

Original Research Article

A prospective study of neuro-cognitive enhancement with carotenoids in elderly adult males with early age related macular degeneration.

The ZVF Study Group (FDA IND #78,973)

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ABSTRACT

BackgroundDiets rich in carotenoids may reduce cognitive impairment.Little is known about dietaryzeaxanthin.**Objective**

Evaluatezeaxanthincarotenoidsupplementationagainstchange in cognitivestatus.

Methods **American Psychological Association (APA)** certified cognitive evaluation from theZeaxanthin and Vision Function Study (**USFDA Investigative New DrugIND#78,973**),a 1 yearprospective **randomized controlled trial (RCT)** of elderly males with mild age related macular degeneration. Neurocognitive testing:**Repeatable Battery for the Assessment of Neuropsychological Status Update RBANS**and Trail Making A & B. Subjects evaluatedatbaseline and 1 yearafter dietary **isomer RR**zeaxanthin (8 mg/d) alone or combined with lutein (9 mg/d) using one way ANOVA, ($P<0.05$) and T testing. **Results** $n=50$ subjects completed both study visits.Delayed memory in the zeaxanthin groupimproved from RBANS score of 91.8 (SD 16) to 99.4 (SD 12), $P = 0.04$.

Conclusions Zeaxanthin, typically minimally present in the US diet, may nonetheless be important in the context of emerging relationships in primates between xanthophyll carotenoids and cognitive function. Additional larger scale RCTs is indicated to investigate the clinical utility of this carotenoid in nutritional neuroscience.

Key Words: age related macular degeneration, carotenoids, macular pigment, cognition

2 Tables

Objectives:

The ZVF primary objectives were to evaluate the visual function properties of zeaxanthin independent of lutein, and to assess the added benefit of dietary RR zeaxanthin to traditional lutein supplementation, in elderly male veterans with AMD[1]. Accordingly, for this segment of the study, the objectives were as follows:

- 1) Evaluate the effect of carotenoid zeaxanthin on cognitive status.
- 2) Evaluate the effect of 8 mg zeaxanthin combined with 9 mg lutein on cognitive status.

Purpose and background:

Age-related macular degeneration (AMD) and cognitive impairment are both neurodegenerative disorders associated with aging and have been hypothesized to share common pathogenic pathways[2]. In a large population based study, Wong et al showed a weak association between cognitive function and early ARM. That is, persons with severe cognitive impairment based on Word Fluency Test scores were more likely to have early AMD (odds ratio (OR: 1.6, 95% confidence interval [CI]: 1.1-2.2) and its components, soft drusen (OR: 1.6; 95% CI: 1.1-2.3) as well as pigmentary abnormalities (OR: 1.5; 95% CI: 0.9-2.5) than those without severe impairment[3]. In a later second large scale trial, persons with low Digit Symbol Substitution Test (DSST) (lowest quartile of scores, ≤ 30) were more likely to have early AMD (odds ratio, 1.38; 95% confidence interval, 1.03-1.85)[4]. The authors conclude that in older populations, cognitive impairment may share common age-related pathogenesis and risk factors with early AMD.

There is a growing body of literature to support the use of dietary carotenoids i.e. lutein and zeaxanthin in the intervention of AMD[5,1]. Research has linked vision loss greater than 20/40 with cognitive impairment[6]. In healthy older adults, macular pigment optical density (MPOD) was only related to visual-spatial and constructional abilities. More recently, for subjects with mild cognitive impairment

(MCI), MPOD was shown to be broadly related to cognition including the composite score on the mini-mental state examination, visual-spatial and constructional abilities, language ability, attention and total scale on the Repeatable Battery for the Assessment of Neuropsychological Status[7].MPOD is related to cognitive function in primates and older adults, and a biomarker of L and Z embedded in all neural tissue[8,9].

Older adults consuming the highest amounts of green leafy vegetables and cruciferous vegetables (both rich sources of lutein) have slower cognitive decline than those consuming the lowest amounts[10,11].Supplementation with lutein has been shown to improve cognitive function in older women[12].As well, there is increasing support for the idea that oxidative stress may play an important role in the pathogenesis of more severe cognitive impairment, specifically Alzheimer's disease (AD), the most common form of cognitive impairment.

Historical evolving evidence has suggested oxidative stress is highly implicated in diseases associated with aging, and lesions typically associated with attacks by free radicals have been found in the brains of persons with AD. The free radicals in the brain that cause oxidative stress, high vascularity and metabolism along with an abundance of fat, provide fodder for free radical attack and protection by endogenous antioxidants[13]. However, early research with the specific antioxidants A and E, thought related to beta amyloid toxicity in AD brains, were found *not associated with* cognitive decline in non-demented subjects[14].

Nonetheless, more recent studies demonstrate that dietary antioxidants may indeed help with slowing the pathogenesis and thereby the progression of cognitive impairment. Observed delays in decline of ADL status, severe dementia, institutionalization and death support this position[13]. Other large scale cross sectional studies¹⁵ suggest that while it is unclear whether low levels of plasma carotenoids precede or are the consequence of cognitive impairment, low carotenoid levels may play a role in cognitive impairment. Plasma analysis of a broad spectrum of antioxidants including carotenoids in elderly groups with unimpaired cognition, **mild cognitive impairment (MCI) and Alzheimer's Disease (AD)**, showed lower levels of peripheral levels and activities of antioxidants in the MCI and AD groups as compared to healthy controls[16]. Therefore, it has been hypothesized that diets rich in antioxidants or supplementation may reduce the risk of cognitive impairment. Recently in the Georgia Centenarian Study, serum lutein, zeaxanthin, and β -carotene concentrations were most consistently related to better cognition ($P < 0.05$) in the whole population as well as centenarians. Only serum lutein was significantly related to better cognition in octogenarians[17].

Obviously, a lifetime of healthy, nutritionally rich foods would be preferable to late life supplementation. Yet, studies have statistically shown significant improvements with verbal fluency and memory scores following lutein and n3 fatty acid supplementation[12], and given that supplementation yields notoriously low negative side effects, this therapy avenue has warranted continued

121 exploration. Accordingly, this study would purport to examine protective factors in
122 the form of dietary supplements that are found in neural tissues, and their effect on
123 both AMD and cognitive functions. While both zeaxanthin and lutein are found
124 throughout body tissues, the highest concentration exists in central neural regions,
125 namely the retina, as well as frontal and occipital cortical regions of the brain[8,9].

126 Study Design:

127 The present study was conducted as a portion of a larger, one-year, prospective
128 randomized, double blind, intention to treat, lutein (faux-placebo) controlled study
129 of patients with early and moderate AMD, as determined by a retinal specialist
130 using the AREDS 1 simplified scale. [1] No patients with advanced AMD were
131 included. The sample included 60 patients, equally assigned to one of two dietary
132 supplement treatment carotenoid pigment arms, or the traditional 9 mg non-
133 esterified lutein (Kemin Health, Des Moines IA) supplement control group. The
134 groups were broken down as follows: 8mg dietary RR zeaxanthin (Chrysantis, Ball
135 Horticulture, Inc, Chicago, IL) (n=25); 8 mg zeaxanthin + 9 mg lutein (n=25) and
136 the faux placebo 10 mg lutein control Group (n=10).

137 Subjects were patients in the Ophthalmology/Optometry Clinic at the North
138 Chicago VAMC with early AMD retinopathy participating in the Zeaxanthin and
139 Vision Function RCT (The ZVF Study Group (FDA IND #78,973)[1]. Those with high
140 risk NEI AREDS retinopathy or who had taken lutein or zeaxanthin supplements or
141 had cataract or retinal surgery within the last 6 months were excluded. Those
142 subjects taking photosensitizing drugs, did not meet ophthalmic/visual entrance
143 criteria, had active comorbidities such as dementia (AD and non-AD type),
144 schizophrenia, severe diabetes, glaucoma, uveitis or optic neuritis or were taking
145 retinotoxic medications were all excluded.

146 Methods:

147 Age range of patients was 54 to 93 (mean of 75.1, SD = 10.0). Education ranged
148 from 6 years of formal education to 20 years (mean of 13.4, SD = 2.6). After
149 subjects were deemed eligible and informed consent was obtained, several
150 assessments were completed and subsequently published, including:

- 151 1. Demographic and Symptom Assessment
- 152 2. Ocular Physical Assessment
- 153 3. Macular Function and Visual Measurements
- 154 4. Neuropsychological Assessment

155 Macular pigment is a well known surrogate marker of plasma levels – more so as it
156 represents end organ tissue accumulation of carotenoids following competition within the
157 duodenum for absorption as well as lipoprotein segregation within the liver. The present
158 article focuses only on the Neuropsychological Assessment portion of the study at
159 baseline and final study visit. Neurocognitive testing consisted of Repeatable

Battery for the Assessment of Neuropsychological Status (RBANS) and the Trail Making Test A& B. RBANS is a brief and comprehensive cognitive assessment tool with age stratified norms, which provides a more global assessment of neuropsychological functioning for adults age 20 and older. Specifically, neurocognitive domains evaluated include immediate and delayed memory, attention, language and visuospatial/constructional abilities. Trail Making Test A & B is a portion of the larger Halstead Reitan Neuropsychological Battery, and measures simple and divided attention respectively. This test also taps speed of information processing, sequencing and visual scanning abilities. Norms from Tombaugh[18] were employed for the Trail Making Test, and norms published with the RBANS were used to interpret those scores.

RBANS and Trail Making Test A& B were administered at baseline and one year, at the conclusion of ZVF, by an APA certified neuropsychologist(KG-H) and her team.

Statistics:

Data were determined to be normally distributed using the Kolmogorov-Smirnoff and Shapiro-Wilk tests. Analysis of variance (ANOVA) was used to assess for between group differences and the test of Scheffe was used to assess specific comparisons. Bartlett's Test was also used to test ANOVA's equal variances assumption. Differential scores were taken (baseline pre-test -1 year post-test) and placed in a correlation matrix. Within the group, changes over time were assessed using a two sample, T test using an equal variances assumption. For all tests an alpha level of 0.05 was considered statistically significant. All calculations were made using STATA™ version 10.1 (College Station, Texas, USA).

Results:

In ZVF, 90% of subjects completed ≥ 2 visits with an initial Age Related Eye Disease Study (AREDS report #18) retinopathy score of 1.4 (1.0 SD) /4.0 (mild macular degeneration), pill intake compliance of 96% and no adverse effects.¹ All testing was administered by the ZVF study neuropsychologist or trained and supervised technicians. Of 60 patients recruited, 60 completed the initial assessment, and 50 completed the second assessment. Data from deceased subjects (n = 2), those who dropped out (n = 7) or those who refused retesting (n = 1) were not analyzed. Therefore, the control group was comprised of 7 subjects of the original 10, 21 of 25 zeaxanthin only, and 22 of 25 in the lutein + zeaxanthin group.

In **Table 1** appear the mean (SD) descriptive statistics (age, education), neurocognitive baseline and final RBANS and Trail Making A, B status. There was initial unequal variance for the RBANS variables 'Attention' and 'Trail Making Factors B' which failed to sustain statistical significance, by study end.

199 As there was no true placebo in the ZVF design, one way analysis of variance
 200 (ANOVA) was performed on the entire data set to determine an effect from
 201 carotenoid supplementation. The RBANS mean sample index and Z scores
 202 indicated that, with the exception of immediate memory, the scores for simple and
 203 divided attention, delayed memory, visuospatial/constructional abilities and
 204 language were all in the average range. Immediate memory fell within the
 205 lowaverage range, in comparison with age and educationally matched normative
 206 samples:

- 207 • Immediate Memory ANOVA yielded $F(2, 49) = 1.58$ with $p = 0.22$.
- 208 • Visuospatial / constructional ANOVA yielded $F(2, 49) = 0.731$ with $p = 0.49$.
- 209 • Language ANOVA yielded $F(2, 49) = 0.052$ with $p = 0.95$.
- 210 • Attention ANOVA yielded $F(2, 49) = 1.35$ with $p = 0.874$.
- 211 • Delayed Memory ANOVA yielded $F(2, 49) = 0.067$ with $p = 0.96$.
- 212 • Total RBANS Score ANOVA yielded $F(2, 49) = 0.383$ with $p = 0.68$.
- 213 • Trails A ANOVA yielded $F(2, 49) = 1.429$ with $p = 0.25$.
- 214 • Trails B ANOVA yielded $F(2, 49) = 0.039$ with $p = 0.96$.

215
 216 **Table 1. Mean (sd) ZVF population descriptive statistics (age, education) and**
 217 **neurocognitive baseline and final RBANS and Trail Making A, B scores. Initial**
 218 **baseline unequal variance for the variables ‘Attention’ and ‘Trail Making Factors B’**
 219 **failed to sustain statistical significance, by study end.**

variable	Baseline				Final			
	n	mean	(sd)	Bartlett's Test for	n	mean	(sd)	Bartlett's Test for
				Equal Variance				Equal Variance
				Between Groups				Between Groups
Age (yrs)	60	75.1	10.0	NS				NS
Education (yrs)	60	13.4	2.6	NS				NS
Immediate Memory	60	88.3	15.3	NS	50	93.9	14.0	NS
Trails Making Factors B	60	-0.8	1.7	0.05	50	-0.9	2.3	NS
Visual Spatial Construction	60	98.4	19.7	NS	50	91.0	17.6	NS

Language	60	97.0	9.9	NS	50	99.1	8.6	NS
Attention Delayed	60	93.2	15.5	0.02	50	96.5	14.0	NS
Memory	60	92.5	14.7	NS	50	95.4	16.0	NS
Total	60	91.6	13.3	NS	50	93.1	13.5	NS
Trails Making Factors A	60	0.1	0.9	NS	50	-0.1	1.3	NS

220

221 In **Table 2**, changes in neurocognitive status over time, for each of the 3
222 intervention groups is presented with a T test $P < 0.05$ considered significant.
223 Individual and Total RBANS parameters as well as Trails A and B were all non-
224 significant with the exception of zeaxanthin, RBANS delayed memory. Delayed
225 memory in the zeaxanthin group improved from a RBANS score of 91.8 (SD 16) to
226 99.4 (SD 12), one way ANOVA, $P = 0.04$.

227 **Table 2. Change in Neurocognitive status over time, for 3 intervention groups: faux**
228 **placebo lutein (left column); zeaxanthin (central column) and zeaxanthin + lutein**
229 **(right column). 1 way Anova, $P < 0.05$ considered significant.**

	n=	Lutein mean (SD)	P value	n =	Zeaxanthin mean (SD)	P value	n =	L + Z mean (SD)	P value
Immediate Memory	Baseline = 10	84.7 (16.5)		Baseline = 25	89.8 (16.0)		Baseline = 25	88.3 (14.5)	
	Final = 7	91.7 (17.5)	NS	Final = 21	96.5 (10.4)	NS	Final = 22	92.0 (16.1)	NS
Visual Spatial Construction	Baseline = 10	102.6 (19.1)		Baseline = 25	99.0 (18.0)		Baseline = 25	96.1 (22.0)	
	Final = 7	99.1 (19.2)	NS	Final = 21	93.6 (15.9)	NS	Final = 22	85.9 (17.8)	NS
Language	Baseline = 10	91.8 (10.9)		Baseline = 25	98.6 (9.5)		Baseline = 25	97.4 (9.6)	
	Final = 7	98.0 (5.2)	NS	Final = 21	98.0 (9.4)	NS	Final = 22	100.5 (8.9)	NS
Attention	Baseline = 10	85.7 (18.2)		Baseline = 25	90.2 (15.9)		Baseline = 25	99.3 (11.8)	
	Final = 7	97.9 (17.9)	NS	Final = 21	94.5 (14.2)	NS	Final = 22	98.0 (12.8)	NS
Delayed Memory	Baseline = 10	93.1 (19.2)		Baseline = 25	91.8 (12.3)		Baseline = 25	93.0 (15.5)	
	Final = 7	97.9 (20.7)	NS	Final = 21	99.4 (11.8)	0.04	Final = 22	90.7 (17.0)	NS
RBAN TOTAL	Baseline = 10	89.0 (16.8)		Baseline = 25	91.4 (11.6)		Baseline = 25	93.0 (13.8)	
	Final = 7	96.0 (17.9)	NS	Final = 21	93.7 (11.4)	NS	Final = 22	91.6 (14.3)	NS

Trails Making A	Baseline = 10	-0.50 (1.2)		Baseline = 25	0.10 (0.8)		Baseline = 25	0.4 (0.9)	
	Final = 7	-0.99 (1.2)	NS	Final = 21	-0.18 (1.5)	NS	Final = 22	0.2 (1.0)	NS
Trails Making B	Baseline = 10	-1.2 (1.7)		Baseline = 25	-0.74 (1.7)		Baseline = 25	-0.65 (1.8)	
	Final = 7	-1.3 (1.8)	NS	Final = 21	-0.65 (1.4)	NS	Final = 22	-0.99 (3.2)	NS

230

231 Discussion:

232 No significant differences were found between the groups except for “Delayed
233 Memory”, suggesting no broad multi factorial effect was seen by supplementing
234 with zeaxanthin or by adding zeaxanthin to lutein. A larger sample size may have
235 been able to show an effect size. Notably, subjects did however maintain their
236 cognitive function during the duration of the study, lending at least modest support
237 for the hypothesis that xanthophyll carotenoids can slow the progression of
238 oxidative stress by containing free radicals and preserving neural tissue, over the 1
239 year ZVF study period, in elderly adult male veterans.

240 The strengths of this study are randomization, double masking and sensitive
241 instruments. The weaknesses were no true placebo and non – generalizable to
242 other populations such as the young, females and those without AMD. Differences
243 with other studies were subtle, and may have been enhanced by subject selection
244 and placement in groups with graded levels of dementia (i.e., unimpaired, mild
245 moderate and severe), that may have highlighted subtle effect(s). Subjects may
246 have also been ‘pre-screened for dementia’ or other comorbid conditions adversely
247 affecting cognitive performance.

248 The presence of a true ‘control group’ receiving a placebo instead of one of three
249 treatment groups may have been useful to underscore any difference that might
250 have been correlated with supplement use. As such, study design or subject
251 selection procedures may have been a factor in masking additional
252 benefit(s). Some studies actually stratify subjects’ education-level[14], which is a
253 known protective factor in the development of cognitive impairment. Since ZVF
254 veterans manifested a broad range of education levels between 6 and 20 years,
255 but overall a higher than average mean level at 13.2 years, this may have had a
256 veiling effect on outcome.

257 Quite recently, serum markers of both oxidation and inflammation have been found
258 to predate the onset of AD[19,20]. It follows that dietary modification /
259 supplementation with carotenoids may take more than 1 year to mount an effect
260 size measurable by our selected instruments.

261 Future studies may also do well to pair serum markers with cognitive screening
262 tools completed by patients or their surrogates, functional visual assessment

measures and new objective structural measures of the human lens and retina. This would yield data that could illuminate any relationship between plasma levels, cognitive performance, objective ocular tissue biomarkers and patient/family observations regarding functional cognitive capacity. Such a global data set would enable researchers to evaluate cognitive performance and ocular functional/structural improvements, enhancing the ecological validity of evaluating the eye and brain together.

In conclusion, cognitive decline and visual decline remain major comorbidities among the aging population, with incidence increasing exponentially from 65 years of age to 85 years of age [21]. With the significant increase in both the number and longevity of older persons, it becomes necessary to identify universal lifestyle choices and treatment options that might slow or reduce the effects of aging on both visual and cognitive disability. Xanthophyll carotenoids will surely play a role.

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