FISH OIL SUPPLEMENTATION INCREASES EVENT-RELATED POSTERIOR CINGULATE ACTIVATION IN OLDER ADULTS WITH SUBJECTIVE MEMORY IMPAIRMENT

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Abstract: *Objectives:* To determine the effects of long-chain omega-3 (LCn-3) fatty acids found in fish oil, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on cortical blood oxygen level-dependent (BOLD) activity during a working memory task in older adults with subjective memory impairment. *Design:* Randomized, double-blind, placebo-controlled study. *Setting:* Academic medical center. Participants: Healthy older adults (62-80 years) with subjective memory impairment, but not meeting criteria for mild cognitive impairment or dementia. *Intervention:* Fish oil (EPA+DHA: 2.4 g/d, n=11) or placebo (corn oil, n=10) for 24 weeks. *Measurements:* Cortical BOLD response patterns during performance of a sequential letter n-back working memory task were determined at baseline and week 24 by functional magnetic resonance imaging (fMRI). *Results:* At 24 weeks erythrocyte membrane EPA+DHA composition increased significantly from baseline in participants receiving fish oil (+31%, $p \leq 0.0001$) but not placebo (-17%, $p=0.06$). Multivariate modeling of fMRI data identified a significant interaction among treatment, visit, and memory loading in the right cingulate (BA 23/24), and in the right sensorimotor area (BA 3/4). In the fish oil group, BOLD increases at 24 weeks were observed in the right posterior cingulate and left superior frontal regions during memory loading. A region-of-interest analysis indicated that the baseline to endpoint change in posterior cingulate cortex BOLD activity signal was significantly greater in the fish oil group compared with the placebo group during the 1-back ($p=0.0003$) and 2-back ($p=0.0005$) conditions. Among all participants, the change in erythrocyte EPA+DHA during the intervention was associated with performance in the 2-back working memory task ($p = 0.01$), and with cingulate BOLD signal during the 1-back ($p = 0.005$) with a trend during the 2-back ($p = 0.09$). Further, cingulate BOLD activity was related to performance in the 2-back condition. *Conclusion:* Dietary fish oil supplementation increases red blood cell omega-3 content, working memory performance, and BOLD signal in the posterior cingulate cortex during greater working memory load in older adults with subjective memory impairment suggesting enhanced neuronal response to working memory challenge.

Key words: Omega-3 fatty acids, working memory, cingulate cortex, functional magnetic resonance imaging, aging.

Introduction

There is increasing theoretical and experimental interest in developing early intervention strategies to delay or prevent progression to dementia and Alzheimer's disease (AD)(1, 2). Evidence from retrospective and prospective studies has identified prodromal cognitive features to identify individuals at increased risk for developing dementia. For example, longitudinal studies suggest that elderly participants with subjective memory impairment are at increased risk for AD (3-5) and may exhibit early neurodegenerative changes (6-8). Based on these and other findings subjective cognitive impairment is now considered an early risk factor in preclinical dementia (9). While support for modifiable risk factors and early intervention strategies is also emerging (10), such strategies remain underdeveloped options and warrant systematic investigation.

One candidate early intervention strategy may involve modification of the diet to increase the intake of beneficial nutrients. Evidence from cross-sectional and prospective longitudinal studies suggests that habitual diets containing higher levels of fish may be protective against cognitive decline and the development of dementia and AD (11-15). Moreover, fish intake is positively correlated with erythrocyte and plasma levels of long-chain omega-3 (LCn-3) fatty acid, including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3)(16), and patients with dementia and non-demented elderly participants with cognitive impairment exhibit significantly lower blood EPA and/or DHA levels (17). Human neuroimaging studies suggest that greater dietary fish consumption or blood LCn-3 fatty acid levels are associated with larger total brain volumes and/or regional gray matter volumes in healthy young and elderly participants (18-24). Prospective LCn-3 fatty acid supplementation studies have observed slower progression of gray matter atrophy in healthy elderly participants (25) but not in patients with mild or moderate AD (26). Together these data suggest that increasing dietary LCn-3 fatty acid intake and biostatus may slow or prevent neurodegenerative processes and cognitive decline in at-risk individuals when initiated early in the course of illness

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progression.

Progressive gray matter atrophy associated with dementia and AD may be due in part to reductions in cortical hemoglobin oxygenation (27-29). Near-infrared spectroscopy studies suggest that higher fish intake (30) or fish oil supplementation (31) increase prefrontal cortical hemoglobin oxygenation in healthy young adults during performance of cognitive tasks. Similarly, DHA supplementation was found to increase blood oxygen level-dependent (BOLD) activity in the prefrontal cortex of healthy children (32) and young adults (33) during performance of cognitive tasks using functional magnetic resonance imaging (fMRI). However, the effects of fish oil supplementation on functional cortical activity in healthy older adults experiencing subjective memory impairment are not known. In the present study, we investigated the effects of 24-week fish oil supplementation on cortical BOLD signal in response to a working memory task using fMRI in older adults with subjective memory impairment.

Materials and Methods

Participants

The study protocol was approved by the University of Cincinnati Medical Institutional Review Board, and each participant reviewed and signed an informed consent document prior to study enrollment. Participants were recruited from the Cincinnati, OH area by means of print advertising in the major daily newspaper soliciting older adults with memory complaints. Participants with diagnosed or suspected dementia, diabetes, kidney disease, liver disease, serious psychiatric condition, substance abuse, and use of medication or dietary supplements that might affect outcome measures or interact with fish oil supplementation (i.e., aspirin, anticoagulants, serotonin reuptake inhibitors, cholinesterarse inhibitors, benzodiazepines, and antioxidants) were excluded. We administered a number of screening instruments to gather demographic and pertinent medical information and to establish level of cognitive function. These included the Academic and Medical History Questionnaire (34), the Clinical Dementia Rating (CDR)(35), the Auditory Verbal Learning Test (AVLT) (36), and Geriatric Depression Scale (GDS)(37). Participants endorsed awareness of memory decline with aging but did not meet criteria for mild cognitive impairment or dementia. This was operationalized as CDR = 0, AVLT cumulative acquisition score between 1.0 standard deviation below and 1.0 standard deviation above the age-corrected mean, and GDS = 12 or less.

Treatments

Participants were randomized to fish oil or placebo (corn oil) provided by the Inflammation Research Foundation, Marbelhead, MA. Each fish oil capsule contained 400 mg EPA and 200 mg DHA. Those assigned to fish oil received a fixed dose of 2.4 g/day (EPA: 1.6 g, DHA: 0.8 g; 4 capsules/d). This EPA+DHA dose (2.4 g/d) was selected based in part on previous studies finding that similar fixed doses were efficacious for improving cognitive functioning in healthy elderly participants (25) and those with mild cognitive dysfunction (38). Placebo and fish oil capsules were identical in size, shape, and color to protect the blind. All participants were required to take 4 capsules each day. To minimize potential gastrointestinal discomfort, participants were advised to take two pills with breakfast and two pills with dinner. All capsules were stored at 4°C, and EPA+DHA content was determined periodically by gas chromatography to assure consistency across the trial. Neither EPA nor DHA content fell below 400 mg and 200 mg per capsule, respectively, during the course of the intervention, and EPA content did not vary more than 3% and DHA content did not vary more the 2.9%.

Participants also received either whole fruit, freeze-dried blueberry powder or matched placebo powder. The blueberry powder dosage was equivalent to one cup whole fruit per day and was administered in individual packets containing 12 g powder with the morning and evening meals.

Participants also were given oral and written instructions to avoid certain foods for the period of the intervention. These forbidden foods included supplements containing fish, canola, walnut or flax seed oil, cold water fish such as tuna, sardines, mackerel, and salmon, walnuts, flax seeds, berries of any kind, red wine, and foods and supplements containing berries and berry extracts.

Functional neuroimaging

Brain imaging data were acquired on a 4.0 Tesla Varian INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA, USA). Prior to entering the scanner room, participants underwent screening for MRI safety to identify hazards such as metallic implants, irremovable body jewelry, and claustrophobia. Visual stimuli for the paradigm were presented through non-ferromagnetic high-resolution video goggles (Resonance Technologies, Inc., Northridge, CA, USA). We acquired four runs of T2*-weighted gradient-echo echo planar images (EPI) consisting of 35 contiguous 4 mm axial slices covering the entire brain (TR/TE 3000/25 ms, FOV 256 x 256 mm, flip angle 85º) with 126 EPI acquisitions, the first two of which were discarded to account for T1 overshoot. A multi-echo reference scan was obtained to correct for ghost and geometric distortions (39). After the fMRI data acquisition, a T1-weighted 3-D anatomical image was acquired using a modified driven equilibrium Fourier transform (MDEFT) sequence (40) (TMD=1.1 s, TR=13 ms, TE=5.3 ms, FOV=25.6 x 19.2 x 19.2 cm, matrix 256 x 192 x 96, flip angle 20º) to provide anatomical co-registration of fMRI data.

Functional magnetic resonance imaging data were acquired while the participants performed a sequential letter, n-back working memory task programmed and administered using EPrime (www.pstnet.com). Working memory paradigms have been extensively studied in healthy (41) and MCI (42) samples. This paradigm involved presentation of 34 stimulus trials per

block. The stimuli included a series of upper case and lower case letters of the alphabet, in silver font on a black screen. Each letter was presented for 500 ms with an interstimulus interval of 2500 ms. Participants were instructed to respond to each letter by pressing one of two response buttons indicating either 'yes' or 'no' as to whether the letter had appeared n items previously. Working memory loading increased with greater values of "n." The 1-back condition required the participant to recognize when the currently displayed letter was the same as the letter displayed one letter previously. In the 2-back condition, the participant was required to recognize when the current letter matched the letter presented two letters back. In the 0-back condition participants were instructed to respond 'yes' when the letter 'X' was displayed. Accordingly, the 0-back condition represented a sustained attention task with minimal working memory load as there was no trial-to-trial retention requirement. Four runs, each consisting of the three n-back blocks, were administered, and the order in which the condition blocks were presented within each run was pseudo-randomized such that each block type appeared once per run. This fixed order was administered to each participant. In order to insure comprehension of the task, all participants performed a practice n-back procedure at a work station outside the scanner prior to fMRI data acquisition.

Stimulus event times were used to model activation in the fMRI data. For each participant, event times were extracted from E-Prime for response time and for hits (correctly identifying a target), misses (failing to identify a target), correct rejections (rejecting a non-target), and false alarms (identifying a non-target as target) for each n-back condition (0-, 1- and 2-back). Misses and false alarms were modeled in the individual activation data but not for the group analyses because of their infrequent occurrence. Instruction screens were displayed for 15 seconds separating blocks of events and served as baseline against which event-related activation was estimated. Slice timing correction was achieved by aligning the midpoint of each TR to the behavioral event times.

MR data post-processing

Raw (binary) MRI data were reconstructed using in-house software developed with the Interactive Data Language program (IDL; www.ittvis.com) with Hamming filtering in the X, Y, and Z planes. Images were subsequently processed, analyzed, and visualized with the Analysis of Functional Neuroimages (AFNI) software (Analysis of Functional Neuroimages, afni.nimh.nih.gov)(43). All datasets were normalized to standard space using tools in AFNI to match each participant's image to the International Consortium for Brain Mapping's ICBM452 template from UCLA's Laboratory of Neuroimaging (www.loni.ucla.edu). Co-registration of functional to anatomical images was completed using the AFNI align_epi_anat python script. The anatomical image was normalized to the TT_icbm452 template, functional runs were co-registered to a sub-brick acquired closest in time to the anatomical image using a six-parameter rigid-body transformation with Fourier interpolation (44), and then all functional EPI datasets were normalized to the anatomical dataset. The transformations were calculated stepwise and then combined into a single transform applied to the EPI data in order to minimize distortion of the fMRI data. Co-registered images were visually inspected and individually censored from further analysis if uncorrectable head movement or image artifacts were detected. This eliminated the need to exclude whole datasets on the basis of global movement characteristics. Binary masking from the anatomical template was applied to each volume of the functional dataset to remove data points outside the brain. Signal scaling was performed on a voxel-wise basis to convert the data to percent signal change on a per-run basis prior to deconvolution. Individual activation maps were then generated for each participant from the stimulus event times using the AFNI program 3dDeconvolve. This algorithm compares the magnitude of the hemodynamic response during stimulus event times of interest, in this case, accuracy (hits and correct rejections for each memory condition (0-, 1- and 2-back), misses, and false alarms. We also included motion correction parameters, as above, as regressors of no interest. This process allowed for the definition of the magnitude of the hemodynamic response relative to the average signal intensity at each location (voxel) in the datasets. These individual maps were then used for group-wise analyses.

Statistical analysis of BOLD signal changes were conducted using 3dMVM, a statistical program developed in R to employ a multivariate modeling approach to neuroimaging datasets with multiple within-subject factors (in this case, visit and n-back condition) at the group level while controlling for inflated false positive rates(45). For the analyses of BOLD response, there were two between-subjects treatment factors identified as oil and berry, each of which had two levels, omega-3 or placebo oil and blueberry or placebo powder, respectively. The within-subject variables were study visit, either enrollment (pre-intervention baseline) or final (24 week), and working memory condition, 0-, 1-, and 2- back. Corrections for multiple comparisons across voxels were conducted using Monte Carlo simulations via 3dClustSim, full-width half-max setting at 7.2; cluster thresholds were set at 46 contiguous (faces touching) voxels and voxel significance threshold was set at $p \leq 0.005$ to obtain a corrected significance level of $p \leq 0.01$ for all clusters.

Erythrocyte fatty acids

At baseline and 24 weeks, whole venous blood (10 ml) was collected into EDTA-coated BD Vacutainer tubes and centrifuged for 20 min (1,500x g, 4°C). Plasma was removed and erythrocytes washed 3 times with 0.9% NaCl and then stored at -80°C. Total erythrocyte membrane fatty acid composition was determined using saponification and methylation methods described previously (32). Samples were analyzed with a Shimadzu GC-2010 equipped with an auto-

Table 1
Baseline demographic variables

	Placebo (n=10)		Fish oil (n=11)		
	Mean	**SD**	**Mean**	**SD**	**P-value***
Age, y	66.4	3.75	70.1	6.12	0.11
Education, y	15.1	1.97	16.5	2.42	0.15
Gender, % female	70.0	n/a	54.5	n/a	0.48
Diagnosis of hypertension, %	40.0	n/a	36.4	n/a	0.89
Blood Pressure Systolic, mmHg	126.2	15.54	122.4	9.56	0.52
Blood Pressure Diastolic, mmHg	79.5	11.93	71.9	10.04	0.14
Pulse, bpm	67.9	10.63	74.2	10.46	0.19
Height, cm	163.8	9.1	164.5	2.84	0.81
Weight, kg	76.6	14.68	75.7	11.69	0.88
Fasting insulin, uU/mL	11.9	5.84	12.4	4.54	0.82
Fasting glucose, mg/dl	103.6	10.14	99.7	9.38	0.38
Triglyceride, mg/dL	108.2	36.90	111.4	42.38	0.86
Total cholesterol, mg/dL	207.4	32.69	207.5	32.25	0.99
CDR sum of boxes score	0.1	0.15	0.3	0.58	0.28
GDS score	3.7	2.83	4.9	4.72	0.48

Note. CDR = Clinical Dementia Rating. GDS = Geriatric Depression Scale; *Two-tailed t-test for continuous variables and chi-square for dichotomous variables

injector (Shimadzu Scientific Instruments Inc., Columbia MD). The column was a DB-23 (123-2332): 30m (length), I.D. (mm) 0.32 wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom CA). The carrier gas was helium with a column flow rate of 2.5 ml/min. Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Analysis of fatty acid methyl esters was based on areas calculated with Shimadzu Class VP 4.3 software and data are expressed as percent weight of total fatty acids (mg fatty acid/100 mg fatty acids). All samples were processed by a technician blinded to treatment. Our primary measure of interest was EPA+DHA. Given evidence for opposing effects of EPA+DHA relative to the omega-6 fatty acid arachidonic acid (AA) on neuroinflammatory processes associated with age-related cognitive decline (46), we also investigated AA and the AA/EPA+DHA ratio.

Statistical analysis

All discrete variables including the working memory performance data, erythrocyte fatty acid composition, ROI values, and demographic variables were analyzed in SPSS v21. Behavioral performance data representing response time (RT) and accuracy on the n-back task were extracted from E-Prime output files. Treatment effects were evaluated by repeated measures ANOVA (RMANOVA) for behavioral and ROI variables. For the behavioral data, accuracy and RT in each memory loading condition were compared by group with baseline and final visit values. Similarly, ROI values were evaluated by RMANOVA between treatment groups using mean percent signal at baseline and final visits. We also assessed the effects of change in erythrocyte fatty acid levels on ROI BOLD signal and on working memory performance with regression analyses.

Results

Participant characteristics and attrition

Twenty-seven participants who met study entry criteria and were randomized to either fish oil (n = 15) or placebo oil (n = 12) qualified for brain imaging studies. Five participants (n = 4 fish oil; n = 1 placebo) chose to discontinue participation because of the burden of maintaining the dietary restrictions prescribed for the study and did not complete the final fMRI visit. One additional participant in the placebo group was excluded because responses on the working memory task indicated poor task engagement. The final sample included 21 participants (n = 10 placebo; n = 11 fish oil) who completed baseline and final visit scans. The fish oil group included 5 participants who also received blueberry powder and 6 who received placebo powder, and the placebo oil group included 6 who received blueberry powder and 4 who received placebo powder. Because we did not observe an effect of blueberry or placebo powder on BOLD signal (described below), the treatment group data were combined into fish oil and placebo oil groups. A comparison of sample characteristics by group is

Table 2
Performance on the n-back task

	Placebo Mean (SD)	Fish Oil Mean (SD)	p-value[1]	p-value[2]
Baseline				
Accuracy				
0-back	0.98 (0.018)	0.97 (0.024)	0.59	-
1-back	0.97 (0.027)	0.94 (0.080)	0.22	-
2-back	0.85 (0.077)	0.86 (0.110)	0.83	-
Response time (ms)				
0-back	685.70 (103.02)	624.09 (143.84)	0.43	-
1-back	835.03 (173.49)	798.02 (155.62)	0.61	-
2-back	1063.50 (179.77)	969.17 (297.74)	0.39	-
Week 24				
Accuracy				
0-back	0.98 (0.011)	0.96 (0.061)	0.25	0.36
1-back	0.96 (0.027)	0.95 (0.052)	0.45	0.42
2-back	0.82 (0.10)	0.90 (0.078)	0.07	0.04
Response time (ms)				
0-back	670.87 (89.42)	654.97 (200.99)	0.82	0.51
1-back	797.64 (104.87)	750.17 (207.67)	0.52	0.85
2-back	1022.37 (209.53)	960.79 (336.75)	0.85	0.69

Note. 1Students t-test (2-tailed). 2Repeated measures ANOVA (group x visit interaction)

shown in Table 1. There was no significant group difference in demographic or clinical variables.

Erythrocyte fatty acid composition

Significant treatment group by visit interactions were observed for DHA ($p = 0.0004$), EPA ($p \leq 0.0001$), and EPA+DHA ('omega-3 index', $p \leq 0.0001$), but not arachidonic acid (AA, 20:4n-6, $p = 0.233$). A significant group by time interaction was also observed for the AA/EPA+DHA ratio ($p \leq 0.0001$). At 24 weeks erythrocyte membrane EPA+DHA composition increased significantly from baseline in participants receiving fish oil (+31%, $p \leq 0.0001$) but not placebo (-17%, $p = 0.06$), and the AA/EPA+DHA ratio decreased significantly from baseline in participants receiving fish oil (-37%, $p \leq 0.0001$) but not in those receiving placebo (+15%, $p = 0.09$, Fig 1).

N-back task performance

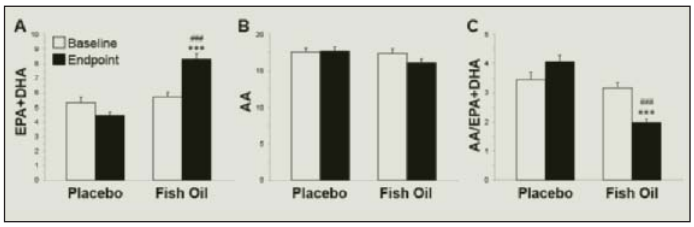
Summary performance data for the n-back conditions are shown in the Table 2. There was no significant group by visit interaction effect for accuracy for the 0-back ($p = 0.36$) and 1-back ($p = 0.42$) memory loading conditions, but there was an effect for the 2-back condition ($p = 0.04$). For reaction time, the group by visit interaction was not significant for the 0-back ($p = 0.51$), 1-back ($p = 0.85$), or 2-back ($p = 0.69$) conditions.

fMRI

Evaluation of the BOLD signal reflecting task activation was assessed by a general linear model within the multivariate model, weighting the factor "Condition" as incrementally increasing with memory-loading condition, that is, 1= zero-back, 2= one-back, and 3= two-back. This identified a pattern of BOLD signal typical of this task in healthy (41) and MCI populations (47). Specifically, a positive relationship between signal change and working memory load was seen in bilateral regions including sensorimotor cortex, posterior and body of the cingulate, insula, and right middle frontal, inferior frontal, and cerebellar regions. An inverse relationship was identified in bilateral lingual, parahippocampal, and lingual regions (Fig. 2A). There was no region of significant interaction identified in a four-way interaction when both oil and berry powder treatment were included in the model. Evaluation of treatment arms, that is, oil and berry powder, with a three-way interaction identified two regions of significant interaction for fish oil, visit, and working memory condition in the right cingulate/BA23,24, and in the right sensorimotor area/BA3,4 (Fig. 2B). No region was identified in the three-way interaction when berry treatment was evaluated.

Figure 1

Erythrocyte membrane EPA+DHA (A) and arachidonic acid (AA)(B) composition, and the AA/EPA+DHA ratio (C), of participants treated with placebo (n = 10) or fish oil (n = 11) at study baseline and endpoint (24 weeks). Values are mean weight percent total fatty acid composition or ratio ± S.E



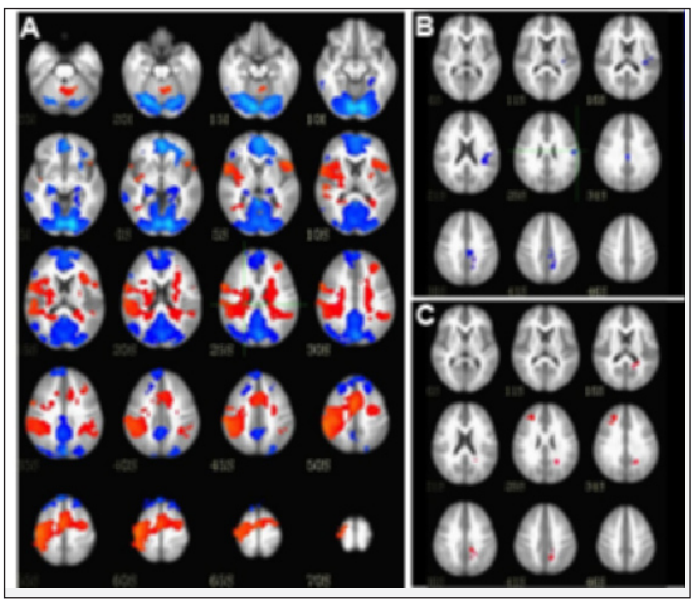
***p ≤ 0.0001 vs. baseline, ###p ≤ 0.0001 vs. placebo endpoint

To evaluate the effect of fish oil supplementation across visits during the working memory task, we contrasted pre- and post- treatment task activation in the fish oil group using a general linear trend. This contrast identified two regions of increased activation during working memory loading at follow-up relative to baseline, the right posterior cingulate and the left superior frontal gyrus (Fig. 2C). To further characterize activation patterns in the 0-, 1-, and 2- back conditions for each group we created a binary mask from the cingulate region identified in the baseline to final visit contrast, and used this mask to extract the mean percent signal change in memory-loading condition for the signal identified for each participant's baseline and final visit scans in this region of interest (ROI). These mean values were then analyzed with repeated measures ANOVA, which identified significant interaction between study visit and treatment group in the 1-back ($p < 0.01$) and 2-back ($p < 0.01$) conditions but not in the 0-back condition. The baseline to final visit change in cingulate BOLD signal was significantly greater in the fish oil group compared with the placebo group during the 1-back ($p = 0.0003$) and 2-back ($p = 0.0005$) conditions, but not the 0-back condition ($p = 0.85$) (Fig. 3).

Regression analyses were conducted to identify relationships of change in red blood cell omega-3 fatty acid status with working memory performance and with cingulate activation during the n-back task. We found that EPA+DHA change was not associated with accuracy in the 0-back and 1-back conditions but was related to performance in the 2-back condition, standardized β (β_{st}) = +0.39, p = 0.01. Similarly, the ratio AA/EPA+DHA was not related to accuracy in the 0-back and 1-back conditions but was inversely related to performance in the 2-back condition, β_{st} = -0.38, p = 0.01. Red blood cell EPA+DHA was not associated with cingulate BOLD signal during the 0-back condition. However, EPA+DHA was significantly related to cingulate BOLD signal in the 1-back condition, β_{st} = +0.60, p = 0.005, and there was a trend in the 2-back condition, β_{st} = +0.39, p = 0.09. Parallel, inverse relationships were found for AA/EPA+DHA with respect to cingulate activation during the 1-back, β_{st} = -0.61, p = 0.004, with a trend during the 2-back, β_{st} = -0.39, p = 0.09. Finally, we investigated whether activation in the cingulate was associated with working memory performance and observed no relationship for the 0-back and 1-back conditions but a positive association for the 2-back condition, β_{st} = +0.35, p = 0.02.

Figure 2

Multivariate modeling evaluating task-specific percent signal change in functional neuroimaging outcomes. (A) Among all participants (n = 21) task-specific contraste identified a positive relationship (red) between BOLD signal change and working memory load in bilateral sensorimotor, posterior and body of the cingulate, insula, and right middle frontal, inferior frontal, and cerebellar regions. An inverse relationship (blue) with working memory load was identified in bilateral lingual, parahippocampal, and lingual areas. (B) Significant 3-way interaction in treatment, visit, and memory loading condition in the right cingulate (BA23/24), and in the right sensorimotor area (BA3/4). (C) Pre- versus post- treatment contrast during memory loading in the fish oil group identified the right posterior cingulate and left superior frontal regions. Corrected significance level of $p < 0.01$ for all clusters



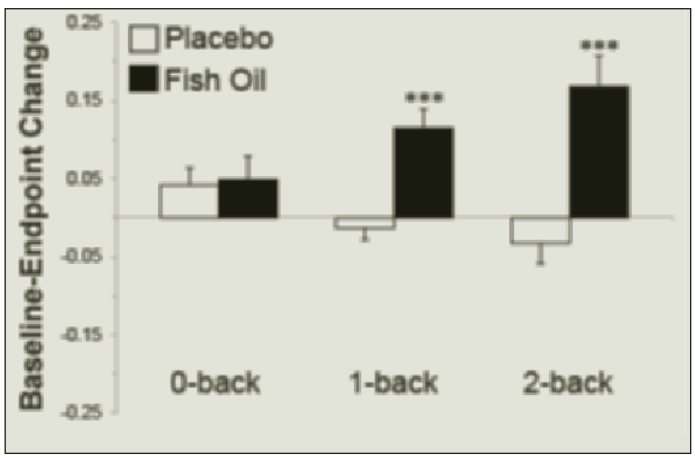
Discussion

This study determined the effects of 24 weeks of fish oil supplementation on event-related prefrontal BOLD responses in healthy older adults with subjective memory impairment using fMRI. Consistent with prior research, fish oil supplementation led to robust increases in erythrocyte DHA and EPA levels. Performance on the n-back task activated and dampened large bilateral networks in frontal and parietal cortices, consistent with previous fMRI studies employing this working memory paradigm in healthy participants (41).

The baseline to final visit increase in BOLD signal in the right cingulate gyrus was significantly greater is the fish oil group compared with placebo during the 1- and 2- back conditions. Among all participants, the change erythrocyte EPA+DHA and in the ratio AA/EPA+DHA was related positively and negatively, respectively, to cingulate BOLD signal during greater working memory challenge. In addition, these changes in measures of erythrocyte LCn-3 status were associated with working memory performance during conditions of greater cognitive demand. These are the first data demonstrating that dietary fish oil supplementation increases BOLD response in the cingulate cortex and enhances performance during working memory loading in older adults with subjective memory impairment.

Figure 3

ROI analysis of baseline-endpoint change in posterior cingulate cortex BOLD signal during increasing working memory load. The fish oil group exhibited a significant baseline-endpoint increase during the 1- and 2-back conditions whereas the placebo group remained relatively unchanged or decreased. Values are mean group baseline-endpoint change in BOLD signal ± S.E.M



***p ≤ 0.0001 vs. placebo

The mean pre-intervention erythrocyte EPA+DHA composition was 5.5%, which is greater than that observed in a large cohort of healthy younger individuals (4.9%) residing in the United States (16). Greater erythrocyte EPA+DHA composition in older adults is consistent with the observation that erythrocyte EPA+DHA levels increase with age (16). Greater DHA content in erythrocyte membranes may be due in part to age-related reductions in phospholipase A2 (PLA2) which releases fatty acids from membrane esterification. Unesterified DHA rapidly diffuses from plasma to brain (48) and age-related reductions in DHA composition have been observed in the human postmortem frontal cortex (49). Moreover, human erythrocyte DHA content is inversely correlated with blood PLA2-activity (50), and reduced PLA2-activity has been observed in blood, cerebrospinal fluid, and postmortem cortex of patients with AD and dementia (51-55). Importantly, fish oil supplementation also increases plasma EPA+DHA composition which would provide a surplus of free EPA+DHA for cortical accrual despite reduced PLA2-activity.

The observation that the baseline to final visit BOLD signal in the right cingulate gyrus increased significantly in the fish oil group is consistent with prior fMRI studies. For example, DHA supplementation was found to increase BOLD signal in the prefrontal cortex of healthy children during performance of a sustained attention task (32) as well as young adults during performance of the Stroop and spatial working memory tasks (33). Moreover, the present findings are consistent with a near-infrared spectroscopy study which demonstrated that fish oil supplementation increased cortical hemoglobin oxygenation in the prefrontal cortex of healthy young adults in response to working memory and executive tasks (31). It is of interest that prior imaging studies have observed a positive relationship between fish consumption and cingulate gyrus gray matter volumes among healthy adults (18) and a recent study found that anterior cingulate gyrus gray matter volume partially mediated the positive relationship between higher blood LCn-3 fatty acid levels and executive function in healthy elderly individuals (56). Together these imaging findings suggest that increased cortical hemoglobin oxygenation in response to fish oil supplementation may promote cingulate cortex structural and functional integrity.

We observed a significant enhancement of accuracy during the 2-back condition in the fish oil group, as well as relationships between omega-3 status and n-back performance and cingulate activation. Enhancement of neurocognitive performance was apparent only during the greater cognitive demands of the 2-back condition which may be more sensitive to detect improvement in this cohort of non-demented elderly participants. In agreement with previous studies observing memory-related activation in the posterior cingulate (57), we found that change in activation in the posterior cingulate was positively associated with working memory performance for the 2-back condition. It is notable that previous event-related MRI studies observed enhanced neural activation in the absence of performance effects following short-term treatment with LCn-3 fatty acids (31-33), as well as other nutritional supplement studies in both younger and older adults (47, 58, 59). Longer treatment with LCn-3 fatty acids and employing more cognitively challenging cognitive tasks may therefore be required to reveal performance improvements with event-related MRI. It is also possible that the early increases in cortical BOLD response following fish oil supplementation may reflect neurophysiological changes that precede neuroplastic-mediated functional improvements emerging over a more extended period.

In conclusion, this double-blind placebo-controlled imaging trial demonstrated that 24 weeks' fish oil supplementation led to robust increases in erythrocyte DHA and EPA levels

and greater baseline to final visit increases in BOLD signal in the right cingulate gyrus in response to a working memory task. This finding is consistent with previous fMRI studies demonstrating that LCn-3 fatty acid supplementation is associated with increases in cortical hemoglobin oxygenation during performance of cognitive tasks, and suggests that increasing dietary LCn-3 fatty acid intake and biostatus may help to mitigate neurocognitive decline and neurodegenerative processes in high-risk individuals when initiated early in the course of illness progression.

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Ethical Standards: The interventional study described in this manuscript was conducted in accordance with the laws of the USA including ethical standards for human research and approved by the University of Cincinnati Institutional Review Board.

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