

1 Running title: effect of DHA and lutein supplements on cognition

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4 **Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein**
5 **supplementation in older women.**

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21 Supported by USDA 1950-5100-065, Mead Johnson Nutritionals, and Martek Biosciences
22 Corporation. Any opinions, findings, conclusions, or recommendations expressed in this
23 publication are those of the authors and do not necessarily reflect the view of the U.S.
24 Department of Agriculture. No supplementary online material has been submitted.

25

26 Word count (text): 2972

27 Number of Figures: 2

28 Number of Tables: 4

29 **ABSTRACT (word count: 174)**

30 Low dietary intake of docosahexaenoic acid (DHA) and or foods rich in lutein may be associated
31 with increased risk of cognitive decline in elders. The cognitive benefit of DHA and lutein in
32 unimpaired elder women was explored in the context of a four-month, double-blind intervention
33 trial of DHA and lutein supplementation for eye health. Forty-nine women (60-80 yrs) were
34 randomized to receive DHA (800 mg/d) (n=14), lutein (12 mg/d) (n=11), a combination of DHA
35 and lutein (n=14) or placebo (n=10). Subjects underwent cognitive tests measuring verbal
36 fluency, memory, processing speed and accuracy, and self reports of mood at randomization and
37 upon completion of the trial. Following supplementation, verbal fluency scores improved
38 significantly in the DHA, Lutein, and combined treatment groups ($p<0.03$). Memory scores and
39 rate of learning improved significantly in the combined treatment group ($p<0.03$), who also
40 displayed a trend toward more efficient learning ($p=0.07$). Measures of mental processing speed,
41 accuracy and mood were not affected by supplementation. These exploratory findings suggest
42 that DHA and lutein supplementation may have cognitive benefit for older adults.

43

44 **KEY WORDS:** docosahexaenoic acid, lutein, cognitive function, elderly

45 INTRODUCTION

46 Cognitive decline and dementia are a major cause of disability among the elderly. The
47 prevalence of cognitive impairment increases exponentially with age from one in ten individuals
48 aged 65 and over to as many as one third of individuals by age 85 (1). The scope of the problem
49 is expected to grow as the population ages (2), making it imperative to identify factors, such as
50 dietary compounds, which might lower the risk of cognitive impairment. The present study
51 explores the possible benefit of dietary supplementation with two candidate compounds, namely
52 docosahexaenoic acid (DHA) and lutein, for enhancing cognition in older women.

53 Deficiency of DHA, a dietary fatty acid that is particularly enriched in fish, and which is
54 required for brain function (3-5) has been implicated in cognitive decline. Brain and plasma
55 content of phospholipids DHA (6, 7) and of plasma cholesterol DHA (8) is reportedly lower in
56 dementia cases than in controls. Moreover, in a prospective study of high-risk elderly subjects,
57 the consumption of at least one fish meal per week decreased the risk of Alzheimer's disease by
58 60% (9). Similarly, increased fish intake has also been associated with decreased risk of mild
59 cognitive impairment (10-12). Several small scale intervention studies provide evidence of
60 improved memory and other cognitive domains in impaired and health elders (13) and similar
61 interventions have demonstrated beneficial effects of DHA on cardiovascular (14) and immune
62 function (15).

63 Lutein, a common dietary carotenoid, is another potentially influential nutrient with regard to
64 brain health and cognitive function. It is well established that lutein is transported across the
65 blood brain barrier where it accumulates in the macula (16) and other neural tissues (17). There
66 is a preferential uptake of lutein over most other carotenoids. Data from the Nurses Health Study
67 in more than 13,000 women found that while higher consumption of fruits and vegetables did not
68 affect the overall decline in cognition due to aging, women with the highest reported

69 consumption of green leafy vegetables and cruciferous vegetables (rich sources of dietary lutein)
70 experienced less cognitive decline than women who ate fewer of these vegetables. This
71 difference was the equivalent of being one to two years younger in terms of cognitive aging (18).
72 In a rat model of age-related cognitive decline, dietary supplementation with spinach, a major
73 source of lutein (19), ameliorated age-related deficits in neuronal receptor-mediated signal
74 transduction (20, 21). Thus, phytochemicals present in spinach, including lutein, may be
75 beneficial in retarding functional age-related cognitive deficits. However, to date, the effects of
76 purified lutein on cognition in humans has not been studied.

77 In light of the above, as well as the fact that both lutein and DHA selectively accumulate in cell
78 membranes of central nervous system (22-25), we evaluated the effects of supplemental DHA
79 and lutein on several cognitive domains in older adults.

80

81 **SUBJECTS AND METHODS**

82 **Subjects.**

83 Fifty-seven healthy non-smoking women (60-80 years) recruited from the general population
84 for a 4 month study in which the primary outcome was the effect of supplemental DHA and
85 lutein on serum lipids and macular pigmentation (26, 27). All subjects underwent a screening
86 examination that included a medical history, a physical examination, and a routine blood clinical
87 chemistry profile. Volunteers with any history or biochemical evidence of lactose intolerance,
88 liver, kidney, or pancreatic disease, anemia, active bowel disease or resection, insulin-dependent
89 diabetes, easy bruising or bleeding, bleeding disorders, hyperglyceridemia,
90 hyperlipoproteinemia, or alcoholism were excluded from the study. Moreover, individuals taking
91 mineral oil or medications suspected of interfering with fat-soluble vitamin absorption were
92 excluded. Other exclusion criteria included current use of steroids or non-steroid anti-

93 inflammatory drugs, antihistamine drugs, vaccinations within the previous 2 weeks, taking any
94 nutrient supplement for the previous 2 months or carotenoid supplements for the previous 6
95 months. Smoking was not permitted during the course of the study.

96 Forty-nine women completed the study (86% of those enrolled). Eight women dropped out of
97 the study for the following reasons: medication use (1); autoimmune disease (1); significant
98 changes in lifestyle throughout the supplementation interval, including a 6.8 Kg weight loss (1);
99 aversion to study protocol (4); unknown (1). The primary complaint was aversion to
100 consumption of the high-calorie breakfast drink. Therefore, the total number of women studied
101 was 49.

102 This study protocol was approved by the Human Investigative Review Committee of Tufts
103 University, Tufts-New England Medical Center and the Schepens Eye Research Institute.
104 Informed consent was obtained from all subjects.

105

106 **Study Design.**

107 Women were randomly assigned to one of four groups: Placebo, DHA, Lutein, and
108 DHA+Lutein. Subjects visited the Jean Mayer USDA Human Nutrition Research Center on
109 Aging at Tufts University on days that supplements were distributed and blood was obtained (0
110 mo-baseline, 2 mo, and 4 mo). Subjects were instructed to take the supplement with a nutritional
111 energy drink (8 oz. BoostPlus[®], Mead Johnson Nutritionals) but were otherwise asked not to
112 alter their diets. This drink was included so that the supplement was consumed together with a
113 known amount of fat, to enhance the digestive uptake of lutein (28). The Boost drink contained
114 10 g protein, 45 g carbohydrate and 14 g fat (360 Kcal/8 oz).

115 Diet was monitored with food frequency questionnaires (29) completed at baseline, 2 months
116 and 4 months to be sure that there were no confounding changes in dietary intake. DHA and

117 lutein intake was assessed using the 100-item Health Habits and History Food Frequency
118 Questionnaire (29). This questionnaire combines lutein with zeaxanthin, another carotenoid of
119 lower concentration in foods (30) Compliance was monitored by interview, compliance calendars
120 and capsule count. The subjects and the experimenter were masked to the experimental groups.
121 Blood samples were collected and serum was separated from red blood cells (800 x g, 10
122 minutes) at 0, 2, and 4 months. Aliquots of serum were stored at -70°C until analyzed. At
123 baseline and 2 months, a two month supply of placebo supplements, DHA (800 mg/d, DHASCO,
124 Martek Biosciences), lutein (12 mg/d plus ~0.5 mg zeaxanthin, Kemin Foods), or DHA + lutein
125 (800 mg/d and 12 mg/d, respectively) and nutritional energy drink was provided. Supplements
126 were provided in capsule form. The placebo supplements (one each for lutein and DHA) were
127 identical in appearance to the test supplements. Previous studies, using comparable doses of
128 DHA and lutein, have found these levels to be safe for human consumption (31-35).

129 At baseline and at 4 months, subjects visited the Schepens Eye Research Institute (Boston,
130 MA) cognitive testing.

131

132 **Cognitive Tests**

133 Subjects underwent cognitive testing upon randomization and completion of the study. The
134 battery of cognitive tests was designed to evaluate several cognitive domains including memory
135 and processing speed or attention and a measure of self-reported mood (**Table 1**). All of these
136 tests or versions of them have been used and validated in aging research settings or have
137 demonstrated sensitivity to drugs and other health variables in intervention and or
138 epidemiological studies (36-39). Alternative forms of verbal fluency and memory tests were
139 administered at subsequent test sessions in order to decrease practice effects.

140

141 **Statistical Analyses**

142 All cognitive outcomes were adjusted for age and education. Differences between cognitive
143 and mood scores at baseline and after supplementation were tested with Student's paired t-test
144 within each treatment group (Systat version 9, Chicago, IL). For those variables where a
145 significant change was found from baseline to end of study, correlations were calculated between
146 age, education, serum levels of DHA and lutein, and test scores when the distribution of test
147 scores were normal or near-normal. Regression analyses were used to further examine significant
148 associations ($p < 0.05$) or those marginally significant ($p \leq 0.10$) found in initial analyses. In
149 those cases in which age or education were significantly related to performance on a particular
150 test, they were entered as covariates.

151 Because this study of cognitive performance is exploratory, unadjusted p values are reported
152 despite the use of multiple statistical tests.

153

154 **RESULTS**

155 **Table 2** presents the age and education characteristics of each of the four study groups. There
156 were no significant differences among groups in age or education. None were there differences in
157 dietary intakes of lutein and DHA at baseline or throughout the study.

158 Compliance for intake of supplements and nutrition drink was $>97\%$ based on our measures
159 (interview, compliance calendar, capsule count). Furthermore, changes in serum concentrations
160 of lutein and DHA indicated adherence to the study protocol (**Figures 1 and 2**). At baseline,
161 neither age nor years of education in the total samples was significantly associated with
162 cognitive test scores or self-report moods. Means and standard deviations of cognitive test scores
163 by subject group at baseline and after supplementation are shown in **Table 3**. There were no
164 differences in cognitive scores at baseline among the groups. The average performance of

165 subjects was close to ceiling (the maximum score) for many cognitive tests (Shopping List
166 Memory, Word List Memory, MIR Apartment, Pattern Recognition).

167

168 **Verbal Fluency**

169 After supplementation, subjects in the DHA ($p=0.03$), lutein ($p=0.000$), and DHA + lutein
170 ($p=0.000$) supplement groups named significantly more items from a category within a minute
171 (“Verbal Fluency”, Table 3) than at baseline. Subjects in the Placebo group did not name
172 significantly more items.

173

174 **Memory and Rate of Learning**

175 There were no significant increases in memory capacity, the number of items (either span or
176 total number of items) subjects recalled on the short-term memory Forward Digit Span or
177 Backward Digit Span tasks. On the Shopping List and Word List memory tests, none of the
178 subject groups significantly increased the number of items they recalled on the first trial during
179 the study.

180 However, on the Shopping List memory test, subjects in the DHA + Lutein supplement group
181 learned all 10 items significantly faster, within five trials or less, after supplementation ($p=0.03$,
182 Table 3). In this group, there was also a trend toward more efficient learning on the Word List
183 memory test, which only had a maximum of three trials in which to learn the list ($p=0.07$) (Table
184 3).

185 On the delayed recall, subjects in the DHA + Lutein ($p=0.02$) supplement group recalled
186 significantly more items on the MIR Apartment memory test, after supplementation (Table 3).
187 However, none of the treatment groups increased the number of items they recalled after a delay
188 on the Shopping List and Word List memory tests.

189

190 Speed and Accuracy

191 On the Pattern Recognition task, only the subjects in the Placebo group ($p=0.04$), who
192 originally had the longest response times on average of all the groups, significantly increased
193 their mean response speed for correct decisions (Table 3). None of the groups increased their
194 accuracy rate significantly. On average, subjects in all groups were close to ceiling in accuracy
195 and, therefore, had little room for improvement.

196 On the computerized version of the Stroop Test, none of the treatments changed mean response
197 times for reading words or naming colors on any of the four lists. As a measure of interference,
198 for each subject, total time to name colors of rectangles (subtask 3) was subtracted from the total
199 time to name colors of color name words that were printed in different colors (subtask 4). None
200 of the treatments changed on this interference measure from baseline to end of study.

201

202 Mood

203 None of the groups reported significantly different moods after supplementation.

204

205 Relations Between Serum Nutrient Levels and Cognitive Performance

206 Correlations between final test scores and possible covariates (age and education), and DHA
207 and lutein serum levels are shown in **Table 4**. Of the test scores that changed significantly after
208 supplementation, Verbal Fluency and Trials to Learn Shopping List scores were the least prone
209 to ceiling effects. Age was the only covariate that was significantly associated with final Verbal
210 Fluency score. Although subjects' scores on the Verbal Fluency test at baseline did not differ
211 significantly by age, younger subjects recalled more instances of a category than older subjects
212 at the end of the study ($p<0.05$).

213 There was a trend toward a significant relationship between DHA serum levels and Verbal
214 Fluency scores after supplementation. With further adjustment of the model for age, DHA serum
215 level remained significantly related to Verbal Fluency score ($p=0.04$). There was also a trend
216 toward a relationship between serum DHA levels and Trials to Learn Shopping List scores, with
217 higher DHA serum levels associated with learning the list in fewer trials.

218 In contrast, the relationships between final lutein serum levels and cognitive scores in the total
219 subject sample were not consistent with the cognitive improvement found in the lutein group
220 after supplementation. Because the distribution of lutein serum level at the end of the study was
221 highly positively skewed, the variable was log-transformed to produce a more normal
222 distribution. No significant relationship was found between final lutein serum levels, with or
223 without log-transformation, and Verbal Fluency scores. Also, in juxtaposition to the findings for
224 DHA, higher lutein serum levels were significantly associated with needing more trials to learn
225 shopping lists. However, Table 3 shows that subjects in the Lutein group had the highest
226 baseline scores for the variable Trials to Learn Shopping List and, therefore, the poorest verbal
227 learning capacity.

228

229 **DISCUSSION**

230 This is the first study to evaluate the effects of supplemental lutein, both alone and in
231 combination with DHA, on cognitive performance in older adults. The positive effects that we
232 observed in this small and relatively short-term randomized trial should encourage investigation
233 of the potential benefits of these compounds in more extensive trials.

234 In this study, supplementation with both DHA and lutein was most reliably associated with
235 significant results toward a significant result on several cognitive tests measuring different
236 aspects of memory. Each of these tests required subjects to retrieve or learn and retrieve

237 information from memory, most often in a time-limited or efficient fashion. In the Verbal
238 Fluency test, subjects had to retrieve instances of a category from long-term memory in a short
239 period of time. In the Shopping List and Word List tasks, subjects were asked to learn all items
240 presented in lists verbally or on a computer screen over several trials. In the MIR Apartment
241 test, subjects had one trial in which to learn common items that they placed in a box that
242 resembled rooms in an apartment.

243 Although subject groups did not show improvement in capacity or span, indicated by the
244 number of items they recalled on the first trial of any test, the DHA and lutein supplementation
245 group improved in efficiency, learning shopping lists or lists of words with fewer trials on
246 average after supplementation. In the MIR Apartment test, which required subjects to remember
247 objects after only one learning opportunity but with control of speed and of how they organized
248 and remembered items as they placed them in the apartment box, subjects in the DHA and lutein
249 supplementation group recalled significantly more objects after a delay. In comparison, subjects
250 did not increase number of items recalled after a delay on other memory tests in which items
251 were presented to them in multiple trials at a constant rate of speed by interviewer or on a
252 computer monitor.

253 On the Verbal Fluency test, subject groups who had been supplemented with either DHA or
254 lutein also showed significant improvement. Because this test evoked one of the least restricted
255 ranges of scores in this subject sample, there is reason to believe that further studies might elicit
256 improvements in cognitive status with either nutrient alone, given subject samples with more
257 variability and possibly tests with similar characteristics. In particular, DHA supplementation
258 might be better assessed in a subject group with scores less close to ceiling; as shown in Table 3,
259 the DHA supplementation group had less room to improve than the other treatment groups.

260 Generally the subjects in this study, although elderly, were competent at the tests. On average,
261 subjects in the Placebo group appear to have been among the strongest performers at baseline on
262 capacity or span measures of memory, and similar to the DHA supplementation group, initially
263 high scores might have limited our ability to detect improvement on some cognitive measures.
264 However, Placebo subject scores were not among the highest at baseline on the Verbal Fluency
265 test, so these subjects would be at least as likely to improve as subjects in other groups.
266 Therefore the lack of significant change on the Verbal Fluency test by the Placebo group
267 suggests that other groups' improvement should be attributed to supplementation.

268 It should be noted that a similar relationship between final serum levels of lutein and Verbal
269 Fluency scores was not found. Perhaps it is not the amount of lutein in the circulation that is
270 important but how lutein is transported in circulating lipoproteins or integrated into tissue. DHA
271 and lutein may interact beneficially through an effect on the transport and uptake of lutein into
272 the neural tissue. In fact, in these same subjects, DHA supplementation resulted in an increased
273 uptake of lutein uptake into the macula (26, 27). These findings demonstrate that DHA enhanced
274 lutein transport across the blood brain barrier into the central nervous system. Although macular
275 pigment densities did not correlate with measures of cognition in this study, the enhancement of
276 DHA of lutein accumulation across the blood brain barrier suggests that lutein content of other
277 cognitively important DHA-rich structures might also have been enhanced.

278 Both animal and epidemiologic studies suggest a beneficial effect of lutein-dense foods on
279 cognition (18, 20). However, there are no previous reports of supplemental lutein improving
280 cognitive function, as has been reported for DHA (13). This present study along with the
281 observation of the preferential accumulation of lutein in the brain (17) warrants further
282 investigation of the role of lutein in brain function.

283

284 Conclusions

285 In conclusion, supplementation of these elderly women with lutein, DHA and the combination
286 of both significantly improved verbal fluency. In addition, the combination of supplements
287 significantly improved subjects' memory, rate of learning, and learning efficiency. Because both
288 DHA and lutein accumulate in the brain, these effects may have occurred through increased
289 concentrations in the brain or influences on metabolic processes that modulate brain function.
290 The data reported from this study suggest that combined intake of DHA and lutein has
291 significant benefit in improving cognitive function in the elderly. Further studies are warranted
292 to confirm and evaluate these potential benefits.

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1 **Acknowledgements**

2 The authors wish to thank the volunteers who participated in this study and the staff of the
3 Metabolic Research Unit and Nutrition Evaluation laboratory at the Jean Mayer USDA Human
4 Nutrition Center at Tufts University for their assistance in subject recruitment and enrollment,
5 dietary instructions and blood drawing.

6

7 EJJ and DMS were responsible for the study design. EJJ and SMC were responsible for data
8 collection. EJJ, KM, SMC and DMS were responsible for interpretation and manuscript
9 preparation. KM was responsible for interpretation of cognitive data. KM and HYC were also
10 responsible for the statistical analysis of the data. SMC was responsible for cognitive testing
11 administration. AMT contributed to a critical revision and writing of the manuscript. The study
12 was conducted under the direction of EJJ and DMS. None of the authors had any advisory board
13 affiliations or financial interest in any organization sponsoring the research. EJJ and DMS have a
14 patent pending (#11/143,966) from the results of this research.

15

Table 1. Cognitive Tests.

Test	Assessment	Task	Reference
Verbal Fluency	Long –term memory retrieval	Subjects name as many items from a category as possible during a one minute period.	(40)
Digit Span Forward and Backward	Short-longer term working memory (auditory presentation)	Subjects repeat numbers in increasing spans in forward sequences, then in backward sequences.	(41)
Shopping List Task	Short-longer term memory (auditory presentation)	Ten associated words (common food items found in a supermarket) are read to the subject in up to five verbally presented serial trials. Verbal recall is tested immediately after each trial and after a delay.	(42)
Word List Memory Test (computer version)	Short-longer term memory (visual presentation , oral reading component)	Ten unassociated words are presented (at a rate of one word every two seconds on a computer monitor) in three serial trials. Verbal recall is tested immediately after each trial and after a delay.	(43)
Memory in Reality (MIR) Apartment Test	Short-longer term memory (hands-on component)	Subjects place common household objects in seven rooms of a model of an apartment. Subjects are asked to recall the names of objects and their locations after a delay.	(44)
NES2 Pattern Comparison Test	Visual processing	Subjects choose the odd pattern from three similar patterns displayed on a computer monitor. The scores are the number of correct responses (maximum 15) and the mean response latency for correct decisions.	(37)
Stroop Test	Processing speed and inhibition	Subjects name words (subtask 1: read words printed in black; subtask 2: read color name words printed in the same color) and colors (subtask 3: name colors of rectangles; subtask 4: name colors in which color name words are printed, in a condition in which colors are different from the color name). Scores for accuracy and response time of answers are collected. This version is presented via computer.	(45)
NES2 Mood Scales	Measure of mood and not cognition	Subjects rate their degree of tension, depression, anger, fatigue, and confusion over the previous seven days, using a computerized format.	(37)

Table 2. Age, Years of Education and Dietary Intake of Lutein/Zeaxanthin and Docosahexaenoic Acid of Treatment Groups, mean (SE).

	Placebo n=10	DHA n=14	Lutein n=11	DHA and Lutein n=14
Age, yrs (SE)	68.0 (1.2)	68.5 (1.3)	66.7 (1.9)	68.6 (1.3)
Education, yrs	13.6 (1.1)	16.0 (1.0)	13.8 (0.5)	14.8 (0.5)
Lutein/zeaxanthin, mg/d	2.2 (0.5)	3.5 (0.7)	3.0 (0.7)	2.7 (0.6)
DHA, mg/d	92 (32)	143 (29)	126 (35)	181 (71)

Table 3. Means (Standard Deviations) of Scores at Baseline (0 mo) and after Supplementation (4 mo).

Test	Placebo (n=10)		DHA (n=14)		Lutein (n=11)		DHA and Lutein (n=14)	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
MEMORY								
<i>Verbal Fluency</i>	12.9 (6.2)	13.8 (3.5)	15.0 (4.9)	17.8 (3.1)**	11.3 (5.1)	15.5 (5.5)**	12.1 (2.8)	16.9 (3.4)**
<i>Forward Digit Span</i>								
Length	7.2 (1.2)	7.2 (1.4)	6.6 (1.5)	6.7 (1.3)	6.6 (1.2)	7.0 (1.5)	7.3 (1.3)	7.3 (1.3)
Total	9.7 (2.5)	9.0 (2.4)	8.4 (2.8)	8.5 (2.7)	8.1 (2.3)	8.7 (2.5)	9.5 (2.5)	9.6 (2.7)
<i>Backward Digit Span</i>								
Length	5.9 (1.4)	5.8 (1.7)	5.4 (1.6)	5.8 (1.6)	5.1 (1.6)	4.7 (1.4)	5.4 (1.4)	5.9 (1.5)
Total	8.2 (2.7)	8.4 (3.3)	7.9 (3.1)	8.4 (3.2)	7.5 (3.1)	6.9 (2.7)	7.4 (2.6)	8.4 (2.6)
<i>Shopping List Memory Test</i>								
Trial 1 Items Recalled (max. 10)	6.5 (1.2)	7.7 (1.5)	7.2 (1.4)	7.7 (1.7)	6.9 (1.8)	6.5 (2.1)	7.0 (1.4)	6.9 (1.6)
Trials to Learn List (max. 6)	3.0 (0.8)	2.8 (0.9)	3.1 (1.3)	2.6 (1.3)	4.2 (1.5)	3.9 (1.4)	3.9 (1.4)	2.9 (1.3)**
Delayed Recall (max. 10)	9.5 (0.9)	9.5 (0.7)	9.0 (0.9)	8.7 (1.7)	8.3 (1.9)	7.6 (3.0)	8.6 (0.6)	8.9 (1.4)
<i>Word List Memory Test</i>								
Trial 1 Items Recalled (max. 10)	6.2 (1.3)	6.6 (1.8)	6.3 (1.7)	5.9 (1.5)	5.8 (1.8)	5.8 (1.8)	5.6 (1.5)	6.2 (1.4)
Trials to Learned List (max. 4)	3.1 (0.9)	2.8 (0.9)	3.0 (1.0)	3.0 (0.7)	3.4 (0.7)	3.5 (0.8)	3.6 (0.6)	3.0 (0.9)*
Delayed Recall (max. 10)	8.1 (1.1)	8.3 (1.8)	8.1 (1.1)	8.6 (1.3)	6.8 (2.9)	7.6 (2.4)	7.6 (1.6)	8.1 (2.0)
<i>MIR Apartment Test</i>								
Delayed Recall (max. 10)	9.3 (0.8)	9.4 (0.7)	9.4 (0.9)	9.4 (0.8)	8.3 (1.6)	8.6 (2.1)	8.3 (1.5)	9.1 (1.2)**
Location Recall (max. 10)	9.7 (0.7)	9.7 (0.7)	9.9 (0.3)	10.0 (0)	9.5 (1.0)	9.5 (0.8)	9.1 (0.9)	9.4 (1.2)

PROCESSING								
<i>Pattern Recognition Test</i>								
Number Correct (max. 15)	14.5 (0.7)	14.9 (0.3)	14.6 (0.9)	14.6 (0.5)	14.5 (0.9)	14.3 (1.8)	14.7 (0.8)	14.0 (1.2)
Mean Response Time-Correct (s)	6.8 (3.0)	5.9 (2.3)**	5.4 (2.1)	5.0 (0.7)	6.1 (2.3)	6.4 (2.3)	5.9 (1.5)	5.9 (1.1)
<i>Stroop Test</i>								
Mean RT, Read Words-Black (ms)	1040(380)	891(222)	879(429)	748(157)	844(239)	945(185)	861(169)	819(165)
Mean RT, Read Words-Color (ms)	788(200)	804(202)	715(181)	727(132)	753(210)	883(213)	754(176)	743(169)
Mean RT, Name Colors (ms)	919(173)	951(220)	838(161)	884(163)	1008(217)	1014(193)	947(150)	965(182)
Mean RT, Name Colors- Words(ms)	1419(308)	1413(508)	1269(215)	1277(226)	1492(329)	1462(221)	1366(225)	1317(241)
Total RT, Interference (NC-C)(s)	25.0(14.8)	23.1(22.0)	21.5(10.0)	19.7(8.3)	24.2(10.9)	22.4(7.1)	21.0(7.8)	17.6(8.6)
<i>Mood Scales</i>								
Tension	2.3 (0.9)	2.2 (0.8)	2.0 (0.8)	2.1 (0.5)	2.1 (0.4)	2.4 (0.9)	2.0 (0.6)	1.9 (0.5)
Depression	1.7 (0.7)	1.9 (0.7)	1.7 (0.7)	1.7 (0.7)	1.5 (0.3)	1.8 (0.7)	1.7 (0.6)	1.6 (0.4)
Anger	1.7 (0.5)	1.5 (0.6)	1.6 (0.7)	1.6 (0.9)	1.4 (0.4)	1.5 (0.5)	1.4 (0.5)	1.5 (0.4)
Fatigue	2.0 (0.7)	2.1 (0.5)	2.1 (0.8)	2.1 (0.7)	2.4 (0.6)	2.9 (0.9)	2.3 (0.8)	2.1 (0.6)
Confusion	1.4 (0.2)	1.7 (0.5)	1.7 (0.5)	1.8 (0.5)	1.9 (0.5)	2.4 (0.9)	1.9 (0.7)	1.7 (0.4)

*p<=0.10: ** p<0.05

Table 4. Correlations Between Variables After Supplementation.

	Verbal Fluency	Shopping List (Trials to Learn)	Word List (Trials to Learn)	MIR Apartment Delayed Recall
Age (N=49)	-0.37**	0.20	0.07	-0.23*
Education (N=49)	0.11	-0.18	-0.19	0.05
DHA serum (N=49)	0.24*	-0.26*	-0.04	0.21
Lutein serum (N=48)	0.03	0.30**	-0.04	-0.16
Lutein serum (log- transformed (N=48)	0.03	0.36**	-0.02	-0.13

* $p \leq 0.10$; ** $p < 0.05$

Figure 1. Change from baseline in serum lutein concentrations in placebo group and each experimental group supplemented with lutein (12 mg) and/or docosahexaenoic acid (DHA, 800 mg), mean \pm se.

Lutein supplementation x DHA supplementation x month interaction, $p=0.3588$ (3 factor repeated measures ANOVA).

Lutein supplementation x month interaction, $p < 0.001$

DHA supplementation x month interaction, $p=0.7302$

*significantly different from baseline ($p < 0.01$) within a group based on paired t test.

Figure 2. Change from baseline in serum DHA concentrations in placebo group and each experimental group supplemented with lutein and/or DHA, mean \pm se.

Lutein supplementation x DHA supplementation x month interaction, $p < 0.05$ (3 factor repeated measures ANOVA).

*significantly different from baseline ($p < 0.0001$) within a group based on paired t test.

**significantly different from baseline ($p < 0.05$) within a group based on paired t test.



