and Harris 2007), compared with controls ($ANOVA_{RM}$, $F_{1,61} = 10.4$, $P = 0.002$; post hoc *t*-test, $t_{(31)} = 2.6$, $P = 0.015$; Fig. 2). Notably, LC-n3-FA supplementation led to significant increases in EPA proportions (Wilcoxon signed rank test, $T = 4.3$, $P < 0.001$), whereas significant decreases were noted in the placebo group (Wilcoxon signed rank test, $T = -3.2$, $P = 0.002$; Table 2). Changes in DHA proportions did not reach significance ($P > 0.05$).

LC-n3-PUFA intake based on fish consumption was moderate in our sample, and comparable between groups, with most of the subjects consuming fish one time per week (see Table 2). These habits did not change in the course of the intervention, neither in the placebo, nor in the LC-n3-PUFA, group (all $P > 0.66$).

Cognitive Changes

We observed a significant interaction effect of group X time on executive functions ($ANOVA_{RM}$, $F_{1,63} = 5.43$, $P = 0.023$; Bonferroni-corrected). Post hoc *t*-tests showed that LC-n3-FA supplementation enhanced executive functions by 26%, whereas performance remained constant after placebo (paired *t*-test, $t_{(31)} = 3$, $P = 0.005$; Fig. 3*A*). Notably, improvements in one of the subtests of the composite score, that is letter fluency, correlated with increases in omega-3 index (trend, $r = 0.34$, $P = 0.06$), and EPA ($r = 0.46$, $P = 0.009$; Fig. 3*B*) after the intervention in the LC-n3-FA group.

Regarding the composite score for memory, both groups showed a similar retest effect at follow-up, with no significant effect of group ($ANOVA_{RM}$, group X time: $P = 0.6$; time:

Table 2

Vascular parameters, anthropometric measures, omega-3 index, dietary fish consumption, physical activity, mood, and fasting serum parameters of participants before and after the intervention period

Parameter	LC-n3-FA			Placebo		
	Pre	Post	*P*-value	Pre	Post	*P*-value
CIMT left, μm	693.2 ± 131.0	662.7 ± 114.3	0.21[a]	639.5 ± 103.8	633.9 ± 126.2	0.81[a]
CIMT right, μm	677.1 ± 132.2	671.1 ± 141.2	0.99[b]	651.5 ± 122.0	645.2 ± 106.1	0.75[a]
Systolic blood pressure, mmHg	141.27 ± 15.8	139.4 ± 15.7	0.53[b]	138.6 ± 14.3	138.9 ± 14.5	0.53[b]
Diastolic blood pressure, mmHg	88.2 ± 7.7	85.2 ± 9.8	**0.047**[b]	86.8 ± 9.4	85.0 ± 7.5	0.51[b]
Weight, kg	84.7 ± 9.7	85.4 ± 10.4	0.17[a]	82.4 ± 8.3	82.2 ± 8.1	0.59[a]
Body mass index, kg/m^2	27.7 ± 1.9	27.8 ± 1.9	0.51[b]	27.6 ± 1.6	27.5 ± 1.6	0.39[b]
Body fat, %	31.1 ± 7.2	31.7 ± 6.8	0.08[b]	29.4 ± 8.7	31.0 ± 7.1	**0.043**[b]
Omega-3 index, %	8.0 ± 2.5	9.7 ± 2.9	**0.014**[a]	7.8 ± 2.6	6.8 ± 2.2	0.057[a]
EPA, %	1.9 ± 0.84	3.2 ± 0.9	**0.000**[b]	1.8 ± 0.7	1.3 ± 0.6	**0.002**[b]
DHA, %	6.1 ± 1.9	6.5 ± 2.2	0.65[b]	5.9 ± 2.2	5.5 ± 1.7	0.21[b]
Dietary fish consumption, *n*%						
Every day	0	0	0.66[c]	0	3	0.81[c]
>1× per week	21.9	21.9		15.2	24.2	
1× per week	43.8	46.9		54.5	39.4	
>1× per month	15.6	15.6		18.2	15.2	
1× per month or less	15.6	9.4		9.1	15.2	
Never	3.1	6.2		3	3	
Physical activity, kcal/week	6765.7 ± 5413.8	5658.1 ± 4222.2	0.16[b]	4299.7 ± 4061.2	4277.3 ± 3631.3	0.28[b]
Positive PANAS score	32.2 ± 7.7	32.1 ± 8.1	0.92[b]	32.8 ± 6.5	30.7 ± 7.1	**0.037**[b,d]
Negative PANAS score	11.7 ± 3.7	12.0 ± 4.2	0.79[a]	12.1 ± 2.9	12.3 ± 2.6	0.78[a]
Triacyglycerides, mg/dL	100.8 ± 37.6	87.3 ± 29.8	**0.009**[a]	101.1 ± 41.6	104.7 ± 49.3	0.57[a]
Total cholesterol, mg/dL	214.6 ± 29.2	218.0 ± 27.9	0.34[a]	222.0 ± 37.3	224.4 ± 39.3	0.54[a]
HDL-to-LDL ratio	2.1 ± 0.5	2.1 ± 0.5	0.64[a]	2.4 ± 0.7	2.4 ± 0.7	0.75[b]
Insulin, mU	9.2 ± 4.9	8.8 ± 3.9	0.56[b]	8.5 ± 4.2	8.2 ± 2.7	0.72[b]
Glucose, mg/dL	91.1 ± 9.7	93.6 ± 8.2	0.11[a]	91.1 ± 9.0	91.9 ± 9.3	0.58[a]
HbA1c, %	5.8 ± 0.3	5.8 ± 0.3	0.82[b]	5.8 ± 0.3	5.9 ± 0.3	0.12[b]
BDNF, pg/mL	4051.7 ± 296.2	4316.1 ± 422.8	**0.003**[a]	4104.2 ± 439.4	4379.1 ± 351.6	**0.016**[a]
IGF-1, ng/mL	152.6 ± 47.9	152.2 ± 48.8	0.72[b]	143.0 ± 45.5	137.2 ± 37.4	0.33[b]
hsCRP, pg/mL	1.9 ± 2.1	1.8 ± 3.1	0.86[a]	2.7 ± 3.4	1.7 ± 1.9	0.09[a]
TNF- α, pg/mL	11.3 ± 2.8	9.3 ± 1.4	**0.001**[b]	14.8 ± 14.4	9.8 ± 2.0	**0.008**[b]
Interleukin-6, pg/mL	4.3 ± 4.2	2.4 ± 1.0	**0.000**[b]	6.1 ± 17.0	2.4 ± 0.9	**0.008**[b]

Note: Significant changes are indicated by bolding the number. Data are given as mean ± SD.

CIMT: carotid intima media thickness; PANAS: The positive and negative affect schedule; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HbA1c: hemoglobin A1c; BDNF: brain-derived neurotrophic factor; IGF-1: insulin-like growth factor 1; hsCRP: high-sensitive C-reactive protein; TNF-α: tumor necrosis factor-alpha.

[a]Paired *t*-test.

[b]Wilcoxon signed-rank test.

[c]Wilcoxon signed-rank test (change in fish consumption frequency pre- vs. postintervention).

[d]Adjusting for changes in PANAS scores did not alter the significant beneficial effect of LC-n3-FA compared with placebo on executive functions ($ANCOVA_{RM}$, $P < 0.05$).

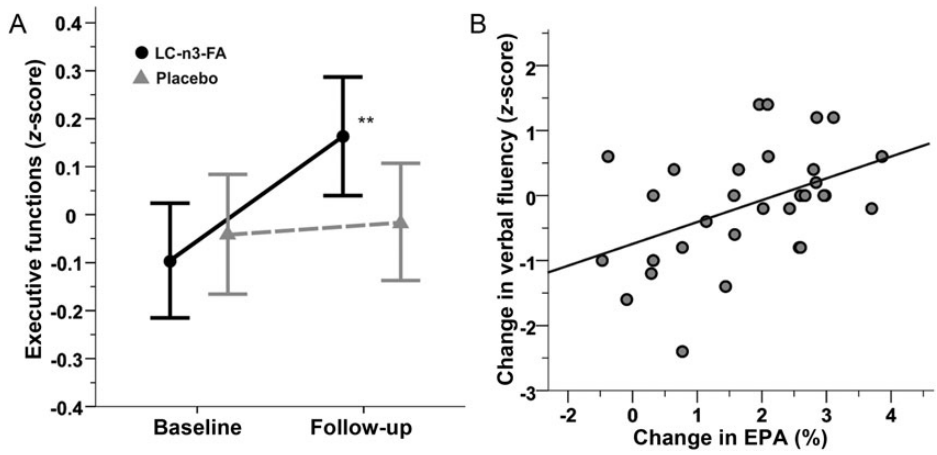

Figure 3. Changes in executive functions due to 26 weeks of supplementary LC-n3-FA or placebo. (*A*) Subjects of the LC-n3-FA-group (black, circles) significantly improved in executive functions compared with controls (dashed, triangles, $ANOVA_{RM}$, $P = 0.023$, Bonferroni-corrected). (*B*) Improvements in a subtest of executive functions, that is verbal fluency, correlated significantly with increases in EPA content in the membranes of erythrocytes after LC-n3-FA supplementation ($P = 0.009$). Error bars indicate standard error. $^{**}P < 0.01$ according to post hoc *t*-test.

$F_{1,63} = 19.8$, $P < 0.001$). When focusing the analysis on those individuals with best response to the intervention/placebo condition (according to changes in omega-3 index, $n = 37$), a selective interaction effect emerged for memory consolidation, showing a trend for improvements after LC-n3-FA but not after placebo ($ANOVA_{RM}$ $F_{1,35} = 3.1$, $P = 0.09$; post hoc *t*-test,

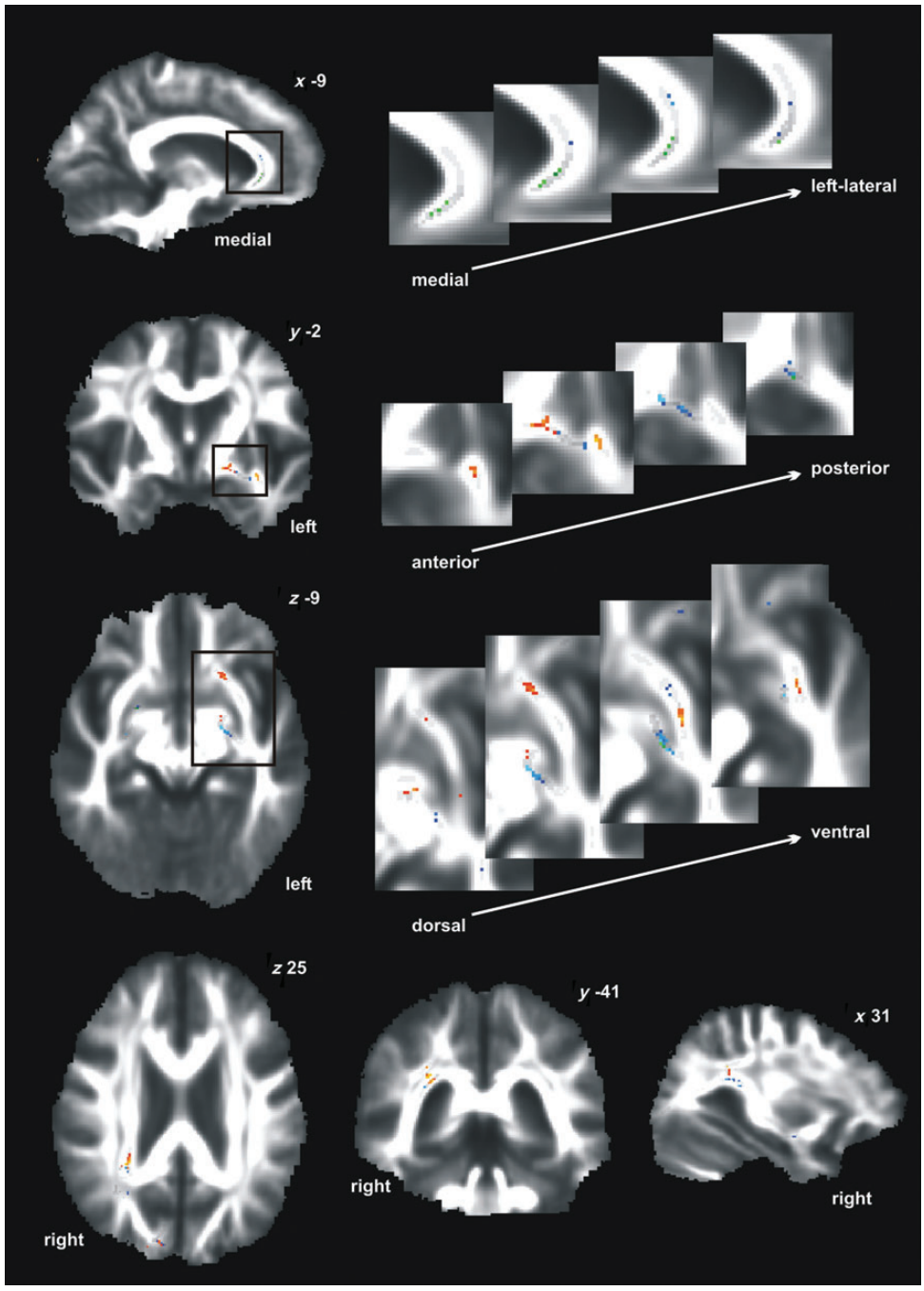

Figure 4. Regional changes in measures of cortical white matter integrity after 26 weeks of supplementary LC-n3-FA, compared with placebo. LC-n3-FA supplementation induced significant increases in FA (red), as well as decreases in MD (green) and RD (blue) within selective white matter tracts. These were located in the left hemisphere, that is in the anterior corpus callosum, in the uncinate fasciculus, and in the inferior-occipital fasciculus, and in the right hemisphere, that is in the superior longitudinal fasciculus and in the superior and inferior fronto-occipital fasciculus. Colors indicate significant voxels ($P < 0.001$, corrected using TFCE), superimposed on a study-specific FA template. Images are displayed in neurological convention, coordinates according to Montreal Neurological Institute (MNI).

$t_{(17)} = 1.55$, $P = 0.14$). Changes in performance correlated with relative changes in DHA after LC-n3-FA supplementation ($r = 0.49$, $P = 0.041$).

For sensorimotor speed, a global retest effect was noticed that did not differ between groups (composite score, $ANOVA_{RM}$, time: $F_{1,63} = 14$, $P < 0.001$). No significant effects were found for attention ($ANOVA_{RM}$, all $P > 0.26$).

Changes in Measures of White Matter Integrity

Voxel-wise permutation testing detected that LC-n3-FA supplementation led to significant increases in FA as well as decreases in MD and RD, indicating superior white matter structural integrity (Alexander et al. 2007), within selective white matter tracts ($P < 0.001$, TFCE-corrected; see Fig. 4). Voxels were located in the anterior corpus callosum, the

uncinate fasciculus, and the inferior-occipital fasciculus in frontal, temporal, and limbic areas of the left hemisphere, and in the superior longitudinal fasciculus and superior and inferior fronto-occipital fasciculus within parietal, temporal, limbic, and occipital areas of the right hemisphere (see Table 3). No selective effects over time could be observed for the inverse contrast (placebo > omega).

Gray Matter Changes

Voxel-wise permutation testing detected a differential effect of LC-n3-FA supplementation, namely that subjects of this group showed significant increases in regional GM volume compared with placebo in the left hippocampus, precuneus, superior temporal, inferior parietal and postcentral gyri, and in the right middle temporal gyrus ($P < 0.001$, TFCE-corrected; Fig. 5; Table 3). No selective effects over time could be observed for the inverse contrast (placebo > LC-n3-FA).

For total GM volume, measured using automated tissue type segmentation of whole-brain images, subjects of the LC-n3-FA group did not show the loss of total GM volume over the intervention/placebo period that was noted in the placebo group (−0.58%, paired *t*-test, $t_{(32)} = -2.2$, $P = 0.037$; Fig. 6).

Table 3
Brain coordinates of changes in FA, MD and RD, and in GM volume

Contrast: LC-n3-FA > placebo	No. of voxels[a]	MNI coordinates (hot voxel)		
		x	*y*	*z*
Brain area				
Frontal lobe, left				
Uncinate fasciculus (FA)	27	−22	20	− 9
Postcentral cortex (GM)	8	−18	−32	74
Corpus callosum, left				
Forceps minor (MD)	5	−7	26	1
	5	−8	24	−2
Forceps minor (RD)	8	−7	26	1
	5	−8	23	−3
Temporal lobe, left				
Superior longitudinal fasciculus (FA)	35	−16	−2	−7
Superior longitudinal fasciculus (RD)	76	−30	−5	−14
	6	−41	−29	−8
	5	−25	−13	−5
Uncinate fasciculus (FA)	43	−34	−1	−13
Uncinate fasciculus (RD)	6	−29	7	−11
Superior temporal cortex (GM)	8	−46	−10	−4
Hippocampus (GM)	6	−24	−16	−14
Temporal lobe, right				
Superior longitudinal fasciculus (FA)	11	53	−16	30
Superior longitudinal fasciculus (RD)	19	48	−59	12
	6	42	−58	18
	19	40	−47	14
	14	37	−56	9
Inferior longitudinal fasciculus (RD)	7	29	−6	−12
	5	40	−13	−26
Uncinate fasciculus (RD)	5	22	4	−10
Middle temporal cortex (GM)	10	64	−18	−6
Parietal lobe, right				
Inferior fronto-occipital fasciculus (FA)	73	29	−42	24
Inferior fronto-occipital fasciculus (RD)	34	32	−40	19
	6	29	−47	24
	5	9	−48	59
Parietal lobe, left				
Inferior parietal cortex (GM)	14	−63	−70	48
Precuneus (GM)	5	−8	−54	50
Occipital lobe, right				
Inferior fronto-occipital fasciculus (RD)	11	8	−79	30
	24	20	−77	33
Inferior fronto-occipital fasciculus (FA)	45	13	−82	26
	12	18	−84	33
	6	18	−84	24
Inferior fronto-occipital fasciculus (MD)	8	11	−83	27
Inferior fronto-occipital fasciculus (RD)	21	11	−82	23
	12	12	−86	16

$P < 0.001$, TFCE-corrected.
[a]Clusters <5 voxels not shown.

Changes in Vascular Markers

A decrease of left CIMT was noted after LC-n3-FA supplementation (−4.4%), which reached significance in women only (paired *t*-test, $t_{(14)} = 2.2$; $P = 0.047$). Moreover, subjects of the LC-n3-FA group showed a significant decrease in diastolic blood pressure after the intervention (−3.4%, $t_{(31)} = 2.1$, $P = 0.044$) that was not observed in controls. For details on vascular parameters, other anthropometric measures, self-reported physical activity, and mood, please see Table 2.

Changes in Fasting Serum Levels

LC-n3-FA supplementation led to a significant decrease in fasting triacylglycerides (paired *t*-test, $t_{(31)} = 2.8$, $P = 0.009$), whereas levels of triacyglycerides remained unchanged in the placebo group. Both groups exhibited higher concentrations of BDNF and lower levels of inflammatory markers [TNF-α and IL-6 (all $P < 0.016$)] and reduced insulin (not significant, all $P > 0.05$). For details of serum parameters, please see Table 2.

In the LC-n3-FA group, changes in cognitive performance were not only associated with increases in omega-3 index, but also with that in BDNF as well as insulin. Specifically, improvements in executive functions were correlated with relative increases in BDNF ($r = 0.46$, $P = 0.024$, Fig. 7*A*). Improvements in executive functions and memory consolidation correlated also with decreases in fasting insulin (executive functions, $r = -0.45$, $P = 0.01$, Fig. 7*B*; memory consolidation, $r = -0.51$, $P = 0.032$).

Increases in total GM correlated inversely with changes in fasting insulin ($r = -0.48$, $P = 0.007$) and glucose ($r = -0.47$, $P = 0.009$). In addition, improvements in mean FA across the white matter skeleton correlated with decreases in HbA1c ($r = -0.41$, $P = 0.03$) and diastolic blood pressure ($r = -0.38$, $P = 0.043$).

Discussion

This interventional study demonstrates enhanced executive functions in healthy older adults after 26 weeks of high levels of supplementary marine LC-n3-FA, compared with placebo. In parallel, LC-n3-FA improved white matter microstructural integrity, GM volume, and vascular parameters. Cognitive improvements correlated with increases in omega-3 index and peripheral BDNF, and with decreases in fasting insulin.

Our findings support previous studies reporting beneficial effects of LC-n3-FA on cognition (Gomez-Pinilla 2008; Hooijmans et al. 2012). For example, a higher omega-3 index correlated with better executive performance (Tan et al. 2012), and 24 weeks of supplementary LC-n3-FA improved executive functions in schizophrenic patients (Reddy et al. 2011). In contrast, a double-blind interventional study in healthy older subjects could not detect specific effects on executive functions (van de Rest et al. 2008). However, LC-n3-FA intake in that study (maximum 1800 mg/day; our study: 2200 mg/day) might not have been sufficient to exert significant effects on cognition. In addition, our study may differ with regard to intake instructions or cohort characteristics (smaller MMSE range, exclusion of brain pathologies by MRI).

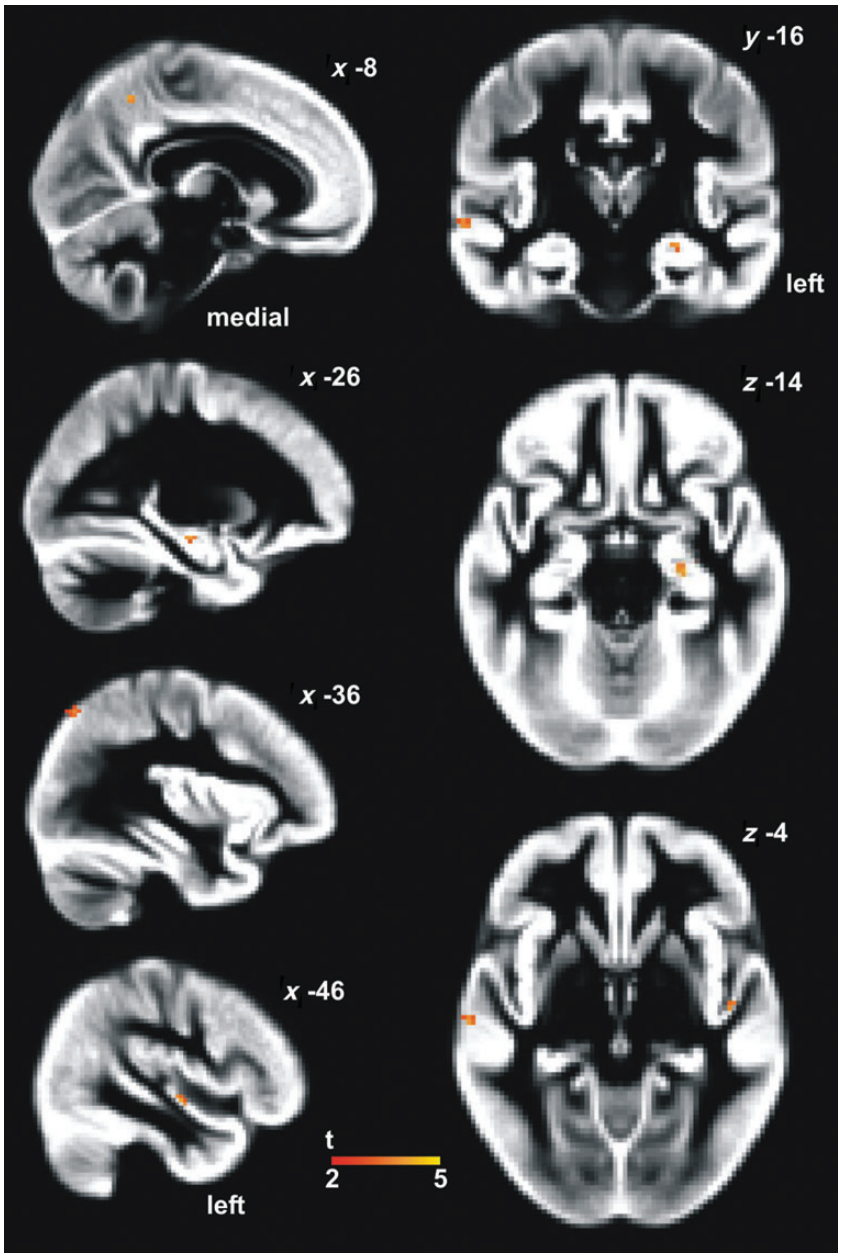

Figure 5. Regional changes of cortical gray matter volume after 26 weeks of supplementary LC-n3-FA, compared with placebo. LC-n3-FA-induced gray matter volume increases were found in areas within the left hippocampus, precuneus, superior temporal, inferior parietal and postcentral gyri, and in the right middle temporal gyrus. Color bar indicates significant voxels ($P < 0.001$, corrected using TFCE), superimposed on a study-specific gray matter template. Images are displayed in neurological convention, coordinates according to MNI.

We could not observe significant improvements after LC-n3-FA on composite memory scores, which is in line with others (van de Rest et al. 2008). However, in a subgroup of those subjects adhering best to the intervention, we did find a trend for better memory consolidation after LC-n3-FA compared with placebo. Positive effects of LC-n3-FA on memory functions have been reported by interventional trials in healthy subjects (Yurko-Mauro et al. 2010), and in subgroups of patients with MCI (Chiu et al. 2008) or mild AD (Freund-Levi et al. 2006). In addition, epidemiological studies suggested that higher intake of fish rich in LC-n3-FA may decrease dementia risk (Fotuhi et al. 2009). Thus, with a longer duration of LC-n3-FA intake, the statistical trend observed in our study for memory consolidation might have reached significance.

Regarding brain structure, we observed LC-n3-FA-induced increases in FA and decreases in MD and RD, which can be interpreted as superior microstructural architecture, due to higher myelination, increased fiber packing density, and reduced axonal damage (Alexander et al. 2007). Thus, improvements in these measures might be linked to better axonal transmission, and studies reported positive correlations with specific cognitive skills (Klingberg et al. 2000). LC-n3-FA-induced microstructural improvements were found in fiber tracts within the left anterior corpus callosum, which primarily connects

prefrontal areas. Executive functioning in older adults is associated with bilateral recruitment of lateral prefrontal areas (Turner and Spreng 2012), and tasks comprising verbal material are predominantly processed in left-hemispheric networks involving frontal, but also temporal, areas (Turken and Dronkers 2011). Thus, LC-n3-FA-induced improvements in microstructural architecture within anterior callosal tracts and within fibers connecting left frontal and temporal areas might have translated into the behavioral advantages in executive processing observed after LC-n3-FA supplementation.

As ventral and dorsal prefrontal cortex is also implicated in memory selecting and verification processes (Fletcher and Henson 2001), improved microstructure of connecting fibers may also exert beneficial effects on memory consolidation. Moreover, we observed increases in regional GM volume within core brain regions of episodic memory, for example, the left hippocampus, precuneus, and temporal areas (Dickerson and Eichenbaum 2010), after LC-n3-FA. In a cross-sectional regions-of-interest analysis, Conklin et al. (2007) likewise observed increased hippocampal GM volume in subjects with higher LC-n3-FA intake. Regional increases in GM volume have been suggested to serve as measures of structural plasticity in the living adult human brain, for example, due to synaptogenesis, neurogenesis, and/or angiogenesis (Dragansky et al. 2004; Thomas and Baker 2013). Thus, beneficial effects of LC-n3-FA supplementation on GM within the medial temporal lobe and precuneus might have led to (marginal) improvements in memory functions, at least in those subjects adhering best to the intervention.

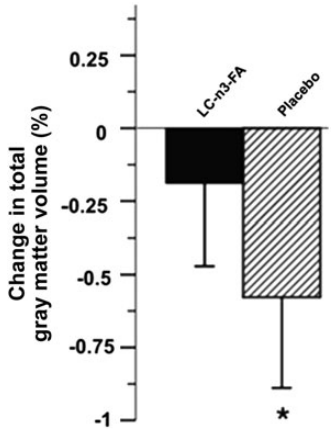

Figure 6. Global structural changes after 26 weeks of supplementary LC-n3-FA or placebo. Subjects of the LC-n3-FA-group (black bars) did not show the significant decrease in total gray matter volume that was observed after placebo (gray striped bars, paired *t*-test $P = 0.037$). Error bars indicate standard error. *$P < 0.05$.

Correlations between increases in cognitive performance and omega-3 index suggest that a better response to the supplementation relates to a larger effect. This might be due to positive effects of LC-n3-FA on neuronal function, for example, via enhancement of synaptic membrane fluidity and plasticity (Cansev and Wurtman 2007; Gomez-Pinilla 2008).In addition, increased expression of myelin-related proteins in the rat brain was reported after EPA injection (Salvati et al. 2008), which points to a more efficient axonal transmission. Stimulation of myelin synthesis might also explain improved white matter microstructure after LC-n3-FA, see Song et al. (2002). In addition, DHA was found to promote neurite outgrowth and neurogenesis in the hippocampus (Kawakita et al. 2006), and to increase synaptic membrane areas and the expression of synaptic proteins (Cansev and Wurtman 2007; Gomez-Pinilla 2008). These molecular changes might have led to the observed improvements in memory consolidation and GM volume, for example, in the hippocampus. Notably, EPA can be converted into DHA by astrocytes and released into the extracellular space (Moore et al. 1991).

DHA was also reported to increase hippocampal BDNF in rats (Wu et al. 2004), and higher BDNF levels have been linked to larger hippocampal and prefrontal GM volume (Pezawas et al. 2004) and superior memory (Egan et al. 2003) and executive processing in humans (Rybakowski et al. 2006). This is in line with our results showing that increases in peripheral BDNF correlated with improved executive functions after LC-n3-FA; however, peripheral levels may not adequately reflect central BDNF concentrations. In addition, we observed an increase in BDNF at postintervention measurements in both the placebo and LC-n3-FA group, as well as a decrease in TNF-α and IL-6, which may be due to potential changes in lifestyle habits such as diet or exercise in both groups. Even though nutrition records and detailed questionnaires at baseline and follow-up did not actually show significant changes in lifestyle habits in our subjects, lifestyle measures were only based on self-reported information and may thus be over- or underestimations.

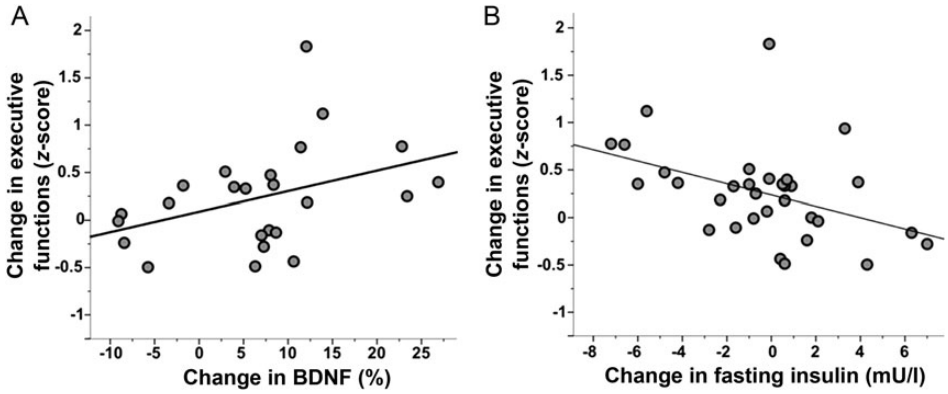

Figure 7. Correlations of cognitive and structural changes after supplementary LC-n3-FA with changes in fasting serum levels. Increases in executive functions correlated with changes in BDNF ($P = 0.024$; *A*) and with decreases in fasting insulin ($P = 0.01$; *B*).

Future studies implementing, for example, physical activity monitors are needed to address this problem in more detail. In addition, potential methodological limitations in laboratory assessments may have contributed to the observed changes in peripheral parameters.

In addition, global measures of total GM volumes suggested an overall protective effect of omega-3 compared with placebo. Several cross-sectional studies in normal aging demonstrate a significant GM volume loss over time, starting in the second decade of life, which regionally magnifies in older age (Good et al. 2001; Jernigan et al. 2001; Walhovd et al. 2005). In the study by Walhovd et al. (2005), a linear cortical volume loss of 26% was suggested from the year 20 to 90, which refers to approximately 0.4% volume loss every year. Longitudinal studies so far even reported greater volume loss than the estimates based on cross-sectional studies, for example, 0.8% for the striatum every year (caudate nucleus; Raz et al. 2003), and 0.8% for the hippocampus after 6 months (control group, left hippocampus; Erickson et al. 2011). Taken together, the observed approximately 0.5% loss of total GM volume in the placebo group over the course of 6 months most likely represent "normal" aging effects. Notably, improvements in both cognitive performance and total GM volume correlated with decreases in fasting insulin and glucose in the LC-n3-FA group. This may be caused by higher peripheral and central insulin sensitivity due to LC-n3-FA (e.g., via fatty acid oxidation; Agrawal and Gomez-Pinilla 2012), leading to improved insulin-related signaling in the brain and in turn to enhanced cognitive processing (Zhao et al. 2004).

In line with cross-sectional results (Sekikawa et al. 2011), LC-n3-FA led to reductions in diastolic blood pressure and CIMT in women, underlining antiatherogenic and antiinflammatory effects of LC-n3-FA, for example, via up-regulation of adiponectin or reduction of proinflammatory cytokines (Fotuhi et al. 2009). These neuroprotective pathways (Agrawal and Gomez-Pinilla 2012) may have additionally contributed to our results. CIMT measurements demonstrated a CIMT reduction in women, only significant for the left side which might indicate a need for longer observation times.

Study limitations include the number of subjects and intervention length. This might account for marginal effects only on memory functions. With a larger sample size and thus greater statistical power, we may have observed significant group-specific effects on memory performance, similar to what we found for executive functions in our cohort. Additionally, changes in structural MR signals may be due several factors, which cannot be fully differentiated in noninvasive studies. Yet, we conducted a full longitudinal analysis stream with placebo-controlled contrasts, and the regional extent of LC-n3-FA-induced changes in FA, MD, and RD did largely overlap, which strengthens the assumption of specific effects (Thomas and Baker 2013). In addition, 5 subjects of the LC-n3-PUFA group noted (fishy) burps following capsule intake in the postevaluation questionnaire, which may have influenced blinding.

The current results provide experimental evidence that marine LC-n3-FA improve executive functions, white matter microstructure, GM volume, and vascular markers in older adults. Cognitive improvements correlated with increases in omega-3-index, BDNF, and with decreases in fasting insulin, underlining positive effects of EPA and DHA on neuronal functioning as postulated by animal experiments. These findings may help to develop new prevention and treatment strategies for maintaining cognitive health into older age.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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