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2022 NMOA Mid-Year Continuing Education

Friday, September 30, 2022

12noon **Registration**
Soda Break Sponsored by MacuHealth

1pm-2pm **OD Education - Professor John Nolan**
Vitreous Antioxidants and Vitreous Floaters: Exploring the Links

2pm-3pm **OD Education - Professor John Nolan**
Targeted Nutrition for the Macula:
Optometry's Role in a New Era of Visual Function

3pm-5pm **OD Education TPA - Dr. Claudio Lagunas**
Utilizing modern tools to diagnose, monitor and treat AMD.

Friday CE Sponsored by Macuhealth

5pm-7pm **NMOA Mid-Year Convention Reception**
Reception Sponsored by Macuhealth

Saturday, October 1, 2022

7:30am **Registration**
Break Sponsored by MacuHealth

8am-10am **OD Education TPA - Dr. Pinakin Davey**
A Guide To Glaucoma Management

10am-11am **OD Education - Dr. Pinakin Davey**
Diagnostic Benefit Beyond OCT: Cone Contrast Testing

11am-12noon **OD Education - Dr. Pinakin Davey**
Carotenoids and its Benefits: More Than Meets the Eyes

12noon End of Mid-Year Convention

2022 NMOA Mid-Year Faculty Biographies

Professor John Nolan

Professor John Nolan is a Fulbright Scholar and currently holds a Chair for Human Nutrition Research at the School of Health Science, Waterford Institute of Technology, Ireland. Prof Nolan is the Founder and Director of the Nutrition Research Centre Ireland (NRCI). His research centre studies the role of nutrition for vision, cognitive function and prevention of age-related diseases. Professor Nolan has published 115 peer-reviewed scientific papers on his area of research (5920 = citations, H index = 47). A major career highlight is his role as Chair of the International Brain and Ocular Nutrition Conference (BON Conference), which is held at Downing College, Cambridge University (www.bonconference.org).

Claudio Lagunas, OD

Dr. Claudio Lagunas has over 25 years experience in both commercial and private practice multiple location ownership and management. He currently has a Vision Source private practice with his wife and 5 associates in The Woodlands, TX. His oldest daughter is a fourth year Optometry Student at the University of Houston College of Optometry. He is the Medical Director and Administrator for Vision Source in the greater Houston area. He is an Alcon speaker, consultant, and key opinion leader for over 11 years. He is faculty for the Practioners Visiting Alcon and Alcon Academy for Eye Care. Excellence programs in Fort Worth, TX. He was part of the Maculogix Speakers Alliance since 2019 and is a speaker and key opinion leader for Macuhealth since 2022. His hobbies include traveling to beautiful beaches around the world and spending time with his family.

Pinakin Davey, PhD, OD, FAAO

Dr. Pinakin Gunvant Davey is a tenured Professor at Western University of Health Sciences, College of Optometry. He holds Doctor of Optometry degree from Southern College of Optometry and a Ph.D. from Anglia Ruskin University in Cambridge, England, in the area of corneal biomechanics and its influence on glaucoma related measurements. His post-doctoral research fellowship at University of Louisville for three years was focused on improving imaging techniques in glaucoma. He has authored over 50 international publications, and has given over 100 conference and invited presentations both nationally and internationally. Dr. Davey research area is focused on retinal and optic nerve physiology and researches methods of improving vision in glaucoma and macular degeneration. Dr. Davey is a fellow of American Academy of Optometry (AAO) and a member of Optometric Glaucoma Society (OGS), Association for research in vision and Ophthalmology (ARVO), American Optometric Association (AOA).

2022 NMOA Mid Year Convention

Handouts for Friday, September 30th Lectures

Drs. John Nolan & Claudio Lagunas

Vitreous Antioxidants and Vitreous Floaters: Exploring the Links

Targeted Nutrition for the Macula: Optometry's Role in a New Era of Visual Function

Utilizing modern tools to diagnose, monitor and treat AMD.

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Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment

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ABSTRACT

This study was designed to investigate the impact of macular carotenoid supplementation on the spatial profile of macular pigment (MP) in subjects where the profile does not exhibit the typical central peak (i.e. peaked MP at foveal epicentre). Thirty one healthy subjects with such atypical MP spatial profiles were assigned to one of three intervention groups: Group 1: ($n = 10$), 20 mg/day lutein (L), 2 mg/day zeaxanthin (Z); Group 2: ($n = 10$), 10 mg/day *meso*-zeaxanthin (MZ), 10 mg/day L, 2 mg/day *Z*; Group 3: ($n = 10$), 17 mg/day MZ, 3 mg/day L, 2 mg/day Z. Subjects were instructed to take one capsule daily over an 8-week period. MP at 0.25°, 0.5°, 1°, 1.75°and 3° was measured using customized-heterochromatic flicker photometry at baseline, four weeks and 8 weeks. Over the study period, we report no statistically significant increase in MP at any eccentricity in Group 1 ($p > 0.05$, for all eccentricities). There was a trend towards an increase in MP at all eccentricities in Group 2, with a significant increase found at 0.25° and 0.50° ($p = 0.000$ and $p = 0.016$, respectively). There was a statistically significant increase evident in MP at 0.25° in Group 3 ($p = 0.005$), but at no other eccentricity ($p > 0.05$, for all other). We report that the typical central peak of MP can be realised in subjects with atypical spatial profiles, following supplementation with a preparation containing all three macular carotenoids, but not with a supplement lacking MZ. The implications of our findings, in terms of visual performance and/or a (photo)-protective effect, warrant additional study.

1. Introduction

The central retina, known as the macula, is responsible for colour and fine-detail vision (Hirsch and Curcio, 1989). A pigment, composed of the carotenoids, lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ), (Bone et al., 1997; Johnson et al., 2005) accumulate at the macula where they are collectively known as macular pigment (MP). MP is a blue light filter (Snodderly et al., 1984b) and a powerful antioxidant, (Khachik et al., 1997) and is therefore believed to protect against age-related macular degeneration (AMD), which is now the most common cause of blind registration in the western world (Klaver et al., 1998). In addition, MP's putative ability to enhance visual performance and comfort is also of interest (Bartlett and Eperjesi, 2008; Engles et al., 2007; Hammond and Wooten, 2005; Kvansakul et al., 2006; Loughman et al., 2010; Nolan et al., 2011; Rodriguez-Carmona et al., 2006; Stringham and Hammond, 2007, 2008; Stringham et al., 2004; Wooten and Hammond, 2002).

Z and MZ are the predominant carotenoid in the foveal region, whereas L predominates in the parafoveal region (Bone et al., 1988; Snodderly et al., 1991). The concentration of MZ peaks centrally, with the MZ:Z ratio of 0.83 (approximately) within the central 3 mm of the macula and 0.25 (approximately) between 11–21 mm from the centre of the macula. (Bone et al, 1997). The above observations are most probably attributable to the fact that retinal MZ is produced primarily by isomerization of retinal L, (Johnson et al., 2005) thus accounting for lower relative levels of L, and higher relative levels of MZ, in the central macula, and vice versa in the peripheral macula, and would also explain why MZ accounts for about one third of total MP, (Bone et al., 1993) in spite of its absence or low concentrations in a typical diet.

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MP represents the most conspicuous accumulation of carotenoids in the human body; however, its concentration has been shown to vary dramatically among individuals (Hammond et al., 1997). Typical MP profiles generally peak at the centre of the macula and decreases in concentration with increasing eccentricity out to the parafovea (Bone et al., 1988; Snodderly et al., 1984a). However, as mentioned above, variations in the distribution of MP have been reported (Berendschot and van Norren, 2006; Delori et al., 2006; Kirby et al., 2009). Recently, for example, it has been shown that atypical MP spatial profiles (i.e. those not exhibiting a typical central peak) are present in some individual MP profiles. More importantly, it has been confirmed that these atypical profiles are real and reproducible features of the MP spatial profile, when measured using customized-heterochromatic flicker photometry (cHFP, a validated technique for measuring MP) (Kirby et al., 2009). The importance of such variations, if any, in the spatial profile of MP (e.g. the absence of a typical central peak) is not yet known, but may be related to the putative protective role of this pigment. For example, reduced MPOD at the centre of the macula may be associated with increased risk of developing AMD (given the lower antioxidant activity and short-wavelength light filtering capacity of such an individual, when compared to an individual with a typical central peak) (Trieschmann et al., 2003). Also, a recent study by our research group has shown that 12% (58 subjects out of a sample database of 484 subjects) of the healthy Irish population exhibit a reproducible atypical MP spatial profile (characterized by the lack of a typical central peak) and that such atypical MP spatial profiles are more common in older subjects and in cigarette smokers (two of the established risk factors for AMD) (Kirby et al., 2010).

In brief, the current study has taken advantage of a unique opportunity, by inviting subjects from the above mentioned database ($n = 58$), (Kirby et al., 2010) who were identified, and confirmed, as exhibiting such an atypical MP spatial profile (see Fig. 1).

2. Methods

2.1. Subjects and study design

Fifty eight subjects with atypical MP spatial profiles (identified from our master MP database; $n = 484$) were invited to revisit our vision science laboratories at the Waterford Institute of Technology (WIT) and Dublin Institute of Technology (DIT), Ireland, to confirm the presence of their atypical MP spatial profile. Of the thirty nine subjects who agreed to come back for testing, thirty subjects were confirmed as still exhibiting an atypical MP profile as defined by our criteria (i.e. MPOD at 0.25° did not exceed MPOD at 0.5° of eccentricity by more than 0.04 optical density units) generated for the purpose of this study, and were therefore enrolled into the 8-week supplementation trial with one of three different macular carotenoid formulations (see below).

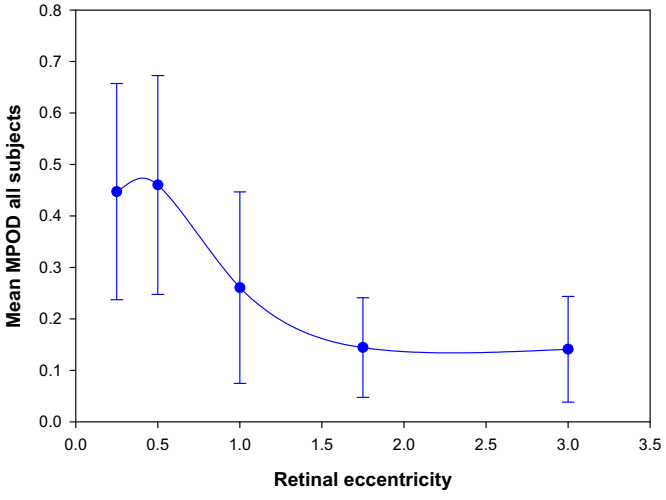

Fig. 1. Mean (±SD) macular pigment optical density spatial profile for the entire study group ($n = 30$) at baseline. The smooth line drawn through the data was achieved using our graphic software Sigma Plot 8.

Of the nine subjects who no longer exhibited an MP spatial profile sufficiently atypical for inclusion in the current study, because of our strict and predefined criteria, seven did exhibit a persistently atypical profile. With respect to the other two subjects, possible explanations as to why they no longer exhibited the previously observed atypical MP profile may rest on the interval between original testing and recall for the purpose of this study and/or changes in dietary habits (including possible supplementation).

All subjects signed an informed consent document and the experimental measures conformed to the Declaration of Helsinki. The study was reviewed and approved by the Research Ethics Committees of Waterford Institute of Technology, Waterford, Ireland, and Dublin Institute of Technology, Dublin, Ireland. Inclusion criteria for participation in this study were as follows: MPOD at 0.25° did not exceed MPOD at 0.5° of eccentricity by more than 0.04 optical density units (thereby defining "atypical" MP spatial profile for the purpose of this study); no presence of ocular pathology; corrected distance visual acuity (CDVA) 20/60 or better in the study eye; no current or prior use of supplements containing L and/or Z and/or MZ.

This was a randomized and double blind clinical trial with three interventions. Subjects were randomly assigned into one of the three groups as follows: Group 1: high L group ($n = 10$; L = 20 mg/day, Z = 2 mg/day); Group 2: combined carotenoid group ($n = 10$; MZ = 10 mg/day, L = 10 mg/day, Z = 2 mg/day); Group 3: high MZ group ($n = 10$; MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day).

All subjects were instructed to take one capsule per day with a meal for 8 weeks. Significant efforts were made to ensure compliance to the study intervention. Weekly text messages and phone calls were made by the research team. In addition, subjects were requested to return their supplement packs at their exit visit, and compliance was checked by tablet counting at this visit.

MPOD, including its spatial profile, i.e. at 0.25°, 0.5°, 1°, 1.75°, 3°, was measured at baseline, four weeks and 8 weeks. The right eye was chosen as the study eye for all subjects, with the exception of one subject whose right CDVA did not meet the criteria for MP testing, and the left eye for that subject was, therefore, chosen as the study eye.

Demographic, lifestyle and vision information was also collected from each subject as follows: name; contact information; age; sex; smoking habits; medication and vision case history. CDVA was measured by logMAR chart. A subject's weekly intake of carotenoid rich foods (eggs, broccoli, corn, dark leafy vegetables) were inputted into the "L/Z screener" to give a carotenoid diet 'score'. Values were weighted for frequency of intake of the food and for the bioavailability of L and Z within these foods and a ranking score reflecting the relative intakes was generated. Evaluation of the L/Z screener against the Willet food frequency questionnaire yielded a positive correlation that was strongly significant ($p < 0.01$). The range of scores from the L/Z screener is 0–75. After adding foods with known concentrations of L and Z into the screener, the following estimates were made. Low dietary carotenoid intake score is from 0 to 15 (i.e. ≤2 mg/d); medium dietary carotenoid intake score is from 16 to 30 (i.e. between >2 and 13 mg/day);

high dietary carotenoid intake score is from 31 to 75 (i.e. >13 mg/day).

2.2. Measurement of macular pigment optical density

The spatial profile of MP was measured using the Macular Densitometer™, a HFP instrument that is slightly modified from a device described by Wooten and Hammond (2005). A detailed discussion of the principle of HFP and its customization to accurately measure MP has also been described by Kirby et al. (2009). All subjects in this study previously had their MP spatial profile measured with the Macular Densitometer™ using the cHFP technique. In addition, further training was provided prior to testing. Therefore, all subjects in the current study were experienced with respect to the device and testing procedure. In order to measure the spatial profile of MP, we performed measurements at the following degrees of retinal eccentricity: 0.25°, 0.5°, 1°, 1.75°, 3° and 7° (the reference point) obtained using the following sized target diameters; 30 min, 1°, 2°, 3.5°, 1° and 2°, respectively. Stimulus 5, our 3° target, was a 1° diameter disc with its centre located 3° from a black fixation point (i.e. the average of the inner arc which defines the disc at 2.5° and the outer arc which defines the disc at 3.5°). Stimulus 6, our reference point, is a 2° diameter disc with its centre located 7° from a red fixation point (i.e. the average of the inner arc which defines the disc at 6° and the outer arc which defines the disc at 8°) as MPOD at this location is optically undetectable and its distribution at this location is essentially flat. Measurement of the spatial profile of MP using cHFP has previously been shown to be highly reproducible (ICC = 0.93–0.96), and therefore does not account for change identified in the spatial profile of MP over time (either following, or without, dietary modification/supplementation) when measured using this technique (Kirby et al., 2009, 2010).

2.3. Statistical analysis

The statistical software package PASW Statistics 17.0 (SPSS Inc., Chicago, Illinois, USA) was used for analysis and Sigma Plot 8.0 (Systat Software Inc., Chicago, Illinois, USA) was used for graphical presentations. All quantitative variables investigated exhibited a normal distribution. Means ± SDs are presented in the text and tables. Statistical comparisons of the three different intervention groups, at baseline, were conducted using one way ANOVA and chi-square analysis, as appropriate.

We conducted repeated measures ANOVA for MPOD, including its spatial profile, for each intervention group, including each study visit, using a general linear model approach, with age as a covariate (as age was significantly different between the groups at baseline, see below). Bonferroni correction was applied as appropriate. We used the 5% level of significance throughout our analysis.

3. Results

3.1. Baseline findings

The demographic, lifestyle, CDVA, and MPOD data of all thirty one subjects recruited into the study, and divided by study arm (i.e. Group 1, Group 2, Group 3), are summarized in Table 1. As seen from this table, most variables under investigation did not differ significantly between these groups at baseline ($p > 0.05$, for said variables). However, a significant baseline difference between these groups with respect to age ($p < 0.01$) was identified, with Group 3 having a significantly lower mean age when compared to Groups 1 and 2, and age was therefore controlled for throughout the remainder of the analysis.

Table 1
Demographic, lifestyle, visual acuity and macular pigment data at baseline.

Characteristic	All ($n = 30$)[a]	Group1 ($n = 10$)	Group 2 ($n = 10$)	Group 3 ($n = 10$)
Age	47 ± 14	51 ± 11[b]	56 ± 11[b]	35 ± 10[b]
Sex				
Male	9	3	3	3
Female	21	7	7	7
Smoking habits[c]				
Never smoker	19	7	7	5
Ex-smoker	10	2	3	5
Current smoker	1	1	0	0
Positive FH of AMD[d]	8	3	2	3
Diet score[e]	27 ± 14	34 ± 15	25 ± 14	22 ± 13
Aided Visual acuity	105 ± 8	105 ± 8	106 ± 6	107 ± 4
MPOD				
0.25°	0.45 ± 0.21	0.46 ± 0.21	0.41 ± 0.27	0.48 ± 0.16
0.5°	0.46 ± 0.21	0.46 ± 0.23	0.44 ± 0.26	0.48 ± 0.15
1°	0.26 ± 0.19	0.20 ± 0.19	0.26 ± 0.23	0.32 ± 0.12
1.75°	0.14 ± 0.10	0.15 ± 0.10	0.18 ± 0.10	0.11 ± 0.09
3°	0.14 ± 0.10	0.14 ± 0.11	0.16 ± 0.12	0.12 ± 0.08

[a] n = sample size.
[b] $p < 0.01$.
[c] Smoking habits: ex-smoker = smoked ≥100 cigarettes in lifetime but none in last 12 months; current smoker = smoked ≥100 cigarettes in lifetime and at least 1 cigarette per week in last 12 months.
[d] Positive FH of AMD: positive family history of age-related macular degeneration (self reported). Values represent mean ± standard deviation; MPOD = macular pigment optical density; 0.25° = MPOD measured at 0.25° retinal eccentricity; 0.5° = MPOD measured at 0.5° retinal eccentricity; 1° = MPOD measured at 1° retinal eccentricity; 1.75° = MPOD measured at 1.75° retinal eccentricity; 3° = MPOD measured at 3° retinal eccentricity; Group 1: high L group (L = 20 mg/day, Z = 2 mg/day); Group 2: combined carotenoid group (MZ = 10 mg/day, L = 10 mg/day, Z = 2 mg/day); Group 3: high MZ group (MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day).
[e] A subject's weekly intake of carotenoid rich foods (eggs, broccoli, corn, dark leafy vegetables) were inputted into an L/Z screener to give a carotenoid diet 'score'. Values were weighted for frequency of intake of the food and for the bioavailability of L and Z within these foods and an arbitrary score were generated and used to adjust for diet, as appropriate.

3.2. Change in MPOD over 8-week supplementation period

As seen in Table 2, increases in MPOD at 0.25° and 0.5° were statistically significant in Group 2. Similarly, a significant increase in MPOD at 0.25° was seen in Group 3. Of note, after Bonferroni correction for multiple testing, the MPOD increase seen at 0.25° in Group 2 was the only observed increase to remain statistically significant.

Changes in MPOD values over time, for each subject and for all eccentricities measured, are presented in Table 3. Change in the spatial profile of MPOD for each group is illustrated in Figs. 2–4. These figures graphically represent mean MPOD spatial profile for each group at baseline (pre supplementation) and at 8 weeks (post supplementation).

At 0.25° of eccentricity, a MPOD increase of >10% was seen in 4 (40%), 10 (100%) and 8 (80%) subjects in Groups 1, 2, and 3, respectively. Further, at this eccentricity, the average increase in MPOD (measured in optical density units) was 0.031 (13%), 0.182 (102%), and 0.094 (22%) in Groups 1, 2 and 3, respectively. At 0.5° of eccentricity, a MPOD increase of >10% was seen in 3 (30%), 7 (70%) and 5 (50%) subjects in Groups 1, 2 and 3, respectively. Further, at this eccentricity, the average increases in MPOD (measured in optical density units) was 0.02 (13%), 0.079 (27%), and 0.019 (6%), for these Groups, respectively.

4. Discussion

This is the first study designed to investigate the effect of macular carotenoid supplementation, with three different

Table 2
Average MPOD values at each degree of retinal eccentricity for all subjects according to group & visit.

Group	MPOD	Baseline	4 wks	8 wks	Time interaction (*p*-value)
Group 1	0.25°	0.46 ± 0.21	0.48 ± 0.21	0.49 ± 0.22	0.220
Group 1	0.5°	0.46 ± 0.23	0.46 ± 0.19	0.48 ± 0.23	0.626
Group 1	1°	0.20 ± 0.19	0.27 ± 0.16	0.25 ± 0.14	0.283
Group 1	1.75°	0.15 ± 0.10	0.16 ± 0.10	0.15 ± 0.09	0.904
Group 1	3°	0.14 ± 0.11	0.16 ± 0.09	0.11 ± 0.08	0.370
Group 2	0.25°	0.41 ± 0.27	0.50 ± 0.27	0.59 ± 0.30	0.000
Group 2	0.5°	0.44 ± 0.26	0.46 ± 0.28	0.52 ± 0.28	0.016
Group 2	1°	0.26 ± 0.23	0.29 ± 0.15	0.34 ± 0.10	0.417
Group 2	1.75°	0.18 ± 0.10	0.19 ± 0.06	0.22 ± 0.06	0.218
Group 2	3°	0.16 ± 0.12	0.14 ± 0.06	0.19 ± 0.11	0.448
Group 3	0.25°	0.48 ± 0.16	0.55 ± 0.19	0.57 ± 0.18	0.005
Group 3	0.5°	0.48 ± 0.15	0.48 ± 0.17	0.50 ± 0.15	0.786
Group 3	1°	0.32 ± 0.12	0.31 ± 0.13	0.34 ± 0.12	0.596
Group 3	1.75°	0.11 ± 0.09	0.12 ± 0.07	0.13 ± 0.08	0.743
Group 3	3°	0.12 ± 0.08	0.15 ± 0.07	0.15 ± 0.07	0.522

Values represent mean ± standard deviation; $n = 31$; MPOD = macular pigment optical density; 0.25° = MPOD measured at 0.25° retinal eccentricity; 0.5° = MPOD measured at 0.5° retinal eccentricity; 1° = MPOD measured at 1° retinal eccentricity; 1.75° = MPOD measured at 1.75° retinal eccentricity; 3° = MPOD measured at 3° retinal eccentricity; Group 1 ($n = 10$): high L group (L = 20 mg/day, Z = 2 mg/day); Group 2: combined carotenoid group (MZ = 10 mg/day, L = 10 mg/day, Z = 2 mg/day); Group 3: high MZ group (MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day); The *p*-values represent repeated measures ANOVA for the 3 study visits (within–subject effects), with Greenhouse–Gesser correction for lack of sphericity as appropriate.

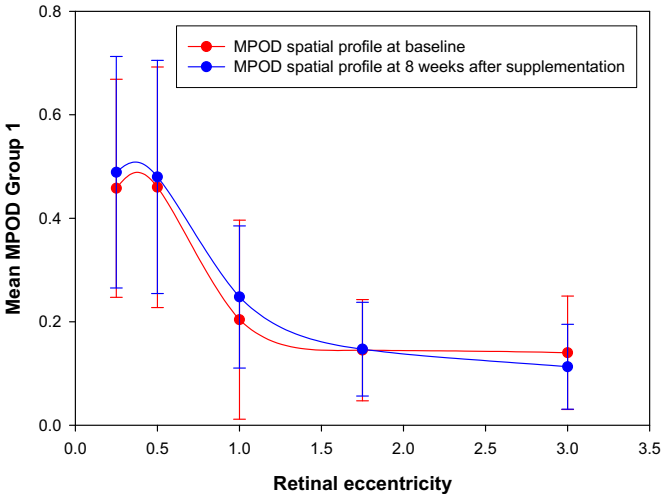

Fig. 2. Mean macular pigment optical density spatial profile of Group 1 at baseline (pre supplementation) and at 8 weeks (post supplementation). Mean ± standard deviation; $n = 10$; Group 1: high *L* group ($L = 20$ mg/day, $Z = 2$ mg/day). The smooth line drawn through the data was achieved using our graphic software Sigma Plot 8.

carotenoid formulations, on the spatial profile of MP in subjects with an atypical MP profile characterized by the lack of the typical central peak. Over an eight week study period, subjects with such a pre-identified and confirmed atypical spatial profile of their MP

Table 3
Individual MPOD values at each degree of retinal eccentricity for all subjects according to group & visit wise.

No.	Group	0.25°			0.50°			1.0°			1.75°			3.0°		
		Baseline	4 wks	8 wks	Baseline	4 wks	8 wks	Baseline	4 wks	8 wks	Baseline	4 wks	8 wks	Baseline	4 wks	8 wks
1	Group 1	0.86	0.88	0.95	0.88	0.74	0.88	0.02	0.43	0.2	0.24	0.21	0.17	0.2	0.13	0.02
4	Group 1	0.47	0.52	0.53	0.43	0.5	0.51	0.11	0.24	0.19	0.07	0.14	0.13	0.09	0.16	0.06
5	Group 1	0.39	0.39	0.39	0.39	0.39	0.39	0.31	0.3	0.21	0.17	0.26	0.2	0.1	0.35	0.15
7	Group 1	0.14	0.16	0.3	0.13	0.18	0.3	0	0	0.2	0.08	0	0.1	0.16	0.2	0.22
8	Group 1	0.31	0.33	0.28	0.28	0.3	0.34	0.12	0.14	0.23	0.12	0.07	0.17	0.06	0.18	0.15
11	Group 1	0.7	0.72	0.7	0.76	0.72	0.75	0.67	0.55	0.57	0.37	0.3	0.32	0.38	0.26	0.26
12	Group 1	0.29	0.31	0.3	0.3	0.32	0.28	0.2	0.33	0.2	0.06	0.1	0.06	0.03	0.11	0.09
15	Group 1	0.35	0.35	0.27	0.31	0.31	0.18	0.13	0.13	0.05	0.17	0.17	0.01	0.08	0.08	0.01
16	Group 1	0.54	0.54	0.57	0.51	0.51	0.51	0.19	0.19	0.26	0.07	0.07	0.08	0.05	0.05	0.1
19	Group 1	0.53	0.6	0.6	0.61	0.66	0.66	0.29	0.37	0.37	0.1	0.23	0.23	0.25	0.07	0.07
2	Group 2	0.55	0.61	0.75	0.57	0.57	0.57	0.73	0.31	0.44	0.29	0.3	0.27	0.34	0.16	0.39
3	Group 2	0.28	0.39	0.45	0.29	0.25	0.38	0.32	0.17	0.3	0.18	0.19	0.17	0.24	0.07	0.05
9	Group 2	0.23	0.28	0.34	0.27	0.31	0.27	0.36	0.17	0.31	0.23	0.14	0.24	0.34	0.1	0.21
10	Group 2	0.04	0.27	0.28	0.12	0.24	0.25	0.02	0.21	0.25	0	0.15	0.19	0	0.08	0.17
13	Group 2	0.35	0.47	0.57	0.36	0.36	0.51	0	0.17	0.33	0.01	0.13	0.21	0	0.07	0.22
14	Group 2	0.68	0.79	0.8	0.74	0.69	0.7	0.43	0.48	0.48	0.24	0.26	0.26	0.14	0.24	0.24
17	Group 2	0.13	0.1	0.23	0.16	0.09	0.2	0.15	0.16	0.33	0.25	0.1	0.24	0.17	0.15	0.28
18	Group 2	0.65	0.82	0.91	0.67	0.88	0.9	0.36	0.57	0.49	0.19	0.24	0.33	0.07	0.22	0.17
20	Group 2	0.86	0.9	1.12	0.88	0.88	1	0.02	0.43	0.2	0.24	0.21	0.17	0.2	0.13	0.02
31	Group 2	0.29	0.39	0.43	0.31	0.37	0.38	0.24	0.25	0.25	0.14	0.16	0.15	0.13	0.15	0.15
21	Group 3	0.64	0.68	0.51	0.63	0.63	0.46	0.44	0.44	0.43	0.26	0.23	0.14	0.29	0.19	0.13
22	Group 3	0.57	0.55	0.61	0.61	0.55	0.58	0.48	0.35	0.31	0.08	0.15	0.11	0.13	0.25	0.14
23	Group 3	0.5	0.59	0.66	0.47	0.48	0.55	0.35	0.38	0.41	0.14	0.15	0.24	0.15	0.24	0.13
24	Group 3	0.55	0.58	0.61	0.56	0.56	0.57	0.32	0.27	0.33	0.12	0.09	0.13	0.12	0.1	0.13
25	Group 3	0.34	0.34	0.4	0.38	0.38	0.38	0.23	0.23	0.23	0	0	0	0.09	0.09	0.09
26	Group 3	0.57	0.7	0.77	0.57	0.69	0.64	0.4	0.29	0.38	0.26	0.19	0.21	0.2	0.18	0.22
27	Group 3	0.29	0.32	0.34	0.29	0.29	0.31	0.2	0.14	0.15	0.06	0.04	0.06	0.06	0.09	0.08
28	Group 3	0.71	0.92	0.93	0.7	0.69	0.79	0.42	0.57	0.59	0.08	0.18	0.2	0.05	0.16	0.3
29	Group 3	0.29	0.37	0.41	0.3	0.23	0.33	0.1	0.16	0.26	0	0.07	0.05	0.03	0.05	0.14
30	Group 3	0.32	0.45	0.48	0.33	0.34	0.42	0.21	0.3	0.28	0.11	0.1	0.11	0.08	0.12	0.11

Values represent macular pigment optical density (MPOD) values; $n = 30$; 0.25° = MPOD measured at 0.25° retinal eccentricity; 0.5° = MPOD measured at 0.5° retinal eccentricity; 1° = MPOD measured at 1° retinal eccentricity; 1.75° = MPOD measured at 1.75° retinal eccentricity; 3° = MPOD measured at 3° retinal eccentricity; Group 1 ($n = 10$): high L group (L = 20 mg/day, Z = 2 mg/day); Group 2 ($n = 10$): combined carotenoid group (MZ = 10 mg/day, L = 10 mg/day, Z = 2 mg/day); Group 3 ($n = 10$): high MZ group (MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day). Of note, at baseline average MPOD at 0.25° was significantly less than average MPOD at 0.5° in the subjects studied here.

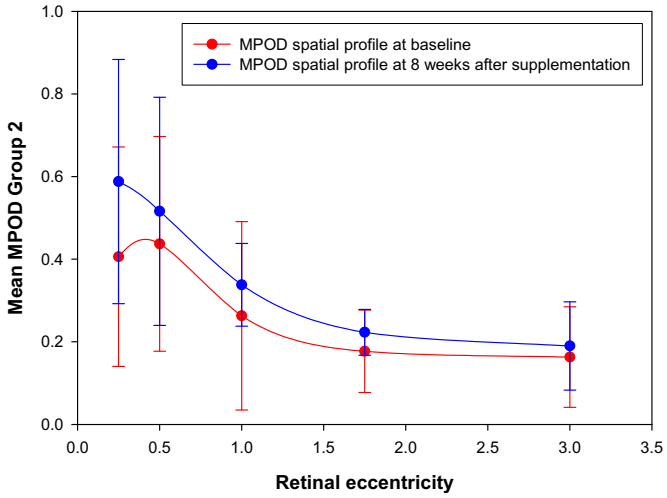

Fig. 3. Mean macular pigment optical density spatial profile of Group 2 at baseline (pre supplementation) and at 8 weeks (post supplementation). Mean ± standard deviation; $n = 10$; Group 2: combined carotenoid group (MZ = 10 mg/day, $L = 10$ mg/day, $Z = 2$ mg/day). The smooth line drawn through the data was achieved using our graphic software Sigma Plot 8.

were supplemented with one of three different carotenoid formulations, as follows: Group 1: high L group (L = 20 mg/day, Z = 2 mg/day); Group 2: combined carotenoid group (MZ = 10 mg/day, L = 10 mg/day, Z = 2 mg/day); Group 3: high MZ group (MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day).

Over the last number of years, reports on the spatial profile of MP have generated debate. In 1997, Hammond, Wooten and Snodderly conducted a study in 32 subjects to investigate individual variations in the spatial profile of human MP, and concluded that an exponential decay with eccentricity explained more variance in the distribution than a Gaussian function (Hammond et al., 1997). The MP spatial profile has since been described as a central peak, which decreases with eccentricity to optically undetectable levels by 10° eccentricity. While it is true that such an exponential decay still describes the MP profile very well (even in subjects with "atypical" profiles), recent study has revealed that there are obvious deviations from a monotonic decline from the central fovea

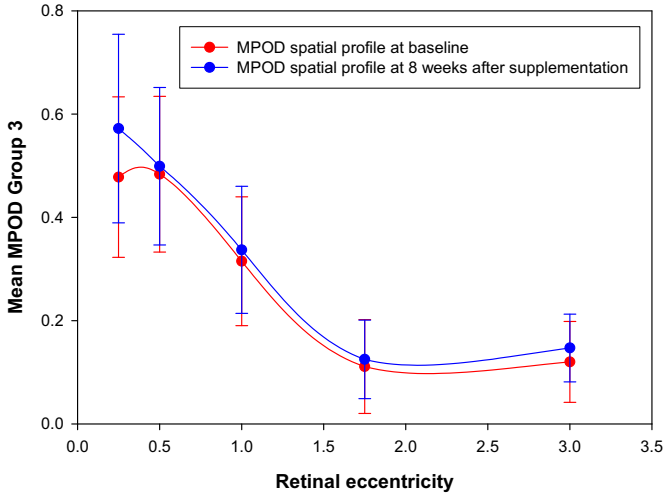

Fig. 4. Mean macular pigment optical density spatial profile of Group 3 at baseline (pre supplementation) and at 8 weeks (post supplementation). Mean ± standard deviation; $n = 10$; Group 3: high MZ group (MZ = 17 mg/day, L = 3 mg/day, Z = 2 mg/day). The smooth line drawn through the data was achieved using our graphic software Sigma Plot 8.

(Berendschot and van Norren, 2006; Dietzel et al., 2011; Kirby et al., 2009, 2010; Trieschmann et al., 2003).

Indeed, even in the publication by Hammond et al. in 1997, the authors noted deviations from an exponential function in 40% of subjects (Hammond et al., 1997). In 2003, Trieschmann et al, reported that the spatial profile of MP, assessed using AF, exhibited four types of distribution, and that MPOD was lower in patients with AMD (Trieschmann et al., 2003). In 2006, Delori et al. described bimodal spatial distributions of MP that were characterized by a central peak of highest MP density surrounded by a ring with high-density values at approximately 0.7° from the fovea. In the same year, Berendschot and van Norren (2006) confirmed this finding and reported that both reflectance and AF maps showed ringlike patterns in the distribution of the MP, and suggested that such patterns follow the distribution of the inner plexiform layer. Indeed, the authors reported a distinct ring pattern in over 50% of subjects, at a mean distance of 0.7° from the foveal centre, and noted that in a few subjects, the orbit of the ring has an even greater optical density than did the central peak. Furthermore, Dietzel et al. reported ringlike structures in circa 20% of subjects, which were less likely to be seen in subjects with AMD. Dietzel et al. also described MP distributions (using AF) as intermediate where there is no strictly monotonic decline from the centre of the fovea to the periphery, but no explicit ringlike pattern of MP, but where an implied plateau exists (Dietzel et al., 2011).

In brief, therefore, there is consensus that inter-individual variability, in terms of the spatial distribution of MP, does exist. However, the terminology used to classify such variations has differed, and the terms employed reflect the methodology used to measure MP.

With HFP, for example, a 2-dimensional profile (silhouette) of MP is generated, prompting terms such as "central dip" (Kirby et al., 2010), "minor flanking peaks" (Hammond et al., 1997) or "shoulder" to describe profiles that do not exhibit the typical monotonic decline with eccentricity and that are seen in about 40% of subjects (Hammond et al., 1997).

Using AF, where an "en face" map is generated, the term "ringlike structure" has been used to describe "...the bimodal pattern of MPOD [is] visible as a ringlike structure with a central peak of MPOD surrounded by a ring of increased density" (Dietzel et al., 2011). We believe the ringlike structures and intermediate profiles described with AF represent the non-monotonic decays of MP that we and others have observed using HFP, given the radial symmetry of MPOD (Hammond et al., 1997). In support of this view is the observation that the former are seen in approximately 50% of cases using AF (Berendschot and van Norren, 2006) and the latter are seen in approximately 40% of cases assessed by HFP (Hammond et al., 1997). For this reason, and for the purpose of this study, we have defined an atypical profile as one where MPOD at 0.25° does not exceed MPOD at 0.5° by more than 0.04 ODU, therefore representing a subgroup of AF-generated ringlike structures or intermediate patterns described by Berendschot and van Norren in a "few" of their subjects (Berendschot and van Norren, 2006).

We report a statistically significant increase in MPOD at 0.25° retinal eccentricity in the combined carotenoid group (Group 2) and the high MZ group (Group 3), but no increase in MPOD at 0.25° in the high L group (Group 1). With respect to individual responses and magnitude of responses within the Groups, it is important to note that the increase in MPOD, whether expressed in terms of the proportion of subjects exhibiting a >10% rise or in terms of average increase in MPOD, at either 0.25 or 0.5° eccentricity, was substantially greater for subjects in Group 2 (i.e. those supplemented with all three macular carotenoids). Further, Group 2 was unique in that all subjects in this Group exhibited an increase of at least 10% (i.e. a clinically meaningful response) at 0.25° eccentricity, and was

also unique in that the average MPOD increase was greater than 100% at this eccentricity (and this compares with only 13% and 22% in Groups 1 and 3, respectively). MP response at this central retinal location is of interest to the current investigation and report, given that the pre-specified hypothesis was that supplementation with appropriate macular carotenoids could realise the typical central peak of MP in subjects selected on the basis that they lacked such (desirable) typical profile at baseline (Kirby et al., 2010). The research question, therefore, was to determine if subjects not exhibiting the typical central peak of MP (at baseline) would respond differently to different macular carotenoid formulations. We hypothesized that supplementation with MZ (as in Groups 2 and 3) may augment central MP in subjects presenting with our predefined and non-peaked MP spatial profiles. The rationale, which informed this hypothesis, was premised on the observation that MZ, which comprises one-third of the human MP, is the most centrally located of the macular carotenoids (Bone et al., 1997) (i.e. the location of the typical central peak of MP (Kirby et al., 2010)).

Of importance to this discussion, macular MZ is produced primarily by isomerization of macular L, (Neuringer et al., 2004) thus accounting for lower relative levels of L, and higher relative levels of MZ, in the central macula, and vice versa in the peripheral macula (Bone et al., 1988). It is possible, therefore, that the mechanism which converts L to MZ at the macula (which may be enzymatic (Bone et al., 1997) and/or light dependent (Nolan et al., 2009)) is defective in individuals with no observable typical central peak of MP. Indeed, the data presented here is consistent with this hypothesis. Importantly, however, we now confirm that subjects without a typical central peak in their MP spatial profiles do respond to a supplement containing MZ (as seen in Groups 2 and 3), but do not respond to a supplement containing high amounts of L (as seen in Group 1).

The above finding is all the more important, given a recent publication by our group which showed that individuals at increased risk of developing AMD (e.g. cigarette smokers and older people) were more likely to lack the typical central peak in their MP spatial profile (see publication by Kirby et al. (2010)). Possible explanations for this observed association between the atypical non central-peaked MP spatial profile and increased risk of AMD may be attributable to this pigment's physical and chemical properties. For example, the absence of a central peak of MP suggests a lack of MZ, and therefore lower antioxidant activity (Foote et al., 1970; Li et al., 2010) and less short-wavelength light filtering capacity, when compared to individuals with the typical peak of MP at the macular epicentre. Indeed, it is these two properties of MP which have been hypothesized to confer protection against AMD, and therefore merit discussion (see below).

Moreover, our data is consistent with previous publications in AMD populations. For example, a study performed by Trieschmann et al., of 400 subjects (253 with signs of early AMD, 147 without AMD), reported that eyes afflicted with AMD were more likely to display low central MPOD when compared to non-AMD subjects (Trieschmann et al., 2003).

Also, we report that enrichment of MP across the full spatial profile (i.e. at 0.25°, 0.5°, 1°, 1.75°, 3°) was achieved only when subjects were supplemented with all three macular carotenoids (as per Group 2), suggesting a beneficial and maybe even an interactively additive effect of supplementing with all three carotenoids. Groups 1 and 3 demonstrated little or no response at the eccentricities beyond 0.25° (i.e. at 0.5°, 1°, 1.75°, 3°). Of interest, the preselected eyes with atypical MP profiles were identified in subjects with high, low and medium levels of baseline MP, thus suggesting that a lack of L is not the cause of a parallel lack of the typical central peak in these subjects, and is consistent with our findings that supplementation with L alone did not increase MP significantly, whereas supplementation with L, Z and MZ increased MP significantly (centrally, in the mid periphery and in the periphery of the macula); whereas supplementation with MZ alone increased MP significantly (but only at the epicenter).

It is important to point out that an 8-week trial of supplemental L represents a relatively short time period for such a purpose. Indeed, other L-supplementation studies have also failed to significantly augment MP over this time period (Nolan et al., 2011; Trieschmann et al., 2007). However, the subjects tested here were atypical by virtue of the fact that they exhibited central dips or plateaus in their MP spatial profiles, and we sought to specifically investigate whether such subjects would respond differently to different carotenoid interventions. Interestingly, only those carotenoid formulations in the current study that contained MZ achieved a rapid response in central MPOD over this time period.

The above findings, however, are consistent with a publication by Connolly et al. who found that enrichment of MP centrally, and across its spatial profile, is achieved in subjects (both normal and AMD-afflicted) supplemented with all three macular carotenoids (Connolly et al., 2010). This notion is also consistent with *in vitro* studies reporting better functionality of the macular carotenoids when in combination rather than in isolation (Li et al., 2010). Possible functional implications of enrichment of MP centrally in subjects lacking the typical central peak of MP (i.e. following supplementation with MZ; Group 2 and Group 3), and across its full spatial profile (Group 3 only) are discussed below. Also, the increase in central MPOD, seen in Groups 2 and 3, is likely to confer optical benefits at this location (i.e. enhanced contrast sensitivity and ameliorated glare disability) (Nolan et al., 2011).

In addition, an increase in central MP will facilitate antioxidant activity at this retinal locus, whether the subject suffers from AMD or is at risk of developing this condition. From an antioxidant perspective, L, Z and MZ are structural isomers of one another and are characterized, biochemically, by their high number of double-bonds (Bone et al., 1993). Their supply of readily available electrons enables these carotenoids to quench reactive oxidative intermediates (ROIs), thus limiting membrane phosopholipid peroxidation and attenuating oxidative injury (Sujak et al., 1999). Kirschfeld was the first to propose the idea that carotenoids protect the macula against oxidative stress, (Kirschfeld, 1982) and in 1997, Khachik et al. confirmed the presence of direct oxidation products of L and Z in human retinal tissue, supporting the hypothesis that MP does indeed protect against oxidative damage in this tissue (Khachik et al., 1997).

Of note, MP is at its highest concentration in the receptor axon layer of the foveola and in the inner and outer plexiform layers of the macula (Snodderly et al., 1984a; Trieschmann et al., 2008). Also, the concentration of the carotenoids within each retinal layer peaks at the foveola (where the ratio of MZ to L and Z is maximum). Importantly, it is at this central retinal location where ROI production is greatest. *In vitro* studies of human RPE cells, subjected to oxidative stress, have demonstrated enhanced survival of these cells in the presence of Z and other antioxidants, when compared with controls (Wrona et al, 2004). Z appears to be a more potent antioxidant than L (Cantrell et al., 2003) and MZ is yet more efficacious, but only in conjunction with its binding protein (Bhosale and Bernstein, 2005). Recently, Li et al. demonstrated that a mixture of L, Z and MZ (in a ratio of 1:1:1) quenches more singlet oxygen than any of these carotenoids individually but at the same total concentration (Li et al., 2010). This collective optimization of antioxidant activity, dependent on the presence of all three macular carotenoids, could prevent depletion of MP in such a high oxidative stress environment. In other words, MP with inadequate quantities of any of the three macular carotenoids may lack sufficient antioxidant potential to stabilize the pigment in a high oxidative stress environment, such as the central retina.

From a light filtering perspective, L is reported to be a superior filter of blue light when compared to Z, due to its orientation with respect to the plane of the phospholipid bilayer of the cell membrane, (Sujak et al, 1999) which is both parallel and perpendicular. In contrast, Z and MZ only exhibit perpendicular orientation to this layer. However, it is important to note that the different absorption spectra of these pigments (L, Z and MZ) result in a collective optimal filtration of blue light at the macula, which would not be achieved by any of these carotenoids in isolation.

In conclusion, we report that the typical central peak of MP can be realised in subjects who do not exhibit such typical and peaked spatial profiles of this pigment, when supplemented with a preparation containing MZ, but not when supplemented with a formulation lacking this carotenoid. In addition, we found that enrichment of MP across its spatial profile can be best achieved following supplementation with all three macular carotenoids (MZ, Z and L). The implications of our findings, in terms of visual performance and/or a (photo)-protective effect, warrant study.

Disclosure

Mukunda C Akkali—None.

Dr. James Loughman does consultancy work for nutraceutical companies in a personal capacity. Dr. John M. Nolan and Professor Stephen Beatty do consultancy work for nutraceutical companies, in a personal capacity, and as directors of Nutrasight Consultancy Limited. Dr. Alan Howard is a Chairman of the Howard Foundation, a foundation that supports research in the field of nutrition and health.

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References

Bartlett, H.E., Eperjesi, F., 2008. A randomised controlled trial investigating the effect of lutein and antioxidant dietary supplementation on visual function in healthy eyes. Clin. Nutr. 27, 218–227.

Berendschot, T.T.J.M., van Norren, D., 2006. Macular pigment shows ringlike structures. Invest. Ophthalmol. Vis. Sci. 47, 709–714.

Bhosale, P., Bernstein, P.S., 2005. Synergistic effects of zeaxanthin and its binding protein in the prevention of lipid membrane oxidation. Biochim. Biophys. Acta 1740, 116–121.

Bone, R.A., Landrum, J.T., Fernandez, L., Tarsis, S.L., 1988. Analysis of the macular pigment by HPLC – retinal distribution and age study. Invest. Ophthalmol. Vis. Sci. 29, 843–849.

Bone, R.A., Landrum, J.T., Hime, G.W., Cains, A., Zamor, J., 1993. Stereochemistry of the human macular carotenoids. Invest. Ophthalmol. Vis. Sci. 34, 2033–2040.

Bone, R.A., Landrum, J.T., Friedes, L.M., Gomez, C.M., Kilburn, M.D., Menendez, E., Vidal, I., Wang, W.L., 1997. Distribution of lutein and zeaxanthin stereoisomers in the human retina. Exp. Eye Res. 64, 211–218.

Cantrell, A., McGarvey, D.J., Truscott, T.G., Rancan, F., Bohm, F., 2003. Singlet oxygen quenching by dietary carotenoids in a model membrane environment. Arch. Biochem. Biophys. 412, 47–54.

Connolly, E.E., Beatty, S., Thurnham, D.I., Loughman, J., Howard, A.N., Stack, J., Nolan, J.M., 2010. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. Curr. Eye Res. 35, 335–351.

Delori, F.C., Goger, D.G., Keilhauer, C., Salvetti, P., Staurenghi, G., 2006. Bimodal spatial distribution of macular pigment: evidence of a gender relationship. J. Opt. Soc. Am. A Opt. Image Sci. Vis 23, 521–538.

Dietzel, M., Zeimer, M., Heimes, B., Pauleikhoff, D., Hense, H.W., 2011. The ringlike structure of macular pigment in age-related maculopathy: results from the Muenster Aging and Retina Study (MARS). Invest. Ophthalmol. Vis. Sci. 52, 8016–8024.

Engles, M., Wooten, B., Hammond, B., 2007. Macular pigment: a test of the acuity hypothesis. Invest. Ophthalmol. Vis. Sci. 48, 2922–2931.

Foote, C.S., Chang, Y.C., Denny, R.W., 1970. Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. J. Am. Chem. Soc. 92, 5216–5218.

Hammond, B.R., Wooten, B.R., Snodderly, D.M., 1997. Individual variations in the spatial profile of human macular pigment. J. Opt. Soc. America A-Optics Image Sci. Vis. 14, 1187–1196.

Hammond Jr., B.R., Wooten, B.R., 2005. CFF thresholds: relation to macular pigment optical density. Ophthalmic Physiol. Opt. 25, 315–319.

Hirsch, J., Curcio, C.A., 1989. The spatial resolution capacity of human foveal retina. Vis. Res. 29, 1095–1101.

Johnson, E.J., Neuringer, M., Russell, R.M., Schalch, W., Snodderly, D.M., 2005. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. Invest. Ophthalmol. Vis. Sci. 46, 692–702.

Khachik, F., Bernstein, P.S., Garland, D.L., 1997. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest. Ophthalmol. Vis. Sci. 38, 1802–1811.

Kirby, M.L., Galea, M., Loane, E., Stack, J., Beatty, S., Nolan, J.M., 2009. Foveal anatomic associations with the secondary peak and the slope of the macular pigment spatial profile. Invest. Ophthalmol. Vis. Sci. 50, 1383–1391.

Kirby, M.L., Beatty, S., Loane, E., Akkali, M.C., Connolly, E.E., Stack, J., Nolan, J.M., 2010. A central dip in the macular pigment spatial profile is associated with age and smoking. Invest. Ophthalmol. Vis. Sci. 51, 6722–6728.

Kirschfeld, K., 1982. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. Proc. R. Soc. Lond B Biol. Sci. 216, 71–85.

Klaver, C.C.W., Wolfs, R.C.W., Vingerling, J.R., Hofman, A., De Jong, P.T.V.M., 1998. Age-specific prevalence and causes of blindness and visual impairment in an older population – The Rotterdam Study. Arch. Ophthalmol. 116, 653–658.

Kvansakul, J., Rodriguez-Carmona, M., Edgar, D.F., Barker, F.M., Kopcke, W., Schalch, W., Barbur, J.L., 2006. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. Ophthalmic Physiol. Opt. 26, 362–371.

Li, B., Ahmed, F., Bernstein, P.S., 2010. Studies on the singlet oxygen scavenging mechanism of human macular pigment. Arch. Biochem. Biophys 504, 56–60.

Loughman, J., Akkali, M.C., Beatty, S., Scanlon, G., Davison, P.A., O'Dwyer, V., Cantwell, T., Major, P., Stack, J., Nolan, J.M., 2010. The relationship between macular pigment and visual performance. Vis. Res. 50, 1249–1256.

Neuringer, M., Sandstrom, M.M., Johnson, E.J., Snodderly, D.M., 2004. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. Invest. Ophthalmol. Vis. Sci. 45, 3234–3243.

Nolan, J.M., O'Reilly, P., Loughman, J., Stack, J., Loane, E., Connolly, E., Beatty, S., 2009. Augmentation of macular pigment following implantation of blue light-filtering intraocular lenses at the time of cataract surgery. Invest. Ophthalmol. Vis. Sci. 50, 4777–4785.

Nolan, J.M., Loughman, J., Akkali, M.C., Stack, J., Scanlon, G., Davison, P., Beatty, S., 2011. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. Vis. Res. 51, 459–469.

Rodriguez-Carmona, M., Kvansakul, J., Harlow, J.A., Kopcke, W., Schalch, W., Barbur, J.L., 2006. The effects of supplementation with lutein and/or zeaxanthin on human macular pigment density and colour vision. Ophthalmic Physiol. Opt. 26, 137–147.

Snodderly, D.M., Auran, J.D., Delori, F.C., 1984a. The macular pigment. II. Spatial distribution in primate retinas. Invest. Ophthalmol. Vis. Sci. 25, 674–685.

Snodderly, D.M., Brown, P.K., Delori, F.C., Auran, J.D., 1984b. The macular pigment .1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest. Ophthalmol. Vis. Sci. 25, 660–673.

Snodderly, D.M., Handelman, G.J., Adler, A.J., 1991. Distribution of individual macular pigment carotenoids in central retina of macaque and squirrel monkeys. Invest. Ophthalmol. Vis. Sci. 32, 268–279.

Stringham, J.M., Hammond Jr., B.R., 2007. The glare hypothesis of macular pigment function. Optom. Vis. Sci. 84, 859–864.

Stringham, J.M., Hammond, B.R., 2008. Macular pigment and visual performance under glare conditions. Optom. Vis. Sci. 85, 82–88.

Stringham, J.M., Fuld, K., Wenzel, A.J., 2004. Spatial properties of photophobia. Invest. Ophthalmol. Vis. Sci. 45, 3838–3848.

Sujak, A., Gabrielska, J., Grudzinski, W., Borc, R., Mazurek, P., Gruszecki, W.I., 1999. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. Arch. Biochem. Biophys. 371, 301–307.

Trieschmann, M., Spital, G., Lommatzsch, A., van Kuijk, F.J.G.M., Fitzke, F., Bird, A., Pauleikhoff, D., 2003. Macular pigment: quantitative analysis on autofluorescence images. Graefes Arch. Clin. Exp. Ophthalmol. 241, 1006–1012.

Trieschmann, M., Beatty, S., Nolan, J.M., Hense, H.W., Heimes, B., Austermann, U., Fobker, M., Pauleikhoff, D., 2007. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. Exp. Eye Res. 84, 718–728.

Trieschmann, M., van Kuijk, F.J., Alexander, R., Hermans, P., Luthert, P., Bird, A.C., Pauleikhoff, D., 2008. Macular pigment in the human retina: histological evaluation of localization and distribution. Eye 22, 132–137.

Wooten, B.R., Hammond, B.R., 2002. Macular pigment: influences on visual acuity and visibility. Prog. Retin. Eye Res. 21, 225–240.

Wooten, B.R., Hammond, B.R., 2005. Spectral absorbance and spatial distribution of macular pigment using heterochromatic flicker photometry. Optom. Vis. Sci. 82, 378–386.

Wrona, M., Rozanowska, M., Sarna, T., 2004. Zeaxanthin in combination with ascorbic acid or alpha-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. Free Radic. Biol. Med 36, 1094–1101.

The Impact of Supplemental Antioxidants on Visual Function in Nonadvanced Age-Related Macular Degeneration: A Head-to-Head Randomized Clinical Trial

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Purpose. The purpose of this study was to evaluate the impact of supplemental macular carotenoids (including versus not including *meso*-zeaxanthin) in combination with coantioxidants on visual function in patients with nonadvanced age-related macular degeneration.

Methods. In this study, 121 participants were randomly assigned to group 1 (Age-Related Eye Disease Study 2 formulation with a low dose [25 mg] of zinc and an addition of 10 mg *meso*-zeaxanthin; $n = 60$) or group 2 (Age-Related Eye Disease Study 2 formulation with a low dose [25 mg] of zinc; $n = 61$). Visual function was assessed using best-corrected visual acuity, contrast sensitivity (CS), glare disability, retinal straylight, photostress recovery time, reading performance, and the National Eye Institute Visual Function Questionnaire-25. Macular pigment was measured using customized heterochromatic flicker photometry.

Results. There was a statistically significant improvement in the primary outcome measure (letter CS at 6 cycles per degree [6 cpd]) over time ($P = 0.013$), and this observed improvement was statistically comparable between interventions ($P = 0.881$). Statistically significant improvements in several secondary outcome visual function measures (letter CS at 1.2 and 2.4 cpd; mesopic and photopic CS at all spatial frequencies; mesopic glare disability at 1.5, 3, and 6 cpd; photopic glare disability at 1.5, 3, 6, and 12 cpd; photostress recovery time; retinal straylight; mean and maximum reading speed) were also observed over time ($P < 0.05$, for all), and were statistically comparable between interventions ($P > 0.05$, for all). Statistically significant increases in macular pigment at all eccentricities were observed over time ($P < 0.0005$, for all), and the degree of augmentation was statistically comparable between interventions ($P > 0.05$).

Conclusions. Antioxidant supplementation in patients with nonadvanced age-related macular degeneration results in significant increases in macular pigment and improvements in CS and other measures of visual function. (Clinical trial, http://www.isrctn.com/ISRCTN13894787).

Keywords: randomized clinical trial, lutein, zeaxanthin, *meso*-zeaxanthin, macular pigment, age-related macular degeneration, visual function, visual acuity, contrast sensitivity, macular pigment, NEI VFQ-25, photostress recovery time, reading performance, glare disability, retinal straylight

AMD is a multifactorial disease characterized by a spectrum of degenerative changes at the macula, ultimately leading to central vision impairment in many cases. Given the growing and aging world population, the number of people suffering from AMD continues to rise. Wong et al.[1] estimated the prevalence of any AMD (globally) to be 8.7% in those aged 45 to 85 years and predicted that the number of people afflicted with AMD worldwide will be 288 million by 2040. In the Republic of Ireland, the current prevalence of (any) AMD among persons aged 50 years and older is estimated to be 7.2%.[2] Beyond the personal suffering of those afflicted with advanced AMD, which includes loss of central vision and associated adverse clinical events such as increased risk of falls, depression, loneliness, suicide, and so on,[3] the growing prevalence of AMD represents a huge socioeconomic burden to society and to health care providers.[4] To address this challenge, preventive, retarding, and vision-optimizing strategies for nonadvanced AMD need to be explored, and prior work in diseased and nondiseased eyes indicates that the enhancement of ocular nutrition is worth pursuing in this endeavor.[5]

Meso-zeaxanthin (MZ), zeaxanthin (Z), and lutein (L) represent the three constituent carotenoids that make up macular pigment (MP), a yellow pigment found in the macula. Their anatomic (central and prereceptorial location), biochemical (antioxidant and anti-inflammatory), and optical (short-wavelength [blue] light-filtering) properties make these compounds ideal candidates to enhance vision and protect against AMD and its progression.[5] The Age Related Eye Disease Study (AREDS) 2, published in May 2013, examined the role of supplementation with two of MP's constituent macular carotenoids (L and Z, in combination with coantioxidants) in patients with intermediate AMD.[6] The primary outcome measure (POM; progression to advanced AMD) in AREDS2 failed to reveal a beneficial effect of supplemental L and Z.[7] However, secondary analysis, where data were dichotomized to those supplemented with L and Z versus those not supplemented with these macular carotenoids, did demonstrate a beneficial effect in terms of progression to the advanced form of the disease, especially in those with a low dietary intake of these carotenoids.[7] It is important to note that AREDS2 was designed and powered to investigate the impact of supplementation with macular carotenoids plus coantioxidants on AMD morphology and on visual acuity, whereas the current trial (Central Retinal Enrichment Supplementation Trial 2 [CREST] AMD - CREST Report 2) was designed and powered to investigate change in psychophysical (visual) function, in patients with nonadvanced AMD, following supplementation with the macular carotenoids plus coantioxidants.

In terms of assessing visual function in patients with retinal disease (including AMD), a number of studies have examined the impact of supplementation with macular carotenoids.[8] Indeed, recent studies have reported favorable outcomes on visual function (e.g., contrast sensitivity [CS] and glare disability [GD]) in patients with AMD and other retinal diseases, following supplementation with the macular carotenoids using a formulation of MZ:L:Z in a ratio (mg/d) of 10:10:2.[9,10] However, given the exploratory nature of those studies, a double-blind randomized controlled trial (RCT) with appropriate methodology was warranted. Originally, the CREST AMD trial planned a placebo-controlled design, but following publication of AREDS2, the CREST Data Safety and Monitoring Committee (DSMC) recommended that the design be amended to reflect the new standard of care and that, accordingly, the placebo group should be replaced with an AREDS2 formula containing a lower dose of zinc (25 mg). In the amended protocol, we chose a lower zinc dose (25 mg) because the AREDS2 study found no efficacy-lowering effect of reducing zinc from 80 mg to 25 mg on either visual acuity or AMD progression.[7]

In summary, CREST AMD was designed and conducted to investigate the impact of macular carotenoid supplementation with coantioxidants on visual function in patients with nonadvanced AMD during a 2-year period (ISRCTN13894787).[11] We also investigated whether the addition of 10 mg of MZ to a formulation containing standard AREDS2 doses of L and Z and in combination with coantioxidants offered advantages/disadvantages in terms of a wide array of measures of visual function and MP response.

Methods

Trial Design

Details of the CREST design and methodology have been reported elsewhere and are briefly summarized here.[11] Ethical approval was granted by the Research Ethics Committee of the Waterford Institute of Technology (reference number 12/CLS/02), Waterford, Ireland, and the Ethics Committee of the European Research Council (reference number 281096). As explained previously, following the AREDS2 report, the CREST protocol was amended from a placebo-controlled design to a double-blind, head-to-head, RCT (ISRCTN13894787) in which participants were randomly assigned to two parallel groups, each receiving active supplements as follows: group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 international units (IU)/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper (Macushield Gold [Alliance Pharma PLC & Alliance Pharmaceuticals Ltd, Chippenham Wiltshire, England, UK]; Macuhealth Plus [MacuHealth Limited Partnership, Birmingham, MI, USA]); and group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper (AREDS2 formula with a lower dose of zinc [25 mg] custom prepared for CREST AMD and not commercially available). The group 2 intervention, therefore, represents the standard of care (AREDS2 formula with a lower dose of zinc [25 mg]), whereas group 1 also represents the same standard of care, but with the addition of 10 mg of MZ. All protocol changes were approved by the DSMC and the Research Ethics Committee of the Waterford Institute of Technology, Waterford, Ireland, and the Ethics Committee of the European Research Council (Saint-Josse-ten-Noode, Brussels, Belgium). In addition, protocol changes were published on the International Standard RCT registration website (www.isrctn.com/ISRCTN13894787) and in the published methodology[11] for this project. Participants in each group were instructed to take the study intervention daily with a meal for 2 years. The trial was conducted at the Macular Pigment Research Group, Nutrition Research Centre Ireland (Waterford, Ireland) from November 2013 (first visit of first participant) to May 2016 (last visit of last participant).

Randomization and Intervention

Participants were randomly assigned to intervention groups using block randomization (block size: 4 and randomization ratio 1:1). The randomization sequence was generated by the study statistician (J.S.), and a pharmacist (C.K.) performed random allocation to intervention groups based on this randomization sequence at Whitfield Clinic, Waterford, Ireland. The study investigator (K.O.A.) received, from the pharmacist, a box of supplements for each study participant, labeled only with the participant identification number. Only at study completion, after a masked database review and following direction from the CREST DSMC, was the randomization sequence revealed to the study investigator and other data analysts.

Participants

Inclusion criteria for the trial were as follows: nonadvanced AMD (1 to 8 on the AREDS 11-step severity scale[12] in at least one eye [the study eye], confirmed by the Moorfields Eye Hospital Reading Centre, London, UK, an accredited retinal grading center); best-corrected visual acuity (BCVA) of 6/12 (20/40) or better in the study eye; no more than five diopters spherical equivalent refraction in the study eye; no previous consumption of supplements containing the macular carotenoids (L and/or Z and/or MZ); no retinal pathology other than AMD; and no diabetes mellitus (by self-report). The study eye could be either the right or left eye. If both eyes exhibited nonadvanced AMD, the eye with the best BCVA was chosen as the study eye. However, if each eye had the same BCVA and nonadvanced AMD, the right eye was selected. Each participant provided written informed consent of their willingness to participate in the trial, and the examination procedures

adhered to the tenets of the Declaration of Helsinki. Clinical assessment was conducted at baseline and at six monthly intervals during a 2-year period by the study investigator (K.O.A.) who was trained in all aspects of the CREST protocol. Retinal photographs were graded in a masked fashion at the Moorfields Eye Hospital Reading Centre, adhering to the AREDS 11-step severity scale.[12]

Outcomes

The POM was change in CS at 6 cycles per degree (cpd) following 24 months of supplementation (letter CS at 6 cpd). The Test Chart 2000PRO (Thomson Software Solutions, Hatfield, UK) was used to assess the POM. Letter CS (instead of grating CS) at 6 cpd was chosen as our primary outcome measure because this measure is close to the peak contrast sensitivity function, and any improvements in CS is best assessed at this spatial frequency. Furthermore, pilot data were only available on letter CS (but not grating CS), and this informed our choice in the current study. Secondary outcome measures included change in CS at the other spatial frequencies, BCVA, GD, photostress recovery time (PRT), MP, retinal straylight, reading acuity, reading speed, subjective visual function (National Eye Institute Visual Function Questionnaire–25 [NEI VFQ-25]), and AMD morphology. For measuring BCVA and letter CS, a Hewlett-Packard monitor LV916AA2211 (Hewlett-Packard, Palo Alto, CA, USA; resolution 1920 × 1080, luminance 250 cd/m^2, dynamic contrast ratio 3,000,000: 1) was used. Prior to use for vision testing, the device was calibrated in accordance with the instructions manual from Thomson Software Solutions. Furthermore, all vision testing was conducted in the same room during the course of the study.

Compliance and Adverse Event Reporting

Compliance was assessed by contacting participants via telephone, by capsule counting, and by serum carotenoid analysis at the end of the study. Participants were also phoned regularly to ascertain whether they had experienced any unusual signs/symptoms during the course of the study. Potential or perceived adverse events were documented and reported to the DSMC.

Statistical Analysis

A previous report described the sample size/power calculation for this study.[11] Based on an effect size of 0.15 logCS units (one line on a letter CS chart) for the POM, and a two-tailed test at the 5% level of significance, we estimated that 56 participants per intervention group were needed to achieve a power of 80% for the comparison of the two intervention groups. One eye (the study eye) of each participant comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, USA). All analyses were conducted as per protocol. However, intention-to-treat (ITT) analysis was also performed, and discrepancies between ITT analyses and per protocol are reported herein. No interim analyses were conducted during the course of the study.

Baseline differences between intervention groups were assessed using independent samples *t*-tests for interval variables and contingency table analyses using the chi-squared tests for categorical variables.

Most of the outcome variables in this study were changes (over time) in interval variables (e.g., CS, MP). To compare the effects of the two intervention groups (on each interval outcome measure, over time), we used repeated measures analysis of variance, with time as a within-participants factor and intervention group as a between-participants factor. In the ITT analysis, the last observation carried forward was used when participant data were missing.

Tests of significance, for all comparisons of intervention groups on interval outcome measures, were two-tailed, and the 5% level of significance was used throughout. We did not correct for multiple tests, as we were anxious to avoid type II errors.

Results

Figure 1 shows the Consolidated Standards of Reporting Trials diagram,[13] summarizing the CREST study design, participant enrolment, randomization, follow-up, and the number of participants included in study analyses. In this study, 121 participants were enrolled at baseline with 98 participants completing final assessment at 24 months. Baseline characteristics (see Table 1) were statistically comparable between interventions, except for letter CS (1.2 and 2.4 cpd) and photopic CS at 3 cpd. Losses to follow-up after 2 years of antioxidant supplementation were statistically comparable between interventions ($P = 0.680$, Pearson chi-square).

Primary Outcome Measure

The repeated measures analysis of change in letter CS at 6 cpd (POM) is presented in Table 2 (as per protocol). There was a statistically significant improvement in the POM during the study period ($P = 0.013$ for time effect), but there was no statistically significant difference between the intervention groups ($P = 0.881$ for the time × group interaction effect). Thus, there is no evidence that the two intervention groups are different with respect to improvement in this measure. Figure 2 graphically illustrates these findings.

Secondary Outcome Measures

Other Visual Function Outcomes From Baseline to 24 Months. Results from the repeated measures analysis, for other visual function variables, are also shown in Table 2. There was a statistically significant improvement ($P < 0.05$, for time effect) in most measures of visual function (75%; 24 of 32 of vision-related outcome measures) during the study period, including CS, PRT, retinal straylight, and GD, and again these improvements were statistically comparable between intervention groups ($P > 0.05$). There was one exception; mesopic GD at 3 cpd ($P = 0.040$ for the time × group interaction effect), which improved to a borderline significantly greater extent in group 2. However, in the subsequent ITT analysis, the disparity between interventions in terms of mesopic GD at 3 cpd was no longer significant ($P = 0.132$ for the time × group interaction effect). Figures 2, 3, and 4 graphically illustrate these findings.

Clinically Significant Contrast Sensitivity Findings

The numbers and proportions of patients exhibiting clinically meaningful changes (one line or more on a letter CS chart) are presented in Table 3, where it is evident (especially for CS at 1.2 and 2.4 cpd, but also for the POM) that the percentage of participants showing a clinically meaningful improvement in CS over time greatly exceeds the percentage showing a clinically significant deterioration, and that this observation is true for each intervention group.

Macular Pigment From Baseline to 24 Months. There was a statistically significant increase in MP for all eccentric-

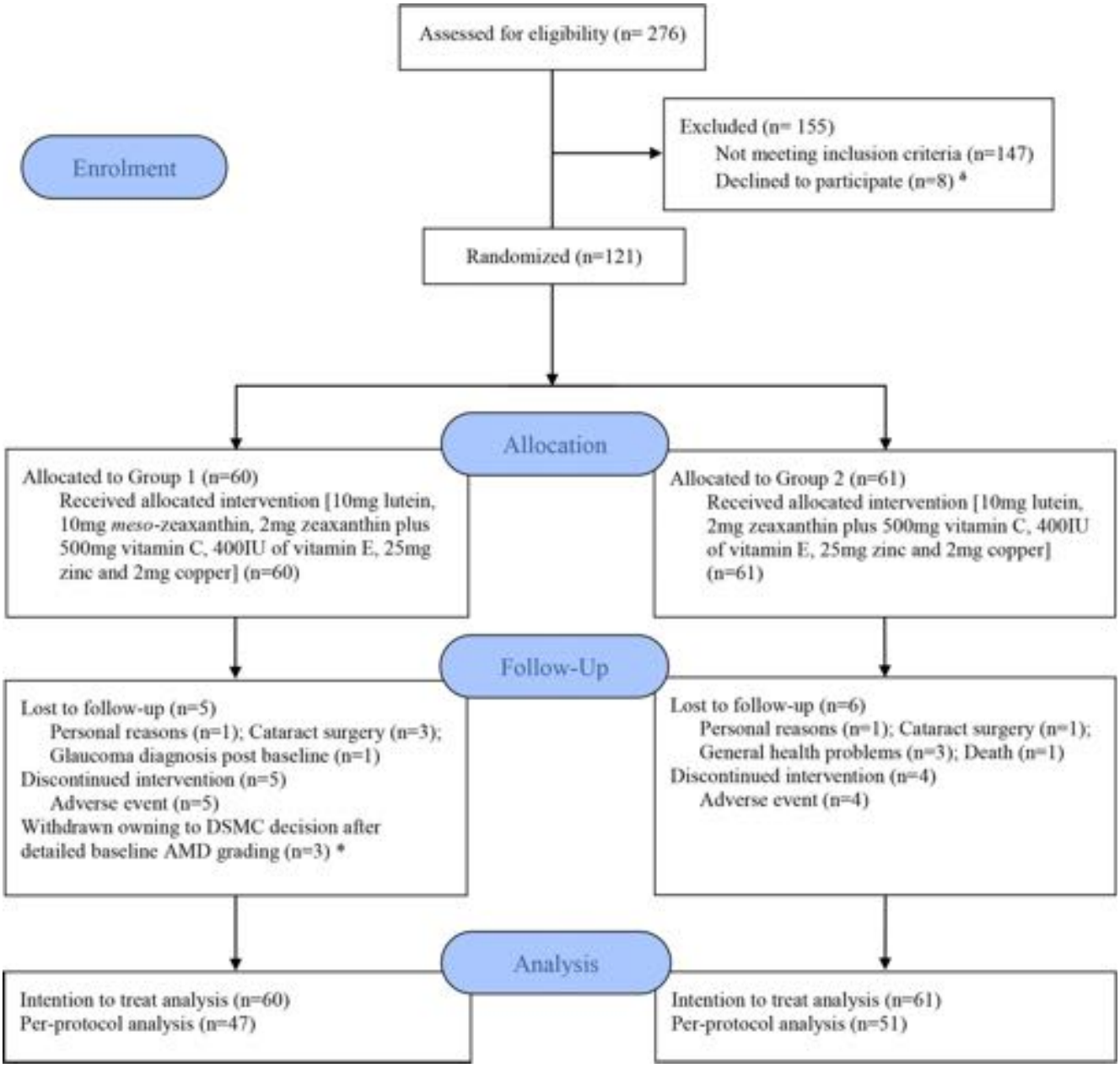

FIGURE 1. CREST AMD consolidated standards of reporting trials flow diagram. δ, Participants declined to participate either due to personal reasons, transportation difficulties, or cataract surgery; *, Participants were initially enrolled based on nondetail grading of retinal photographs obtained at screening visit, confirming eligibility by the Moorfields Eye Hospital Reading Centre. However, detailed grading of baseline retinal photographs showed some participants had AMD grades > 8 on the AREDS 11-step severity scale and therefore these participants were excluded based on a decision by the DSMC.

ities during the course of the study ($P < 0.0005$, for all time effects), but this increase was statistically comparable between intervention groups ($P > 0.05$ for time × group interaction effect at all retinal eccentricities; Table 4). Figure 5 graphically illustrates these findings.

Serum Carotenoids From Baseline to 24 Months. There was a statistically significant increase in serum concentrations of L, Z, and MZ during the course of the study ($P < 0.0005$, for all time effects; Table 4). The repeated measures analysis of change in serum L concentrations over time did not show significant differences between intervention groups ($P = 0.111$ for the time × group interaction effect). Observed increases in serum Z concentrations were significantly greater in group 2 when compared with group 1 ($P = 0.005$ for the time × group interaction effect). Significant increases in serum MZ concentrations were observed in group 1, but not in group 2 ($P < 0.0005$ for the time × group interaction effect). In terms of observed increases in total (composite) serum macular carotenoid concentrations (i.e., L, Z, and MZ combined), this measure increased significantly over time, and no significant difference between intervention groups ($P = 0.241$ for the time × group interaction effect) was observed. Figure 6 graphically illustrates these findings.

Grade of AMD From Baseline to 24 Months. Table 5 shows, within each intervention group, the transition between these grades from baseline to final study visit at 24 months.

Table 1. Baseline Characteristics by Intervention Group in the CREST AMD Study (Per Protocol)

Variables	Group 1, $n = 57$*	Group 2, $n = 61$†	Sig.
Demographic, lifestyle, and health			
Age, y	65.09 ± 8.59	64.34 ± 9.50	0.657
Body mass index, kg/m^2	28.27 ± 4.30	27.78 ± 4.57	0.551
Blood pressure, mm Hg			
Systolic	142.07 ± 20.98	138.00 ± 24.35	0.334
Diastolic	82.65 ± 11.21	79.12 ± 9.81	0.070
Sex			
Male	18 (45.0)	22 (55.0)	0.607
Female	39 (50.0)	39 (50.0)	
Education			
Primary	7 (43.8)	9 (56.3)	0.766
Secondary	29 (51.8)	27 (48.2)	
Tertiary	21 (45.7)	25 (54.3)	
Smoking			
Never	29 (50.0)	29 (50.0)	0.933
Past	23 (46.9)	26 (53.1)	
Current	5 (45.5)	6 (54.5)	
AMD family history			
Yes	16 (53.3)	14 (46.7)	0.406
No	31 (44.3)	39 (55.7)	
Cardiovascular disease			
Yes	5 (50.0)	5 (50.0)	0.804
No	50 (47.6)	55 (52.4)	
Hypertension			
Yes	17 (48.6)	18 (51.4)	0.970
No	40 (48.2)	43 (51.8)	
AMD grades			
1-3	13 (43.3)	17 (56.7)	0.528
4-8	44 (50.0)	44 (50.0)	
Diet score	26.90 ± 12.00	26.26 ± 12.03	0.776
Serum carotenoids*			
Serum L, μmol/l	0.35 ± 0.20	0.34 ± 0.22	0.710
Serum Z, μmol/l	0.07 ± 0.05	0.07 ± 0.05	0.639
Serum MZ, μmol/l	0.00 ± 0.01	0.01 ± 0.02	0.205
Macular pigment			
Densitometer*			
0.25°	0.79 ± 0.24	0.72 ± 0.26	0.179
0.5°	0.65 ± 0.22	0.60 ± 0.21	0.204
1.0°	0.45 ± 0.16	0.45 ± 0.17	0.927
1.75°	0.32 ± 0.12	0.31 ± 0.15	0.933
Vision			
Best corrected visual acuity, VAR			
Study eye	100.04 ± 5.83	100.08 ± 5.62	0.965
Fellow eye	94.63 ± 10.95	95.92 ± 12.20	0.549
Letter contrast sensitivity, LogCS			
1.2 cpd	1.77 ± 0.17	1.85 ± 0.16	0.007
2.4 cpd	1.76 ± 0.21	1.83 ± 0.18	0.045
6 cpd, POM	1.49 ± 0.25	1.56 ± 0.21	0.108
9.6 cpd	1.23 ± 0.30	1.32 ± 0.25	0.082
15.15 cpd*	0.86 ± 0.35	0.94 ± 0.29	0.160
Mesopic contrast sensitivity, LogCS			
1.5 cpd	1.53 ± 0.22	1.61 ± 0.21	0.065
3 cpd	1.62 ± 0.23	1.68 ± 0.18	0.106
6 cpd	1.21 ± 0.35	1.33 ± 0.35	0.065
12 cpd	0.78 ± 0.27	0.85 ± 0.28	0.132
18 cpd	0.33 ± 0.12	0.32 ± 0.11	0.749

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Table 1. Continued

Variables	Group 1, $n = 57$*	Group 2, $n = 61$†	Sig.
Photopic contrast sensitivity, LogCS			
1.5 cpd	1.46 ± 0.19	1.52 ± 0.16	0.061
3 cpd	1.72 ± 0.22	1.80 ± 0.19	0.047
6 cpd	1.58 ± 0.31	1.68 ± 0.31	0.079
12 cpd	1.19 ± 0.38	1.27 ± 0.35	0.279
18 cpd	0.51 ± 0.34	0.62 ± 0.34	0.081
Mesopic glare disability, LogCS			
1.5 cpd	0.91 ± 0.32	0.99 ± 0.29	0.193
3 cpd	1.11 ± 0.37	1.19 ± 0.32	0.241
6 cpd	0.93 ± 0.25	0.93 ± 0.23	0.977
12 cpd	0.66 ± 0.15	0.63 ± 0.11	0.355
18 cpd	0.30 ± 0.00	0.31 ± 0.04	0.336
Photopic glare disability, LogCS			
1.5 cpd	1.40 ± 0.21	1.46 ± 0.17	0.082
3 cpd	1.67 ± 0.22	1.73 ± 0.18	0.130
6 cpd	1.51 ± 0.32	1.58 ± 0.31	0.210
12 cpd	1.11 ± 0.36	1.19 ± 0.36	0.206
18 cpd	0.52 ± 0.35	0.56 ± 0.31	0.583
Retinal Straylight	1.30 ± 0.18	1.33 ± 0.25	0.381
Photostress recovery time, s	15.98 ± 8.72	15.97 ± 7.99	0.996
Reading performance			
Reading acuity, LogRAD	0.12 ± 0.13	0.09 ± 0.12	0.165
Mean reading speed, w/min	154.48 ± 26.82	156.45 ± 27.53	0.694
Maximum reading speed, w/min	199.61 ± 31.58	201.56 ± 34.44	0.749
National Eye Institute Questionnaire-25			
Overall vision score	87.80 ± 9.96	90.38 ± 9.22	0.147

Data displayed are mean ± standard deviation for interval data and percentages, *n* (%), for categorical data; the percentages displayed are row percentages. Sig., significance set at $P < 0.05$. Education, highest level of education; Smoking, Never (<100 cigarettes in lifetime), Past (smoked ≥100 cigarettes in lifetime and none in past year), current (smoked ≥100 cigarettes in lifetime and at least one in the last year). *, $n \neq 57$ in group 1 and/or $n \neq 61$ in group 2 as certain tests/measures were not obtained. VAR, visual acuity rating. VAR = 100 − 50 LogMAR, a score of 100 corresponds with 20/20 (6/6); LogCS, logarithm of contrast sensitivity units. Family history of AMD means having a first degree relative, that is, parent or sibling, with AMD AREDS 11-step scale. Diet score, estimated dietary intake of lutein and zeaxanthin using the "L/Z screener" developed by Professor Elizabeth Johnson, Tufts University. Macular pigment measured using the Macular Densitometer (Macular Metrics Corp.). Serum macular carotenoids analyzed by HPLC. Best-corrected visual acuity measured with the Test Chart 2000 Xpert (Thomson Software Solutions). Letter contrast sensitivity measured using the Test Chart 2000 PRO (Thomson Software Solutions). Mesopic and photopic contrast sensitivity measured using the Functional Vision Analyzer (Stereo Optical Co.). Mesopic and photopic glare disability measured using the Functional Vision Analyzer (Stereo Optical Co.). Retinal straylight measured using the Oculus C-Quant (Oculus GmbH, Wetzler, Germany) and recorded in logarithms (judged reliable when estimated standard deviation (ESD) ≤ 0.08 and Q ≥ 1). Photostress recovery time measured by assessing the time of recovery after a 10-second exposure to a 300-watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions GmbH, Berlin, Germany) with a low-pass glass dichroic filter. Reading performance assessed using the English version of the standardized Radner reading chart at a distance of 40 cm with reading correction. Reading acuity recorded in logarithm of the reading acuity determination (LogRAD). The following formula was used to calculate the LogRAD-score: logRAD + total number of incorrectly read syllables × 0.005. Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14 words × 60 seconds divided by reading time in seconds). National Eye Institute Visual Function Questionnaire–25 overall vision scores range from zero (worst) to 100 (best).

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

Importantly, no participant from Group 1 (the intervention containing MZ) and only one participant from Group 2 progressed to advanced AMD over the study period.

Compliance

The compliance to study intervention (as measured by capsule counting) was not significantly different between intervention groups during the course of the study ($P = 0.342$ for the time × group interaction effect). In addition, serum carotenoid assessment indicated good compliance to study intervention (see Fig. 6).

Adverse Events

The distribution of potential or perceived adverse events reported during the course of the study is shown in Table 6. Some participants reported more than one adverse event. The proportion of participants experiencing any adverse event was statistically similar between interventions: 15 (26%) of 57 from group 1 and 10 (16%) of 61 from group 2 ($P = 0.187$, Pearson chi-squared test). No serious adverse event relating to the study intervention was reported in either intervention group during the course of the study.

Discussion

This RCT was designed to compare the impact of two different macular carotenoid formulations, in combination with coantioxidants, on visual function in patients with nonadvanced AMD. The AMD disease status of participants was graded using the AREDS 11-step severity scale[12] and included only eyes

Table 2. Repeated Measures Analysis of Visual Function Outcomes From Baseline to 24 Months in the CREST AMD Study by Intervention Groups

	Group 1*					Group 2†				Time	Time × Group
	Baseline		24 Months			Baseline		24 Months		Effect	Interaction
Variable	*N*	Mean	SD	Mean	SD	*N*	Mean	SD	Mean	SD	Sig.	Sig.
Vision											
Best corrected visual acuity, VAR	46	101.22	5.16	100.91	5.80	51	100.78	5.08	101.31	5.20	0.746	0.233
Letter contrast sensitivity, LogCS											
1.2 cpd	46	1.79	0.17	1.89	0.20	51	1.86	0.14	1.91	0.16	<0.0005	0.058
2.4 cpd	46	1.78	0.22	1.86	0.22	51	1.85	0.16	1.91	0.18	<0.0005	0.582
6 cpd, POM	46	1.53	0.24	1.57	0.29	51	1.58	0.18	1.61	0.23	0.013	0.881
9.6 cpd	46	1.29	0.28	1.31	0.30	51	1.36	0.21	1.38	0.26	0.154	0.925
15.15 cpd	46	0.92	0.33	0.95	0.34	51	0.96	0.27	1.01	0.33	0.082	0.747
Mesopic contrast sensitivity, LogCS											
1.5 cpd	46	1.55	0.22	1.62	0.24	51	1.63	0.21	1.70	0.23	0.007	0.982
3 cpd	46	1.63	0.24	1.76	0.27	51	1.69	0.18	1.84	0.27	<0.0005	0.523
6 cpd	46	1.25	0.35	1.48	0.45	51	1.34	0.34	1.49	0.42	<0.0005	0.228
12 cpd	46	0.81	0.29	0.94	0.36	51	0.87	0.28	0.96	0.35	0.002	0.605
18 cpd	46	0.33	0.13	0.39	0.23	51	0.31	0.08	0.41	0.25	<0.0005	0.369
Photopic contrast sensitivity, LogCS											
1.5 cpd	46	1.47	0.19	1.60	0.23	51	1.53	0.16	1.64	0.21	<0.0005	0.862
3 cpd	46	1.75	0.23	1.84	0.23	51	1.82	0.18	1.91	0.21	<0.0005	0.986
6 cpd	46	1.63	0.28	1.74	0.39	51	1.70	0.29	1.81	0.34	<0.0005	0.934
12 cpd	46	1.25	0.37	1.34	0.43	51	1.30	0.33	1.34	0.37	0.015	0.468
18 cpd	46	0.56	0.36	0.71	0.44	51	0.65	0.34	0.69	0.36	0.008	0.174
Mesopic glare disability, LogCS											
1.5 cpd	46	0.98	0.32	1.08	0.44	51	1.01	0.29	1.20	0.45	<0.0005	0.172
3 cpd	46	1.19	0.36	1.22	0.43	51	1.22	0.30	1.38	0.41	0.001	0.040
6 cpd	46	0.97	0.27	1.05	0.35	51	0.94	0.23	1.09	0.35	<0.0005	0.222
12 cpd	46	0.67	0.16	0.68	0.22	51	0.64	0.12	0.69	0.18	0.133	0.412
18 cpd	46	0.30	0.00	0.32	0.10	51	0.31	0.04	0.31	0.08	0.197	0.486
Photopic glare disability, LogCS											
1.5 cpd	46	1.43	0.21	1.55	0.26	51	1.47	0.18	1.55	0.24	<0.0005	0.364
3 cpd	46	1.70	0.22	1.82	0.25	51	1.74	0.18	1.83	0.24	<0.0005	0.542
6 cpd	46	1.56	0.31	1.65	0.40	51	1.61	0.29	1.70	0.34	0.001	0.987
12 cpd	46	1.18	0.34	1.26	0.41	51	1.23	0.33	1.31	0.38	0.011	0.913
18 cpd	46	0.58	0.37	0.60	0.39	51	0.57	0.31	0.62	0.33	0.179	0.646
Retinal Straylight, Logs	41	1.29	0.18	1.25	0.19	43	1.33	0.20	1.26	0.16	0.004	0.359
Photostress recovery time, s	46	16.93	9.19	12.47	6.79	51	16.00	8.51	10.96	6.05	<0.0005	0.757
Reading performance											
Reading acuity, LogRAD	46	0.09	0.12	0.09	0.08	51	0.07	0.10	0.06	0.10	0.637	0.759
Mean reading speed, w/min	46	154.61	27.11	189.89	26.53	51	158.75	27.00	192.82	28.54	<0.0005	0.765
Maximum reading speed, w/min	46	200.44	32.25	244.00	35.02	51	204.74	33.40	245.38	37.90	<0.0005	0.606
National Eye Institute Questionnaire-25											
Overall vision score	46	89.24	7.95	89.27	9.61	50	90.83	9.66	91.93	7.01	0.408	0.434

N, participants with data at all study visits; Sig., significance set at $P < 0.05$. *P* values obtained from repeated measures analysis of variance. Best-corrected visual acuity measured with the Test Chart 2000 Xpert (Thomson Software Solutions). Letter contrast sensitivity measured using the Test Chart 2000 PRO (Thomson Software Solutions). Mesopic and photopic contrast sensitivity measured using the Functional Vision Analyzer (Stereo Optical Co.). Mesopic and photopic glare disability measured using the Functional Vision Analyzer (Stereo Optical Co.). Retinal straylight measured using Oculus C-Quant (Oculus GmbH) and recorded in logarithms (judged reliable when ESD $\leq$ 0.08 and Q $\geq$ 1). Photostress recovery time measured by assessing the time of recovery after a 10-second exposure to a 300-watt tungsten spotlight (ARRI 300 Plus lamp) with a low-pass glass dichroic filter. Reading performance assessed using the English version of the standardized Radner reading chart at a distance of 40 cm with reading correction. Reading acuity recorded in logarithm of the reading acuity determination (LogRAD). The following formula was used to calculate the LogRAD score: logRAD + total number of incorrectly read syllables × 0.005. Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14 words × 60 seconds divided by reading time in seconds). National Eye Institute Visual Function Questionnaire–25 overall vision scores range from zero (worst) to 100 (best).

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

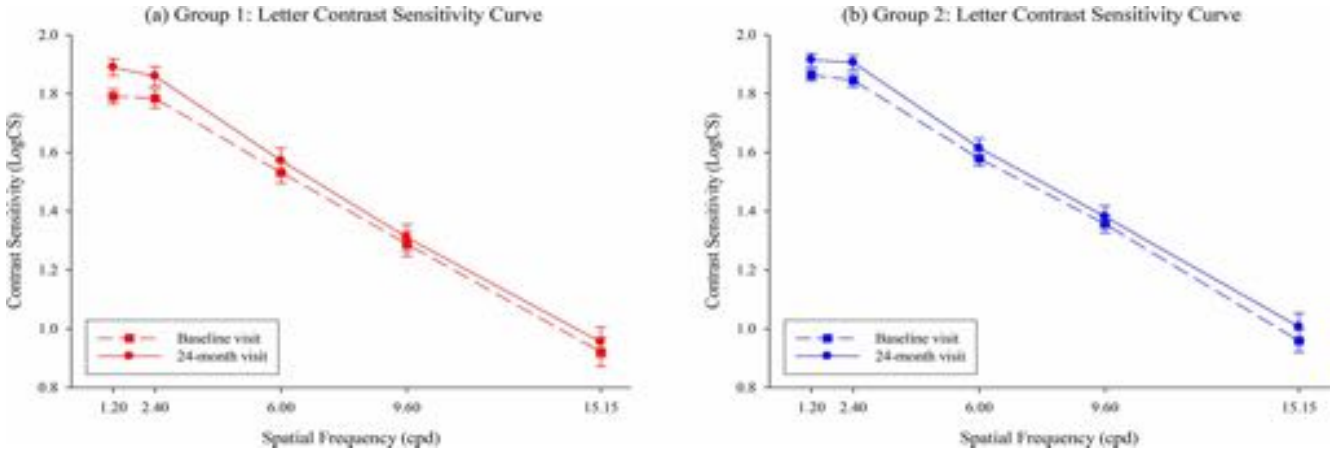

Figure 2. Letter contrast sensitivity function using the Test Chart 2000 PRO (Thomson Software Solutions) in the CREST AMD study. Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper; group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper. *Error bars* represent standard error of mean.

classed as grade 1 to 8 at baseline (referred to as nonadvanced AMD for the purpose of the current study). We did not include eyes with noncentral geographic atrophy (AMD grade 9 on the AREDS 11-step severity scale). Given the biologically plausible rationale that benefits, in terms of vision and in terms of MP augmentation, are more likely to extend to participants with earlier disease (before irreversible damage has occurred, such as in noncentral geographic atrophy [grade 9 AREDS 11-step severity scale]), we purposely recruited eyes at an earlier stage of disease. We report improvements in a range of measures of visual function (i.e., CS, GD, PRT, reading speed) following supplementation with the macular carotenoids in combination

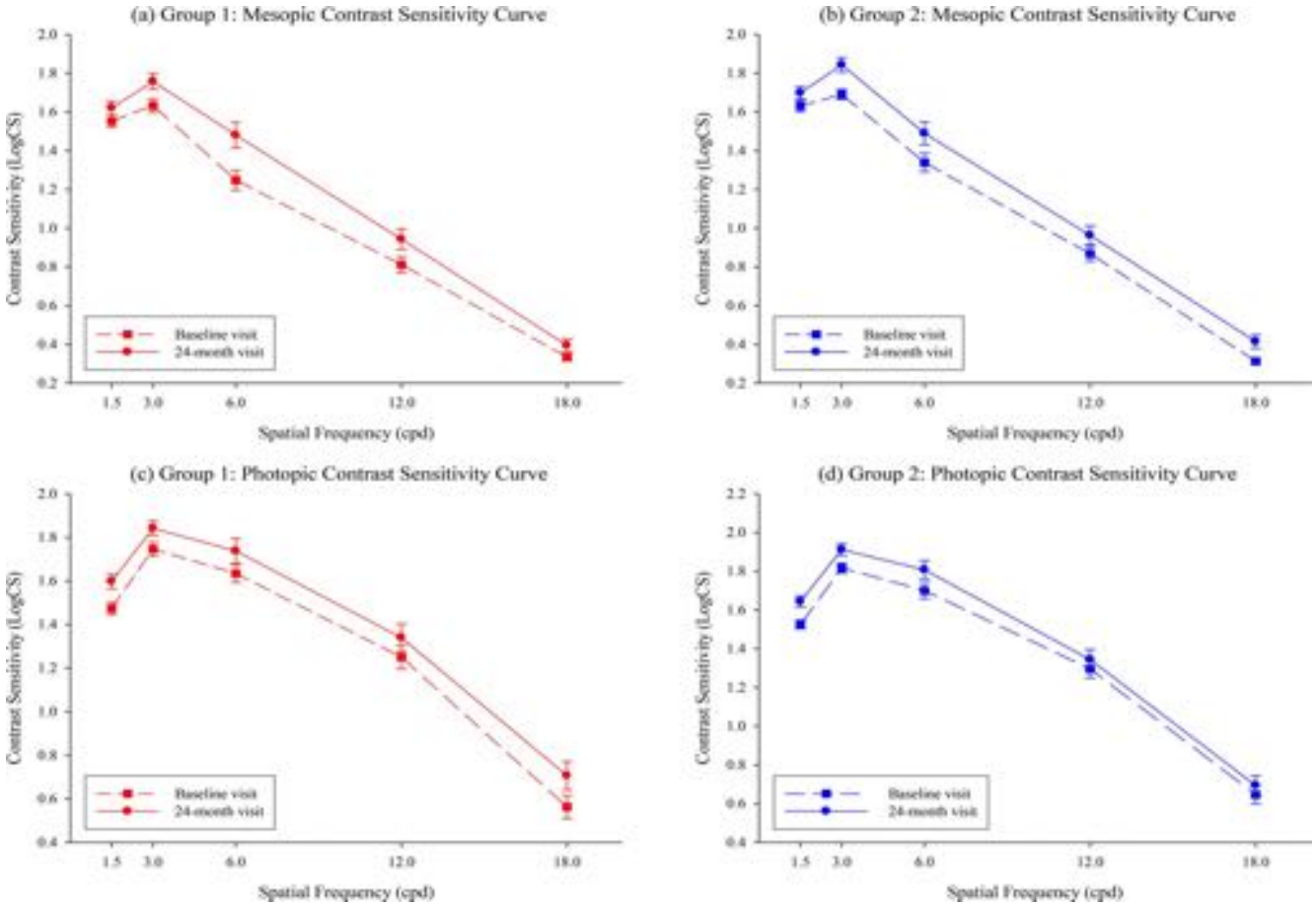

Figure 3. Mesopic and photopic contrast sensitivity function using the Functional Vision Analyzer (Stereo Optical Co., Chicago, IL, USA) in the CREST AMD study. Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper; group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper. *Error bars* represent standard error of mean.

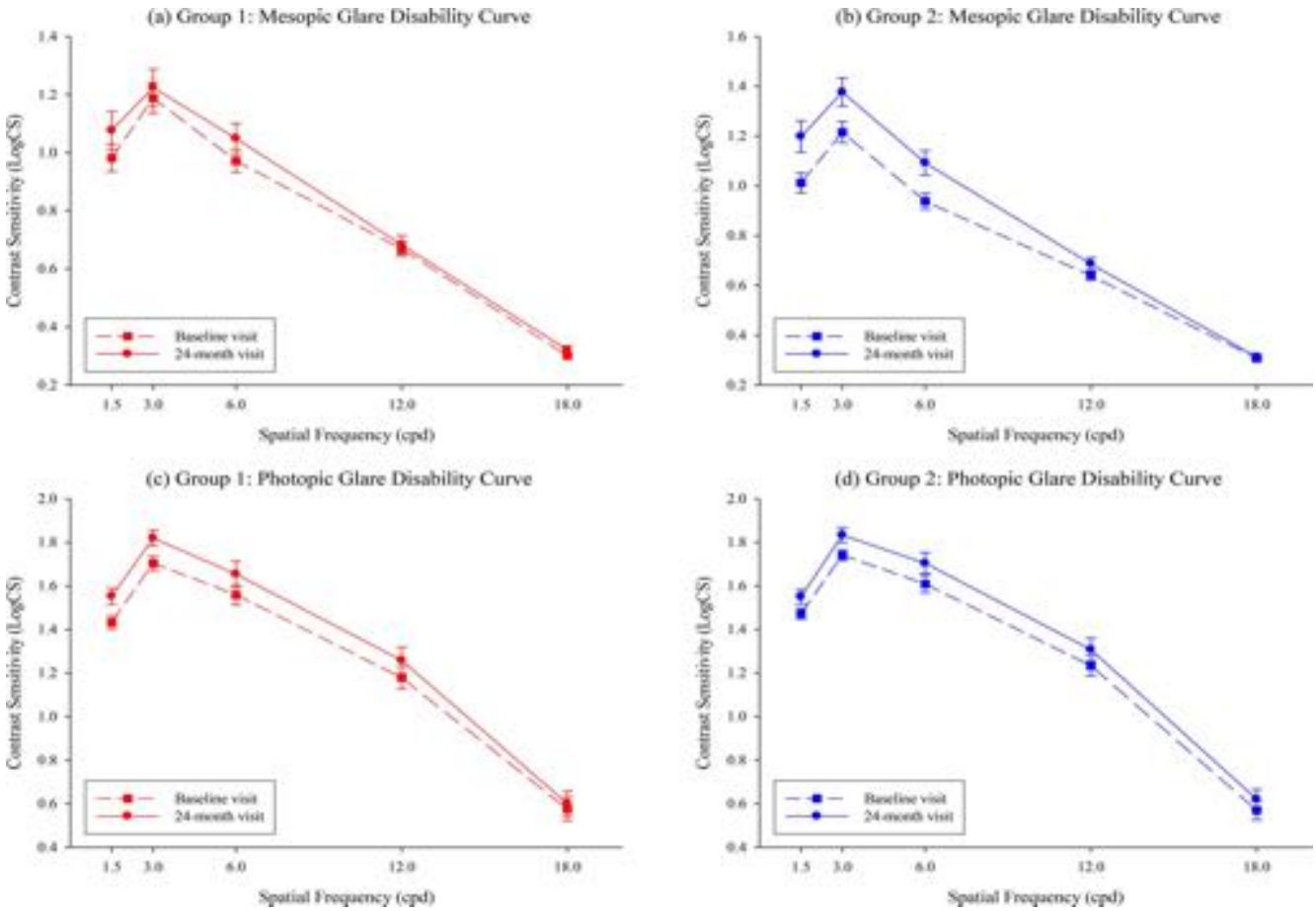

Figure 4. Mesopic and photopic glare disability using the Functional Vision Analyzer (Stereo Optical Co.) in the CREST AMD study. Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper; group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper. *Error bars* represent standard error of mean.

with coantioxidants, and our results are consistent with previous studies.[9,14,15]

A possible explanation for the role that MP plays in optimizing CS rests on the visibility hypothesis of MP, which posits that this prereceptorial pigment enhances visualization of a target's detail by the absorption of blue haze.[16] Blue haze is a subjective experience and is caused by scattered short-wavelength dominant air light (blue light), which results in a veiling luminance when we view objects at a distance.[16] MP accentuates the luminance of an object relative to its background by attenuating the impact of this scattered (veiling) short-wavelength visible blue light on the

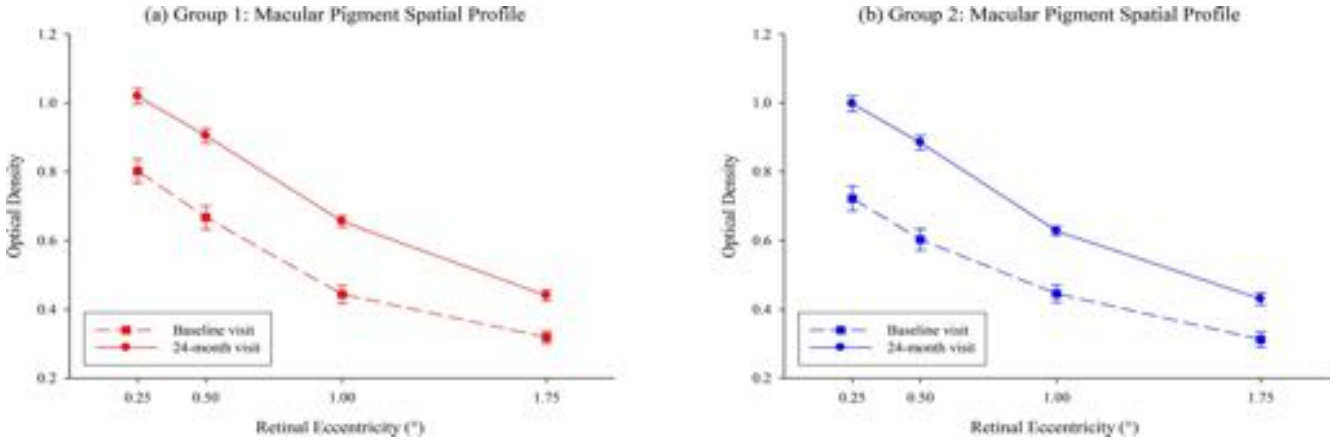

Figure 5. Macular pigment response in the CREST AMD study. Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper; group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper. Macular pigment measured using a Macular Densitometer (Macular Metrics Corp., Providence, RI, USA). *Error bars* represent standard error of mean.

Table 3. Change in Contrast Sensitivity of ≥1 Line of CS

	% Showing Clinical Improvement		% Showing Clinical Deterioration	
Variable	Group 1*	Group 2†	Group 1*	Group 2†
Letter CS 1.2 cpd	34.8	19.6	2.2	3.9
Letter CS 2.4 cpd	26.1	21.6	4.3	3.9
Letter CS 6 cpd	26.1	21.6	13	11.8

Clinical significance, which for present purposes we defined as one line or more on a letter CS chart. Letter CS measured using the Test Chart 2000 PRO (Thomson Software Solutions).

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

Table 5. Change in AMD Morphology in the CREST AMD Study by Intervention Group

Study Visit	Intervention	Low Risk	High Risk	Advanced AMD	Total
Baseline	Group 1*	13	44	0	57
	Group 2†	17	44	0	61
24 months	Group 1*	11	35	0	46
	Group 2†	11	38	1	50

Low risk, AMD grades 1 to 3 on the AREDS 11-step scale; high risk, AMD grades 4 to 8 on the AREDS 11-step scale; advanced AMD, AMD grades 9 to 11 on the AREDS 11-step scale.

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

just noticeable differences of luminance required for discernibility and, by consequence, extends the visual range.[17] Indeed, the visibility hypothesis has been tested empirically and is supported by two studies that have demonstrated the beneficial effect of MP in this respect under simulated blue haze conditions.[18,19] Beyond this optical effect, the macular carotenoids may also favorably influence lateral inhibitory mechanisms[20] and may thereby have contributed to the observed improvements in CS following supplementation.

Importantly, we believe that the observed improvements in CS in this trial are clinically meaningful. Recently, Maynard et al.[21] demonstrated that, when compared with age-similar healthy eyes, patients with nonadvanced AMD exhibit significantly worse CS (reflecting a deterioration of −0.007 log CS/year), consistent with the findings of a major review by Neelam et al.[22] In terms of visual performance, visual acuity is a measure of the ability to correctly identify targets (of variable size) at 100% contrast, whereas CS is a measure of the ability to detect/identify targets (of variable sizes [spatial frequencies]) at varying contrast (i.e., faintness). Furthermore, CS (but not BCVA) can effectively predict how well patients see targets typical of everyday life, which has important implications for quality of life.[23] Consequently, good visual acuity in the presence of poor CS (e.g., nonadvanced cataract) results in reports of visual complaints,[24] particularly for real-world tasks and targets,[23] but the following question remains: what degree of change in CS will have a clinically meaningful impact for the patient?

For VA, a one-line change (0.1 log MAR) is considered clinically meaningful.[25] For CS, the available data indicate that a 0.1 log change in the percentage threshold contrast required for the detection of a target/pattern is equally (if not more) devastating to visual performance than a deterioration of one line of BCVA.[23,26] In brief, threshold contrast is the contrast required to see the target reliably; the reciprocal of threshold is called sensitivity, which is expressed as a percentage (e.g., see Michelson contrast).[27] For example, for spatial frequencies that are near the peak of the contrast sensitivity function (i.e., 4–6 cycles/degree), younger and middle-aged patients have contrast thresholds of, on average, circa 2.5%. A 0.1 log unit deterioration from this value yields a contrast threshold of 3.2%, which is classed as visual impairment.[28] Moreover, contrast thresholds > 5% are associated with increased risk of driving accidents.[28] Accordingly, a decrease/increase in CS of 0.1 log unit is deemed clinically meaningful; in this study, 19.6% to 34.8% of participants exhibited at least this magnitude of improvement at three spatial frequencies, whereas this

Table 4. Repeated Measures Analysis of Macular Pigment and Serum Carotenoid Outcomes From Baseline to 24 Months in the CREST AMD Study by Intervention Group

		Group 1*					Group 2†				Time	Time × Group
		Baseline		24 Months			Baseline		24 Months		Effect	Interaction
Variable	N	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD	Sig.	Sig.
Macular pigment												
0.25°	45	0.80	0.23	1.02	0.15	50	0.72	0.25	1.00	0.16	<0.0005	0.247
0.5°	45	0.67	0.23	0.90	0.14	50	0.60	0.22	0.88	0.15	<0.0005	0.334
1.0°	45	0.44	0.17	0.66	0.11	50	0.45	0.18	0.63	0.09	<0.0005	0.444
1.75°	45	0.32	0.12	0.44	0.10	50	0.31	0.16	0.43	0.13	<0.0005	0.924
Serum carotenoids												
Serum L, μmol/l	41	0.34	0.16	1.40	0.83	47	0.33	0.21	1.72	1.06	<0.0005	0.111
Serum Z, μmol/l	40	0.07	0.04	0.14	0.07	46	0.07	0.05	0.19	0.11	<0.0005	0.005
Serum MZ, μmol/l	40	0.00	0.01	0.10	0.08	46	0.00	0.01	0.00	0.01	<0.0005	<0.0005
Serum TC, μmol/l	40	0.41	0.20	1.65	0.96	46	0.40	0.26	1.91	1.18	<0.0005	0.241

Macular pigment measured using the Macular Densitometer (Macular Metrics Corp.). Serum macular carotenoids analyzed by HPLC. Total carotenoids represent the total (composite) serum macular carotenoid concentrations (i.e., L, Z, and MZ combined). *N*, participants with data at all study visits. Sig., Significance set at $P < 0.05$.

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

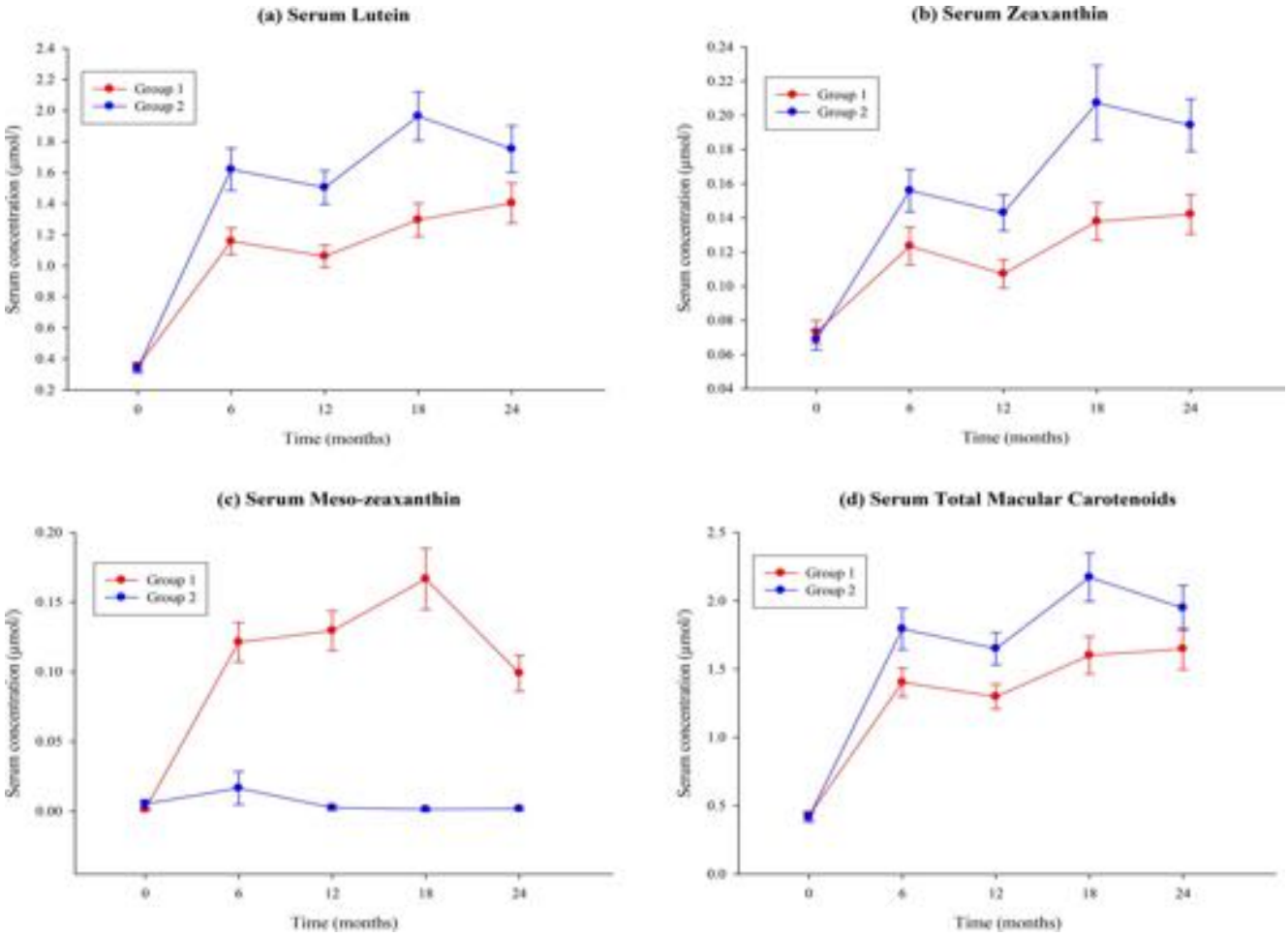

FIGURE 6. Serum carotenoid response in the CREST AMD study. Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper; group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper. Serum macular carotenoids analyzed by HPLC. Serum total macular carotenoids represent the addition of serum lutein, zeaxanthin, and *meso*-zeaxanthin concentrations obtained at each study visit. *Error bars* represent standard error of mean.

magnitude of deterioration was demonstrable in only 2.2% to 13% of participants at the same three spatial frequencies.

GD, defined as reduction in visual function caused by a glare source, results in retinal contrast loss secondary to retinal straylight.[29,30] Clinically, GD can be measured by assessing the impact of a glare source on visual function (BCVA or CS) or by the measurement of retinal straylight.[30] Of note, the Commission Internationale de l'Eclairage defines GD in terms of retinal straylight.[29] For the purposes of this study, GD was measured using each of these aforementioned methods (i.e., by assessing CS under conditions of glare [in both mesopic and photopic conditions] using the Functional Vision Analyzer and by measuring retinal straylight using the Oculus C-Quant). Mechanisms put forward to explain the observed improvements in CS following MP augmentation in patients with nonadvanced AMD apply also to the observed improvements in GD in this population, but with the possibility of an additional element, which relates the glare hypothesis of MP.[31] The glare hypothesis of MP posits that MP augmentation should improve GD and PRT via its optical (blue light) filtration properties.[31] Of note, the absorption spectrum of MP[32] accounts for one third of the visible spectrum, and wavelengths of light responsible for GD are those in MP's absorption range.[31] Therefore, and given that MP filters short-wavelength light at a prereceptorial level, thereby reducing the adverse impact of retinal straylight (caused by the glare source) that casts a veiling luminance on the retina, the observed improvements in CS under conditions of glare (GD) are unsurprising.[31] Also, improvements in PRT following supplementation may also be explained, at least in part, by the glare hypothesis of MP.[31] In brief, MP attenuates short-wavelength light from the glare source before it reaches the photoreceptors, thereby reducing its impact on photopigment bleaching, and, consequently, reducing the recovery time (i.e., the time it takes for vision to be restored).

The observed improvement in reading speed as a consequence of supplementation may be attributed to visual and/or nonvisual (neurocognitive) factors. In terms of the visual factors, reading speed is a function of both spatial and temporal CS,[33] and we have already discussed the mechanisms whereby antioxidant supplementation resulted in an improvement in two aspects of spatial vision (CS and GD). In terms of temporal vision, it has been shown that MP is positively related to critical flicker fusion frequency and to the full temporal CS function measured at the fovea but not the parafovea.[34] Furthermore, supplemental macular carotenoids have been shown to increase critical flicker fusion frequency thresholds and visual motor reaction time in young healthy participants.[35] Thus, MP could improve reading speed by its effects on temporal vision (i.e., increasing temporal processing speeds). Indeed, Stringham and Stringham[36] have suggested that temporal visual mechanisms compensate for MP's optical

Table 6. Distribution of Adverse Events in the CREST AMD Study by Intervention Group

Adverse Events	Group 1, $n = 57$*	Group 2, $n = 61$†
Any adverse event	15	10
Ocular		
Watery eyes	1	1
Transient blurred vision	1	0
Gritty eyes	1	0
Ocular pain	1	
Bloodshot eyes	1	0
Nonocular		
Nausea	2	3
Tiredness	2	1
Vomiting	3	0
Itchy skin	1	1
Metallic taste in mouth	1	1
Heat rash	0	2
Irritable bowel syndrome	1	0
Night-time urination	1	0
Headaches	1	0
Weight gain	1	0
Overactive kidney	0	1
Leg cramps	1	0
Knee ache	1	0
Red and swollen arms and legs	0	1
Dizziness	1	0
Neck stiffness	1	0
Abdominal pains	0	1
Pancreatitis	0	1
Palpitations	1	0
Sleep disturbance	1	0
Swollen face	0	1
Hallucinations	0	1
Swollen ankle	0	1
Loss of appetite	0	1

Data expressed as number of participants. Some participants reported more than one adverse event.

* Group 1, 10 mg/d MZ, 10 mg/d L, and 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

† Group 2, 10 mg/d L, 2 mg/d Z plus 500 mg/d vitamin C, 400 IU/d of vitamin E, 25 mg/d zinc, and 2 mg/d copper.

filtration properties by reducing temporal input from the short-wavelength cone system and increasing temporal processing by the middle/long wavelength cone system. These aspects of temporal vision may be enhanced following supplementation with the macular carotenoids[35] and may lead to subsequent improvements in reading speed.

Vision-related quality of life questionnaires are known to correlate with subjective measures of visual function (e.g., CS and reading speed),[37] and, therefore, it is likely that improvements in these parameters will result in improved quality of life. Scilley et al.[38] reported that persons with nonadvanced AMD have good visual acuity, but are likely to have problems with night driving, near vision tasks, and GD when compared with persons with no retinal disease (age-matched controls with normal retinal health). Visual acuity, CS, and reading speed are known determinants of vision-related quality of life in patients with nonadvanced AMD,[39] reflected in the findings of the Los Angeles Latino Eye Study, where nonadvanced AMD lesions (i.e., soft indistinct drusen and pigmentary abnormalities) were associated with a lower self-reported vision-related quality of life.[40] Therefore, the observed improvements in visual function parameters (in the current study) are likely to impact favourably on quality of life of patients with nonadvanced AMD. However, our vision-related quality of life instrument (NEI VFQ-25) did not show any statistically significant improvements following supplementation with macular carotenoids (in combination with coantioxidants), and we suspect that a larger number of participants will be required to do so with such an instrument. For instance, to detect a two-point difference between interventions in the NEI VFQ-25 overall score (assuming a 5% level of significance, 80% power ,and two-tailed test), the required sample size would be 3136 participants (1568 per intervention group).[41]

Eye care professionals should be aware of the observed visual benefits afforded to patients with nonadvanced AMD as a result of supplementation with macular carotenoids (and coantioxidants) in the short, medium, and long terms, and the indication for recommending such supplements should no longer be limited to risk reduction for disease progression in the long term. Also, and importantly, further augmentation of MP and further improvements in psychophysical function are realized in patients with nonadvanced AMD after 24 months of sustained supplementation, and it may well be that the improvements observed in this study (duration of 24 months) understate the visual improvements that patients can expect.[9]

Given that psychophysical function is compromised in nonadvanced AMD in a way that is commensurate with the stage of nonadvanced AMD and given that AMD is a progressive disease, our findings of visual improvements in a condition where visual deterioration is expected is as interesting as it is welcome. If psychophysical visual function can be improved in a progressive condition (such as nonadvanced AMD), it is tempting to hypothesize that improvements in psychophysical function herald regression of the morphological changes that underpin them. However, longer term studies with larger numbers of patients with nonadvanced AMD, and with regular monitoring of MP and psychophysical function as well as morphological changes, are required to confirm or refute this hypothesis.

It is possible that some of our reported improvements in psychophysical measures of visual function (e.g., reading speed) may be due to learning effects, but given that we had no placebo group (which represents a limitation of our study) it is difficult to ascertain to what level (if any). It is also important to point out that reading speed was not a primary outcome measure in this trial. However, given the long periods of time between study visits, we feel that these learning effects are likely to be minimal. It should also be appreciated that these improvements in for example, reading speed, were observed in patients suffering from a condition associated with progressive visual deterioration and at a time of life when speed of neural processing declines.

Of note, the MP levels reported at baseline may be considered high.[42] This suggests that the current study was representative of a very well-nourished population and this may have, in fact, resulted in understating the benefits of supplementation that may have been seen in a less well-nourished population (as was the case in subgroup analyses of the AREDS2 cohort).[43]

In this study, we measured MP using two devices; namely the Densitometer (Macular Metrics) and the Spectralis HRA-OCT MultiColor (Heidelberg Engineering, GmbH, Heidelberg, Germany). In a previous report, using data from the current study, we found that measures of MP using these two devices are not impressively concordant, although each of these two devices is capable of detecting statistically significant changes in MP over time, within a given eye, following supplementation with MP's constituent carotenoids.[44] Furthermore, another recent study has found that MP measurement using the

Spectralis is affected by cataract.[45] Thus, in the current study, which included patients with varying severity of lens opacification, we elected (following advice from the DSMC) to use the MP measures from the Densitometer, which are robust to cataract.[46,47]

The strengths of this study include its randomized, controlled, and double-masked design, the range of parameters of visual function assessed, the fact that MP was measured and monitored using an established and validated technique, and the determination of serological responses and that AMD was graded in a masked fashion by an accredited reading center. Finally, the study was overseen by an independent DSMC.

A study limitation (albeit slight) is the failure to reach the intended sample size of 56 participants per group; actual samples sizes were 51 and 46. However, because we elected to use repeated measures analysis of variance, rather than the independent-samples *t*-tests on which the original sample size calculations had been based, our statistical tests (of time and time × supplement interaction effects) were based on the t-distribution with more than 90 degrees of freedom, that is, these tests were more than adequately powered.

Another study limitation is the absence of a placebo arm. However, as already noted, the original study protocol had a true placebo, but that protocol had to be revised on ethical grounds, following publication of the AREDS2 findings. We did not measure the serum concentrations of any of the co-antioxidants (vitamin C, E, zinc, and copper) in this RCT. We do, however, report serum response of the macular carotenoids, which was important in the assessment of compliance, and that allowed us to investigate whether participants were responding to the nutrients of interest. Assessing the concentrations of the coantioxidants may have yielded insights into the interrelationships/interactions between these compounds and the macular carotenoids, and future studies may consider adopting such an approach. Correction for multiple testing was not performed in the current study. It is therefore possible that some of our reported significant results may be attributable to type 1 errors. However, many of the reported *P* values in this study would still be significant after Bonferroni adjustment.

In summary, supplementation with a formulation that contains the macular carotenoids (with or without MZ), in combination with coantioxidants, results in improvements in contrast sensitivity and other measures of visual function in patients with nonadvanced AMD.

Acknowledgments

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References

1. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Global Health*. 2014;2:e106–e116.
2. Akuffo KO, Nolan J, Stack J, et al. Prevalence of age-related macular degeneration in the Republic of Ireland. *Br J Ophthalmol*. 2015;99:1037–1044.
3. Williams RA, Brody BL, Thomas RG, Kaplan RM, Brown SI. The psychosocial impact of macular degeneration. *Arch Ophthalmol*. 1998;116:514–520.
4. Brown GC, Brown MM, Sharma S, et al. The burden of age-related macular degeneration: a value-based medicine analysis. *Trans Am Ophthalmol Soc*. 2005;103:173–184.
5. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retin Eye Res*. 2016;50:34–66.
6. Chew EY, Clemons T, SanGiovanni JP, et al. The Age-Related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 report number 1). *Ophthalmology*. 2012;119:2282–2289.
7. Age Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309:2005–2015.
8. Liu R, Wang T, Zhang B, et al. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:252–258.
9. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902–912.
10. Crosby-Nwaobi R, Hykin P, Peto T, Sivaprasad S. An exploratory study evaluating the effects of macular carotenoid supplementation in various retinal diseases. *Clin Ophthalmol*. 2016;10:835–844.
11. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111–123.
12. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS report no. 17. *Arch Ophthalmol*. 2005;123:1484–1498.
13. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Trials*. 2010;11:32.
14. Ma L, Yan SF, Huang YM, et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology*. 2012;119:2290–2297.
15. Huang YM, Dou HL, Huang FF, Xu XR, Zou ZY, Lin XM. Effect of supplemental lutein and zeaxanthin on serum, macular pigmentation, and visual performance in patients with early age-related macular degeneration. *Biomed Res Int*. 2015;2015:564738.
16. Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Prog Retin Eye Res*. 2002;21:225–240.
17. Hammond BR Jr, Fletcher LM. Influence of the dietary carotenoids lutein and zeaxanthin on visual performance:

application to baseball. *Am J Clin Nutr*. 2012;96:1207S–1213S.

18. Fletcher LM, Engles M, Hammond BR Jr. Visibility through atmospheric haze and its relation to macular pigment. *Optom Vis Sci*. 2014;91:1089–1096.
19. Hammond BR Jr, Wooten BR, Engles M, Wong JC. The influence of filtering by the macular carotenoids on contrast sensitivity measured under simulated blue haze conditions. *Vision Res*. 2012;63:58–62.
20. Stringham JM, O'Brien KJ, Stringham NT. Contrast sensitivity and lateral inhibition are enhanced with macular carotenoid supplementation. *Invest Ophthalmol Vis Sci*. 2017;58:2291–2295.
21. Maynard ML, Zele AJ, Feigl B. Mesopic Pelli-Robson contrast sensitivity and MP-1 microperimetry in healthy ageing and age-related macular degeneration. *Acta Ophthalmol*. 2016;94:e772–e778.
22. Neelam K, Nolan J, Chakravarthy U, Beatty S. Psychophysical function in age-related maculopathy. *Surv Ophthalmol*. 2009;54:167–210.
23. Owsley C, Sloane ME. Contrast sensitivity, acuity, and the perception of "real-world" targets. *Br J Ophthalmol*. 1987;71:791–796.
24. Charalampidou S, Loughman J, Nolan J, et al. Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol*. 2011;129:1155–1161.
25. Kaiser PK. Prospective evaluation of visual acuity assessment: a comparison of snellen versus ETDRS charts in clinical practice (an AOS thesis). *Trans Am Ophthalmol Soc*. 2009;107:311–324.
26. Leat SJ, Legge GE, Bullimore MA. What is low vision? A re-evaluation of definitions. *Optom Vis Sci*. 1999;76:198–211.
27. Pelli DG, Bex P. Measuring contrast sensitivity. *Vision Res*. 2013;90:10–14.
28. Owsley C, McGwin G Jr. Vision impairment and driving. *Surv Ophthalmol*. 1999;43:535–550.
29. Vos JJ. Disability glare—a state of the art report. *Commission Internationale de l'Eclairage J*. 1984;3:39–53.
30. Mainster MA, Turner PL. Glare's causes, consequences, and clinical challenges after a century of ophthalmic study. *Am J Ophthalmol*. 2012;153:587–593.
31. Stringham JM, Hammond BR Jr. The glare hypothesis of macular pigment function. *Optom Vis Sci*. 2007;84:859–864.
32. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
33. Legge GE, Rubin GS, Luebker A. Psychophysics of reading—V. The role of contrast in normal vision. *Vision Res*. 1987;27:1165–1177.
34. Renzi LM, Hammond BR Jr. The relation between the macular carotenoids, lutein and zeaxanthin, and temporal vision. *Ophthalmic Physiol Opt*. 2010;30:351–357.
35. Bovier ER, Hammond BR. A randomized placebo-controlled study on the effects of lutein and zeaxanthin on visual processing speed in young healthy subjects. *Arch Biochem Biophys*. 2015;572:54–57.
36. Stringham NT, Stringham JM. Temporal visual mechanisms may mediate compensation for macular pigment. *Perception*. 2015;44:1400–1415.
37. Orr P, Rentz AM, Margolis MK, et al. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:3354–3359.
38. Scilley K, Jackson GR, Cideciyan AV, Maguire MG, Jacobson SG, Owsley C. Early age-related maculopathy and self-reported visual difficulty in daily life. *Ophthalmology*. 2002;109:1235–1242.
39. Maguire M. Baseline characteristics, the 25-Item National Eye Institute Visual Functioning Questionnaire, and their associations in the Complications of Age-Related Macular Degeneration Prevention Trial (CAPT). *Ophthalmology*. 2004;111:1307–1316.
40. Choudhury F, Varma R, Klein R, Gauderman WJ, Azen SP, McKean-Cowdin R. Age-related macular degeneration and quality of life in Latinos: The Los Angeles Latino Eye Study. *JAMA Ophthalmol*. 2016;134:683–690.
41. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol*. 2001;119:1050–1058.
42. Akuffo KO, Nolan JM, Peto T, et al. Relationship between macular pigment and visual function in subjects with early age-related macular degeneration. *Br J Ophthalmol*. 2016;101:190–197.
43. Chew EY, Clemons TE, SanGiovanni JP, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report no. 3. *JAMA Ophthalmol*. 2013;132:142–149.
44. Akuffo KO, Beatty S, Stack J, et al. Concordance of macular pigment measurement using customized heterochromatic flicker photometry and fundus autofluorescence in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:8207–8214.
45. Akuffo KO, Nolan JM, Stack J, et al. the impact of cataract, and its surgical removal, on measures of macular pigment using the Heidelberg Spectralis HRA+OCT multicolor device effect of cataract on Spectralis MP measurement. *Invest Ophthalmol Vis Sci*. 2016;57:2552–2563.
46. Ciulla TA, Hammond BR Jr, Yung CW, Pratt LM. Macular pigment optical density before and after cataract extraction. *Invest Ophthalmol Vis Sci*. 2001;42:1338–1341.
47. Nolan JM, O'Reilly P, Loughman J, et al. Augmentation of macular pigment following implantation of blue light-filtering intraocular lenses at the time of cataract surgery. *Invest Ophthalmol Vis Sci*. 2009;50:4777–4785.

Investigative Ophthalmology & Visual Science

Enrichment of Macular Pigment Enhances Contrast Sensitivity in Subjects Free of Retinal Disease: Central Retinal Enrichment Supplementation Trials – Report 1

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Purpose. The high-performance visual function associated with central vision is mediated by the macula (the central retina), which accumulates three diet-derived pigments (the carotenoids lutein [L], zeaxanthin [Z], and meso-zeaxanthin [MZ]). Our study sought to investigate the impact on visual function, including contrast sensitivity (CS), of supplementation with these naturally occurring carotenoids, in individuals with low retinal concentrations.

Methods. Subjects consumed daily a formulation containing 10 mg L, 2 mg Z, and 10 mg MZ (active group; $n = 53$) or placebo ($n = 52$) for a period of 12 months. Study visits were at baseline, 3, 6, and 12 months. Contrast sensitivity at 6 cycles per degree (cpd) was the primary outcome measure (POM). Secondary outcome measures included CS at other spatial frequencies, best-corrected visual acuity (BCVA), glare disability, photostress recovery, and light scatter. Macular pigment optical density (MPOD) was measured using dual-wavelength autofluorescence, and serum carotenoid concentrations were analyzed using high performance liquid chromatography (HPLC).

Results. Compared to placebo, statistically significant improvements from baseline CS were detected at 6 ($P = 0.002$) and 1.2 ($P = 0.004$) cpd in the active group. Additionally, improvements in CS were commensurate with the observed increases in retinal concentrations of these carotenoids ($r = 0.342$, $P = 0.002$ at 6 cpd).

Conclusions. These results indicate that dietary fortification with the macular carotenoids can have meaningful effects on visual function.

Keywords: macular pigment, contrast sensitivity, meso-zeaxanthin, lutein, visual function, visual acuity, glare disability, randomized clinical trial

Macular pigment (MP), a yellow pigment concentrated at the macula, is composed of the xanthophyll carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ; Fig. 1).[1–3] Studies on this pigment, and its constituent carotenoids, have intensified over the last two decades, with researchers hypothesizing, investigating, and reporting on its origins and functions.[4] Specifically, research has been conducted on the role of supplementation with MP's constituent carotenoids (L, Z, and MZ) on clinical course[5,6] and vision[7] in patients with established nonadvanced age-related macular degeneration (AMD). These studies were prompted by the observations that MP is a powerful antioxidant[8,9] and also acts as a filter of short-wavelength visible (blue) light[10] (given that AMD is attributable, at least in part, to oxidative stress and that irradiation with blue light induces oxidative stress in the retina).[11]

In 2013, the AREDS2 study concluded that supplementation with at least two of MP's constituent carotenoids (L and Z, along with coantioxidants, vitamin C, vitamin E, zinc, copper) is beneficial in terms of reducing disease progression and in terms of visual outcomes in patients with nonadvanced AMD.[6] However, from an evolutionary perspective, it is unlikely that humans have evolved to selectively accumulate three carotenoids (L, Z, and MZ) in the central retina to retard the natural course of an age-related disease.[12] In other words, it seems intuitive that the primary role of MP is other than protection against age-related macular disorders.

Accordingly, many have postulated that MP is important for vision in a nondiseased eye, and this view was first proposed by Schultze et al. in 1866.[13] In brief, it is proposed that MP's prereceptorial filtration of short-wavelength visible (blue) light optimizes and/or enhances visual function by its attenuation of chromatic aberration and by its attenuation of the visual impact of light scatter, phenomena that are largely restricted to short wavelengths of visible light (i.e., blue light).[12,14–17] However, there are optical effects of the eye that reduce overall chromatic aberration.[18] Moreover, visual acuity is largely driven by middle- and long-wavelength sensitive cones.[19] Both of these effects serve to reduce the capacity of short-wavelength light (and thereby limit MP's ability) to influence visual acuity.

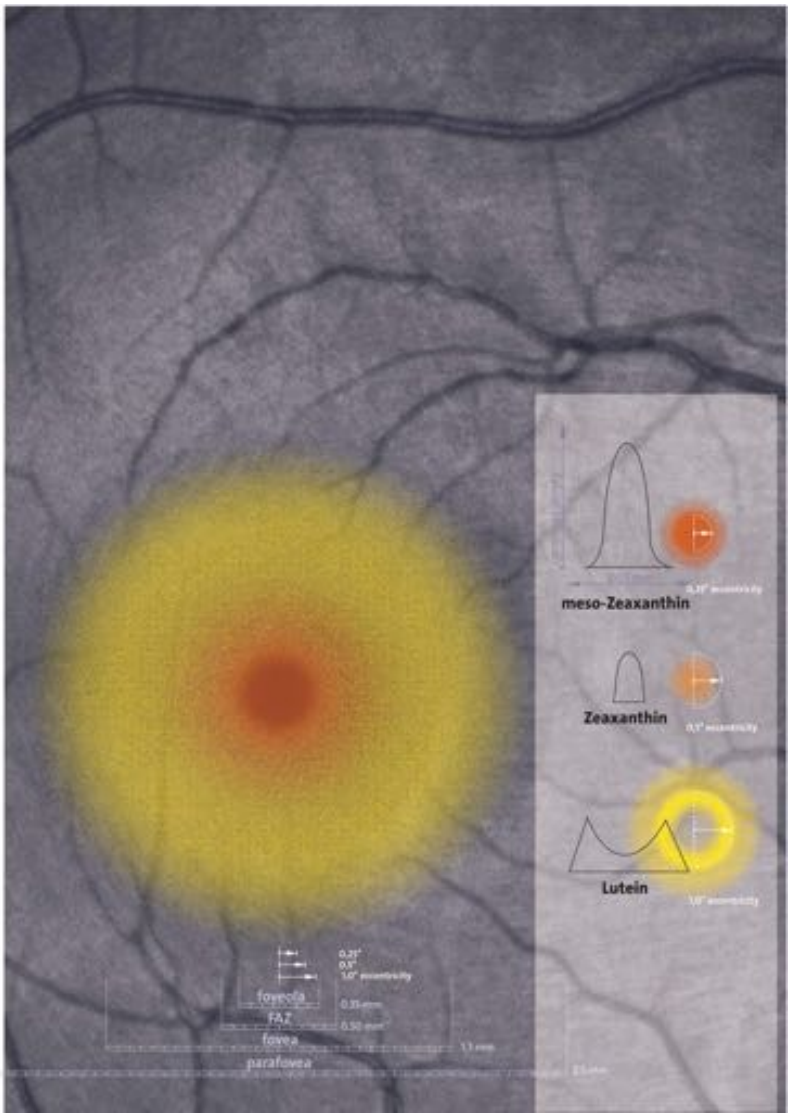

Figure 1. Distribution of MP's constituent carotenoids presented in scale onto a photograph of a healthy human retina. Figure courtesy of John Nolan, Robert Kuchling, and Kristiane Nöbel.

However, studies performed to date to test the above hypotheses have been limited in terms of their design (e.g., single-blind),[20] methodology (e.g., measurement devices not optimal or validated),[21,22] outcome measures (e.g., assessing visual function using, for instance, best corrected visual acuity [BCVA] only),[21] and interventions used (e.g., trials using supplements containing either low amounts of carotenoids [thereby limiting bioavailability][23] and, in most cases, the supplement formulations used only one or two of the three macular carotenoids [typically L and/or Z],[24,25] thereby precluding comment on the impact of supplementation with all three macular carotenoids, a desirable endeavor given that L, Z, and MZ are found in equal amounts at the macula[26]).

In July 2011, the European Research Council (ERC) awarded funding of €1,493,342 to support and conduct the Central Retinal Enrichment Supplementation Trials (CREST).[27] The CREST project was funded under the ERC "Ideas" Framework 7 program. The objective of CREST was to use a gold standard clinical trial design to study the "protective" and "visual function" hypotheses of MP. In brief, two clinical trials were established to investigate the impact of supplementation with a combined carotenoid formulation of MZ, L, and Z on visual function in normal subjects with low MP at baseline (Trial 1, the focus of the current report) and in subjects with early AMD (Trial 2, report to follow).

A novel and important feature of the CREST trials was the inclusion of MZ in the study intervention. Indeed, recent published data from our laboratory have shown that the addition of MZ to the carotenoid formulation, resulting in a MZ:L:Z (mg) ratio of 10:10:2, on a daily basis, results in optimal response in terms of: (1) total circulating serum carotenoid concentrations,[28] (2) enrichment of MP centrally and across its spatial profile,[28,29] (3) enhancements in visual function in subjects free of retinal disease,[30] and (4) enhancement of visual function in subjects with retinal disease (i.e., subjects with established nonadvanced AMD).[7,31] However, while these earlier and exploratory studies have added greatly to knowledge in the field regarding the importance (or not) of including all three of the macular carotenoids in a formulation, we felt that a gold-standard clinical trial, with optimal study design and appropriately informed outcome measures, was merited. With this objective in mind, the CREST study was designed and the findings of the CREST Normal trial (CREST Trial 1) are presented and discussed here.

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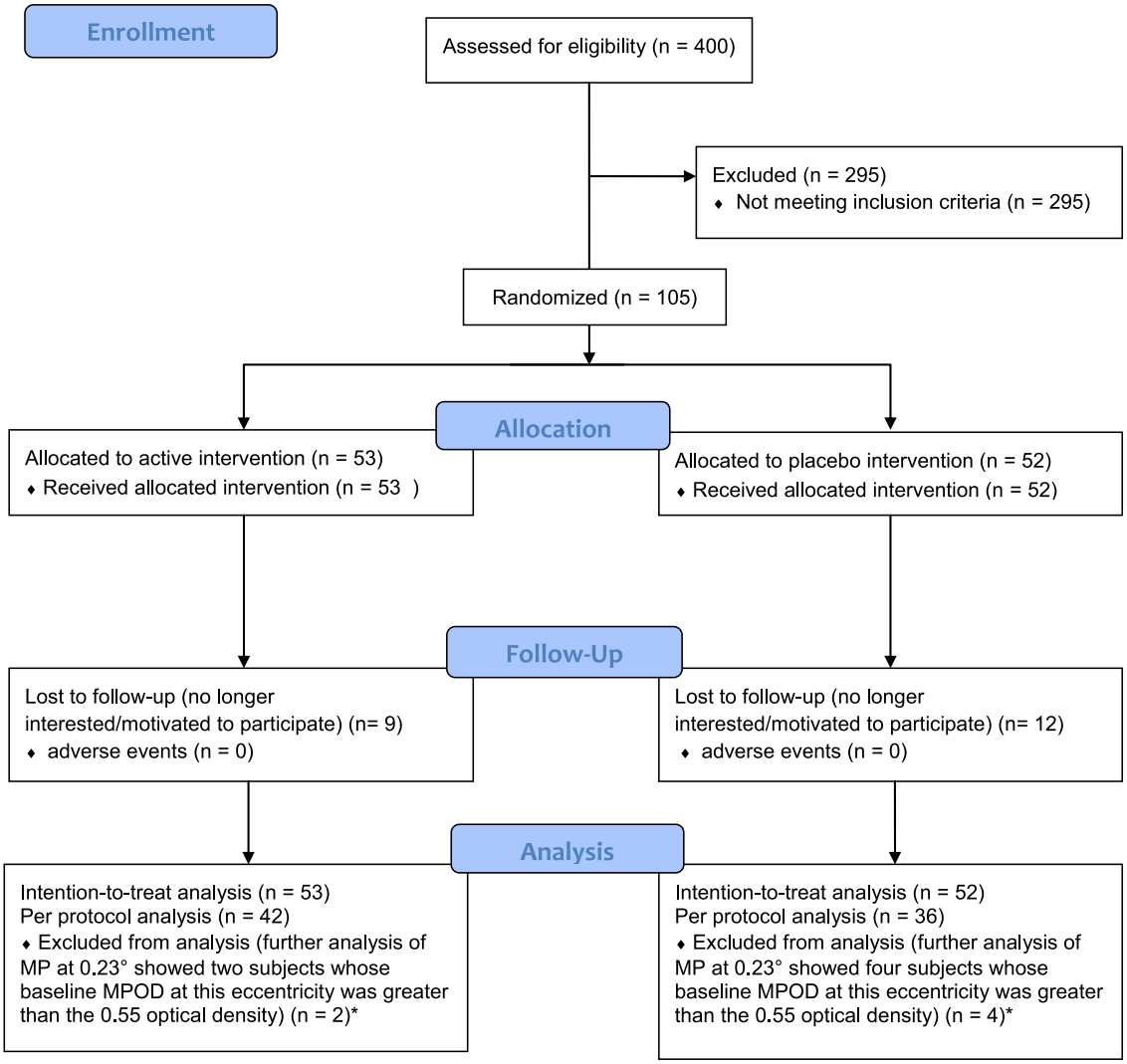

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram for CREST Trial 1.

Materials and Methods

The design and methodology of this study have been described in detail previously.[27] A summary of the methodology used in the CREST Trial 1 is presented below. In brief, CREST Trial 1 is a parallel group, double-blind, placebo-controlled, block-randomized trial investigating the impact of macular carotenoid supplementation on visual function in normal subjects with low MP at baseline (Trial registration No. ISRCTN68270512). The trial commenced in October 2012 (i.e., the first subject visit) and concluded in June 2015 (i.e., last subject 12-month visit).

Of 105 subjects (52 male, 53 female) originally recruited into the study, 10 were excluded before statistical analysis, as the threshold for defining "low" MP was set at 0.55 optical density units (for MP measured at 0.23° eccentricity, measured on the Heidelberg Spectralis [Heidelberg Engineering GmbH, Heidelberg, Germany], see Fig. 2). Before enrollment, all subjects provided written informed consent. Ethical approval was granted by the Research Ethics Committee of the Waterford Institute of Technology, Waterford, Ireland, and the Ethics Committee of the ERC. The CREST study adhered to the tenets of the Declaration of Helsinki, and followed the full code of ethics with respect to subject recruitment, subject testing, and data protection.

Inclusion criteria for participation in this study were as follows: age 18 years or older, monocular BCVA of 6/6 or

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better, no more than ±5 diopters (D) spherical equivalence of refraction, no previous consumption of supplements containing the macular carotenoids (L, Z, and/or MZ), no ocular pathology, and MP at 0.23° of eccentricity ≤ 0.55 optical density units. A subject was defined as "normal" when he/she exhibited no vision-related abnormalities, which was assessed as follows: clinical examination, which consisted of ocular and medical history and general health questionnaire, BCVA measurement, MP measurement, optical coherence tomography (OCT), fundus photography, and completion of a general health questionnaire. This assessment battery was performed as part of a screening visit, which took place on a separate day, before a subject's baseline study visit (visit 1).

Subjects who passed the eligibility assessment were assigned to intervention groups in a ratio of 1:1 with no stratification using block randomization.[32] We randomly assigned 53 subjects to the active intervention, which contained 10 mg L, 10 mg MZ, and 2 mg Z in a sunflower oil suspension. There were 52 subjects randomly assigned to the placebo intervention, which contained just sunflower oil. Subjects were instructed to take one capsule daily with a meal. The intervention and placebo supplements were identical in external appearance and, therefore, the two treatments were indistinguishable from each other. Frequent phone calls and reminder text messages were sent to subjects to ensure compliance with consumption, and capsule counting was implemented at follow-up visits. Of note, capsule count was comparable between the active and placebo groups for each time point in the study.

Study visits occurred at baseline, and at 3-, 6-, and 12-month intervals. Study visits were conducted by one of two researchers (RP or JD). Statistical analysis by the research group statistician (JS) found no evidence of systematic difference in measurements, for any study outcome measure, between the two researchers. This was a single-site study, which presents advantages and limitations. The advantages of a study which involves only a single site include governance and validity/reproducibility of measurements, each being important in terms of standardization of methodology, quality control, and compliance with study visits/interventions. However, the principal disadvantage rests on the fact that, typically, a single site attracts only subjects from a given geographic area, and, therefore, is not necessarily generalizable to the overall population.

Demographic, Lifestyle, Medical, and Ophthalmic Assessment

Questionnaires were used to obtain demographic and lifestyle information at baseline. Medical and ocular histories also were documented. Body mass index (BMI) was calculated (kg/m^2) from height (m) and weight (kg) measurements recorded using the Leicester Height Measure and SECA weighing scales (SECA, Birmingham, UK), respectively. Weekly intake of carotenoid-rich foods (eggs, broccoli, corn, dark green leafy vegetables) was recorded using a dietary LZ screener previously used by our group and developed by Elizabeth Johnson.[33]

Assessing Visual Function

The eye with the best visual acuity was selected as the study eye for assessment. Where both eyes had the same BCVA, the right eye was chosen. Best corrected visual acuity was measured with a computerized LogMAR Early Treatment Diabetic Retinopathy Study (ETDRS) test chart (Test Chart 2000 Xpert; Thomson Software Solutions, Hatfield, UK). Letter contrast sensitivity (CS) was assessed using the computerized ETDRS test chart (Test Chart 2000 PRO) at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, 15.15 cycles per degree [cpd]). Both visual performance tests used the Sloan optotypes and were viewed at a distance of 4 m. Contrast sensitivity also was assessed using the Optec Functional Vision Analyzer[34] (Stereo Optical Co., Inc., Chicago, IL, USA), which uses the functional acuity contrast test to assess CS at five different spatial frequencies (1.5, 3, 6, 12, 18 cpd). These methods have been described in more detail previously.[30]

The amount of intraocular straylight on the retina was measured using the C-Quant Straylight Meter (Oculus GmbH, Wetzler, Germany). Photostress recovery time was measured by assessing CS and investigating the impact of a light stress using a 300-watt tungsten spotlight (ARRI 300 Plus lamp; ARRI Lighting Solutions GmbH, Berlin, Germany) with a low-pass glass dichroic filter. A CS value of 0.30 log units (i.e., two lines on Letter CS) above the individual's contrast threshold was used. The time taken for the subject's study eye to recover (nonstudy eye was covered with an eye patch) and see all five letters on the chart after the 10-second exposure was taken as the photostress recovery time (seconds). Visual function was also assessed subjectively (at baseline and 12 months only) via questionnaire.[35]

Fundus Photography and Grading

All photography was performed by trained and certified photographers. Standard color fundus photographs centered on the macula were taken using the Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany) at a 45° magnification setting, following pupil dilation. These fundus photographs were reviewed by an ophthalmologist (SB) to exclude any ocular pathology.

Macular Pigment Measurement

Macular pigment was measured using the Heidelberg Spectralis HRA+OCT MultiColor (Heidelberg Engineering GmbH). Pupillary dilation was performed before measurement. This technology uses confocal scanning laser ophthalmoscopy (cSLO) imaging with diode lasers and uses dual-wavelength autofluorescence (AF) for measuring MP.[36] Dual-wavelength AF in this device uses two excitation wavelengths, one that is well-absorbed by MP (486 nm, blue), and one that is not (518 nm, green). A 30-second video was taken in simultaneous blue AF and green AF imaging mode for MP measurement acquisition. The video images were aligned and averaged using the Heidelberg Eye Explorer software (HEYEX, version 1.7.1.0), from which a MP density map was created. Central MP at 0.23° eccentricity and MP volume (calculated as MP average times the area under the curve out to 7° eccentricity) are reported here.

Serum Carotenoid Assessment

Nonfasting blood samples were collected at each study visit by standard venipuncture techniques in 9 mL vacuette tubes (BD Vacutainer SST Serum Separation Tubes; Becton, Dickinson and Company, Plymouth, UK) containing a "Z Serum Sep Clot Activator." All collection tubes were inverted a minimum of five times to ensure appropriate mixing of the clot activator. The blood samples were allowed to clot at room temperature for 30 minutes, after which they were centrifuged for 10 minutes at 725*g* in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. After centrifugation, serum was transferred to light-resistant microtubes and stored at circa –80°C until the time of batch analysis. Serum carotenoid analysis was done by

high performance liquid chromatography (HPLC) as described previously.[27,37]

Statistical Analysis

The statistical package IBM SPSS version 22 was used for all analyses. Contrast sensitivity at 6 cpd was the primary outcome measure (POM) of this study. Secondary outcome measures included CS at other spatial frequencies, visual acuity, glare disability, photostress recovery, light scatter, MP, serum carotenoid concentrations, and subjective visual function.

This was a double-blind, placebo-controlled, block-randomized clinical trial. Sample size was estimated as 45 in each of the active and placebo groups, based on an effect size of 0.15 log CS units in the POM (equivalent to one line on Letter CS [Thomson Test Chart 2000 PRO]), 80% statistical power, and a 1-tailed test at the 5% level of statistical significance. Estimates of standard deviations and pre–post correlation, needed for the sample size calculation, had been obtained from an earlier pilot study. The decision to use a 1-tailed test, in sample size calculation, also was based on the results of this pilot test, where we had found clear evidence that the POM improved significantly in the active supplement group relative to the placebo group. We had targeted to recruit 120 subjects into this trial (30 more than indicated by the sample power calculations), and we screened a total of 400 subjects to achieve this target of 120. In the event, only 95 subjects (24% of those screened [see Fig. 2]) eventually were deemed eligible (met all inclusion criteria, including MP at 0.23° of eccentricity $\leq$ 0.55 optical density units); 10 more subjects participated in the study but were excluded from statistical analysis due to exceeding the MP threshold.

No adjustment was made for multiple comparisons. Standard statistical tests, such as the independent samples *t*-test for quantitative variables, and the contingency table χ^2 test for categorical variables, were used to compare active and placebo groups at baseline. Repeated measures ANOVA was used for the between-group comparisons of change in outcome variables over time. As specified in the CREST methodology study,[27] subjects who failed to complete the full 12 months of trial were not included in final between-group analysis; however, we performed additional intention to treat analysis using Last Observation Carried Forward (LOCF), for purposes of comparison whenever the main analysis, excluding missing values, produced statistically significant results. Statistical significance was set at the standard $P < 0.05$ for all analyses.

Results

Baseline

Table 1 presents baseline summary statistics for demographic, health, lifestyle, and vision study variables, in the active and placebo intervention groups. There were no statistically significant differences between treatment groups for any of these variables at baseline.

Change in Outcome Variables Over Time

Change in Contrast Sensitivity. Table 2 shows (for active and placebo study groups) changes, over the 12-month study period, in mean CS at five different frequencies, as well as changes in mean BCVA. The final column of Table 2 displays the *P* values for the time-group interaction effects; that is, it identifies those outcome variables for which the mean change, after 12 months, was significantly different between active and placebo groups. Figure 3 displays graphically the mean CS curves for the active and placebo groups at baseline and 12 months. As seen in Figure 3 and Table 2, mean changes in two CS outcome measures (CS at 1.2 and 6 cpd [POM]) were statistically significantly different between the active and placebo intervention groups by 12 months. These statistically significant differences constituted an improvement in CS in the active treatment group. Of note, intention to treat analysis also gave statistically significant results for both of these CS outcome measures.

Change in Serum Carotenoids and MP. Figure 4 shows (for the active and placebo groups) mean change in serum L, MZ, and Z, over the 12-month study period. Of note, for each carotenoid analyzed, a drop in serum concentration was seen at V4, which may reflect the influence of a regulatory process governing uptake of circulating carotenoids.

Figure 5 shows the corresponding results for MP at 0.23° and MP volume. The error bars do not overlap, indicating statistically significant increases in the active intervention group compared to the placebo group, with the exception of serum Z at 12 months.

Change in Other Outcome Variables: Visual Acuity, CS at Other Eccentricities, Glare Disability, Photostress Recovery, Light Scatter and Subjective Visual Function. Repeated measures ANOVA of all other study variables (Table 1) did not reveal any statistically significant differences, over the 12-month study period, between active and placebo intervention groups.

When Did Significant Change Occur?

In this study, the statistically significant differential in CS in the active treatment group versus the placebo group was not observed until 12 months (i.e., with no significant change in CS by 3 or 6 months). However, the statistically significant increases in serum L, Z, and MZ, and in MP (at 0.23° and MP volume) all occurred by 3 months ($P < 0.0005$ for all, repeated measures ANOVA).

Relationship Between Change in Serum Concentrations of L, Z, and MZ, Change in MP, Versus Change in CS

We also investigated the relationship of change in CS (at 6 and 1.2 cpd) versus change in MP, measured over the 12-month study period. We did this for placebo and active intervention groups combined, using Pearson correlation analysis, and found positive and statistically significant relationships between change in MP and change in CS.

The following relationships were positive and statistically significant: change in central MP and change in CS at 6 cpd (POM, $r = 0.342$, $P = 0.002$), change in MP volume and change in CS at 6 cpd (POM, $r = 0.255$, $P = 0.024$), change in central MP and change in CS at 1.2 cpd ($r = 0.249$, $P = 0.028$), and change in MP volume and change in CS at 1.2 cpd ($r = 0.293$, $P = 0.009$). Thus, in general, greater changes in subjects' MP were associated with greater changes in CS.

Of note, the relationship between change in each of the serum carotenoids, and change in MP, over the 12-month study period, was positive and statistically significant ($P < 0.01$ for all). Thus, in general, greater changes in subjects' serum carotenoid concentrations were associated, in this study, with greater changes in MP. However, no statistically significant relationships were observed between change in serum concentrations of L (or MZ) and change in CS at any spatial frequency ($P > 0.05$, for all).

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Table 1. Demographic, Health and Lifestyle, Vision, and MP Data of the Active and Placebo Intervention Groups

Variables	Active Intervention, $n = 48$	Placebo Intervention, $n = 47$	Sig.
Demographic and health			
Age, y	44.83 ± 11.46	46.49 ± 13.07	0.513
BMI, kg/m^2	27.32 ± 4.69	26.32 ± 4.58	0.319
Exercise, min/wk	288.72 ± 306.51	286.63 ± 296.98	0.973
Diet, estimated intake of L and Z	24.13 ± 14.69	21.5 ± 12.8	0.357
Sex, % male	47.9	53.2	0.607
Education, highest level %			0.903
Primary	2.1	2.1	
Secondary	22.9	19.1	
Higher, third level	75	78.7	
Smoking, %			0.720
Never smoked	45.8	46.8	
Past smoker	35.4	31.9	
Current smoker	16.7	21.3	
Alcohol frequency, %			0.103
Never	6.4	2.1	
Special occasions	6.4	19.1	
1–2 times/mo	23.4	21.3	
1–2 times/wk	63.8	51.1	
Everyday	0	6.4	
AMD family history, % yes	10.4	17	0.370
Vision			
BCVA	105.67 ± 3.79	106.41 ± 4.26	0.373
CS 1.2 cpd	1.96 ± 0.10	1.98 ± 0.12	0.398
CS 2.4 cpd	1.94 ± 0.12	1.95 ± 0.17	0.919
CS 6 cpd	1.68 ± 0.15	1.69 ± 0.21	0.841
CS 9.6 cpd	1.47 ± 0.14	1.51 ± 0.21	0.254
CS 15.15 cpd	1.17 ± 0.19	1.19 ± 0.25	0.512
Light scatter	1.17 ± 0.17	1.22 ± 0.22	0.285
PRT, seconds	22.88 ± 17.04	23.96 ± 16.40	0.753
MPOD 0.23°	0.38 ± 0.08	0.38 ± 0.10	0.925
MPOD volume	3992.98 ± 1288.81	3792.94 ± 1597.32	0.504

BCVA was reported in visual acuity rating, CS was reported using the Thomson Test Chart 2000PRO, and PRT was reported in seconds. Data displayed are mean ± SD for numerical data and percentages for categorical data. Variables, variables analyzed in the study; Active Intervention, group supplemented with 10 mg L, 10 mg MZ, and 2 mg Z in a sunflower oil suspension; Placebo Intervention, group supplemented with sunflower oil; Sig., the statistical difference (*P* value) between the groups; BMI, the body mass divided by the square of the body height, expressed in units of kg/m^2; Exercise, total exercise measured as minutes per week engaged in physical or sporting activity; Diet score, estimated dietary intake of lutein and zeaxanthin; Education (highest level %), highest level to which subject was educated; Smoking (%), current smoker (smoked ≥100 cigarettes in lifetime and at least one in the last year), past smoker (smoked ≥100 cigarettes in lifetime and none in past year), or nonsmoker (smoked < 100 cigarettes in lifetime); Alcohol frequency, frequency of consumption of Alcohol; AMD family history (% yes), the percent of subjects with a confirmed family history of AMD for a first degree relative; MPOD 0.23°, MPOD at 0.23° of retinal eccentricity; MPOD volume, the volume of macular pigment out to 7° of retinal eccentricity.

Discussion

The CREST Trial 1 is a double-blind, placebo-controlled, block-randomized clinical trial, designed to investigate the impact of supplementation with all three macular carotenoids (in a MZ:L:Z [mg] ratio of 10:10:2) on visual function in individuals free of retinal disease, but with low MP at study baseline. The design and methodology of this study have been informed by the published literature, and in consultation with the world's leading macular carotenoid vision scientists.[27] Of note, to our knowledge this is the first study of rigid and gold standard design and with published a priori outcome measures (POM, CS at 6 cpd) designed to investigate the impact, if any, of supplemental macular carotenoids on visual function in nondiseased eyes. The principal finding was that, following supplementation with the macular carotenoids in a MZ:L:Z (mg) ratio of 10:10:2 for 12 months, the POM (CS at 6 cpd) exhibited significant improvement.

Visual performance, in spite of the multifaceted composite that it represents, is typically oversimplified to measures of visual acuity, even by eye health professionals. Although visual acuity is, indeed, an important aspect of visual performance, it is by no means a surrogate for an individual's visual performance and experience, and other variables relating to visual function should be investigated when conducting a scientific study of this nature.

Vision is a composite of optical, physiologic, and neural processes. One could argue that visual acuity is largely determined by the optics of a healthy eye, reflected in the observation that optical resolving power is the principal determinant of acuity.[38] Some parameters of visual performance, however, such as dark adaptation,[39] are mediated primarily by physiologic processes, whereas others, such as color constancy,[40] are the result of visual processing at higher levels (i.e., cortex). Interestingly, the results of our study suggest an outcome governed by some or all of the determinants of visual performance: for example, visual acuity did not change over the study period for subjects in the active supplement group, whereas CS did (Fig. 3B; Table 2), and this

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Table 2. Repeated Measures Analysis of Visual Function Variables From Baseline to 12 Months Showing the Time Group Interaction

	Active Intervention				Placebo Intervention				Time X Group Interaction
	Baseline		12 Mo		Baseline		12 Mo		
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*
BCVA	105.67	3.79	105.7	4.25	106.41	4.26	106.12	3.56	0.398
CS 1.2 cpd	1.96	0.1	2.08	0.12	1.98	0.12	1.98	0.12	0.004*
CS 2.4 cpd	1.94	0.12	2.00	0.15	1.95	0.17	1.98	0.12	0.13
CS 6 cpd	1.68	0.15	1.76	0.16	1.69	0.2	1.68	0.2	0.002*
CS 9.6 cpd	1.47	0.14	1.46	0.18	1.51	0.21	1.51	0.21	0.761
CS 15.5 cpd	1.17	0.19	1.16	0.23	1.2	0.25	1.22	0.22	0.967

Contrast sensitivity was assessed using the Thomson Test Chart 2000PRO.

* Significant difference between groups at the 0.05 level; only subjects with MP at 0.23° eccentricity ≤ 0.55 optical density units were included. Per protocol analysis $n = 42$ in the active arm and $n = 36$ in the placebo arm (see Fig. 2 for full breakdown).

observation indicates that CS (although a correlate of visual acuity)[41] is influenced by factors other than the determinants of acuity, including retinal and/or cortical factors, thereby explaining the disparity of the results in terms of visual acuity versus CS.

In this study, we reported statistically significant improvements in CS at 1.2 and 6 cpd, associated with changes in MP, but a discussion on the clinical significance of this finding is merited. Clinical meaningfulness is difficult to define. However, having based our study sample size calculations on an effect size of 0.15 log CS units—one line on a contrast sensitivity chart—it seems reasonable also to define clinical significance in these terms. For CS at 6 cpd (POM), 28.5% of subjects in the active supplement group, and just 3.1% of subjects in the placebo group, improved CS by at least one line on a chart. The corresponding percentages for CS at 1.2 cpd were 37.5% in the active supplement group and 6.2% in the placebo group, who also improved CS by at least one line on a chart. Given that the carotenoid status of our subjects' retinas was significantly augmented over the study period, it is safe to assume that the observed impact on CS is attributable to the observed augmentation of MP.

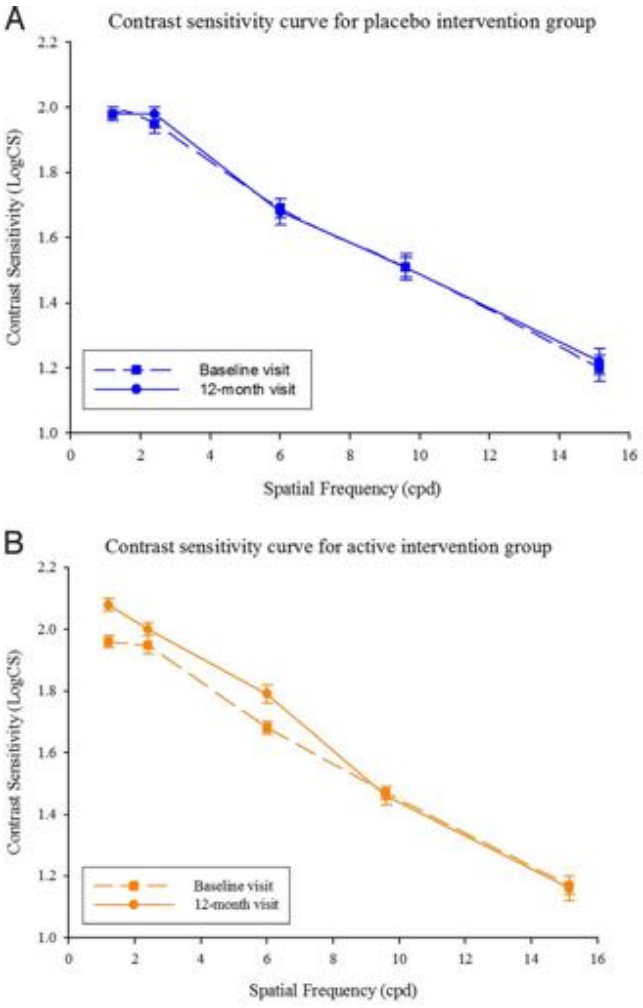

Figure 3. (**A**) Letter CS function for placebo intervention group. (**B**) Letter CS function for active intervention group.

All subjects in the active intervention arm of the study exhibited augmentation of MP, reflected in a mean (± SD) increase in MP volume of 2436 (± 1451), and a range of observed increases in MP volume of 738 to 6464. In percentage terms, MP volume increased by a mean (± SD) of 73% (± 62%), with a range of increases of 16% to 337%. This is an important observation, given that circa 20% of supplemented subjects do not normally exhibit any rise in MP in studies that did not include MZ in the formulation,[35,42] consistent with the view that some individuals lack the capacity to bioconvert retinal L to retinal MZ.[43,44]

We believe that the visual improvements observed herein are the result of at least one of two mechanisms. First, the prereceptoral filtration of blue light could reduce chromatic aberration and also reduce the impact of any (albeit mild) light scatter. These effects could plausibly improve CS, but arguments against the observed improvement being attributable to prereceptoral filtration of visible blue light include the fact that light and dark bars would be equally affected, thereby negating any perceived differences in luminance that would serve to enhance CS. Further, given the moderate light levels during testing in the current study, scattered light would not be expected to appreciably affect visual performance in an adverse way. Lastly, if prereceptoral absorption of blue light was driving the observed visual benefits reported herein, the effect would be at higher spatial frequencies than those that we observed because MP optical density (MPOD) peaks centrally, where the density of photoreceptors averages 200,000/mm^2 and where it can be much higher[45] (an observation that is responsible for very fine visual resolution [including CS for high spatial frequencies] at this locus). However, because CS improved only for frequencies near the peak of the contrast sensitivity function, and not for high spatial frequencies, it is likely that the observed visual benefits are primarily physiologic/retinal/cellular in origin, rather than

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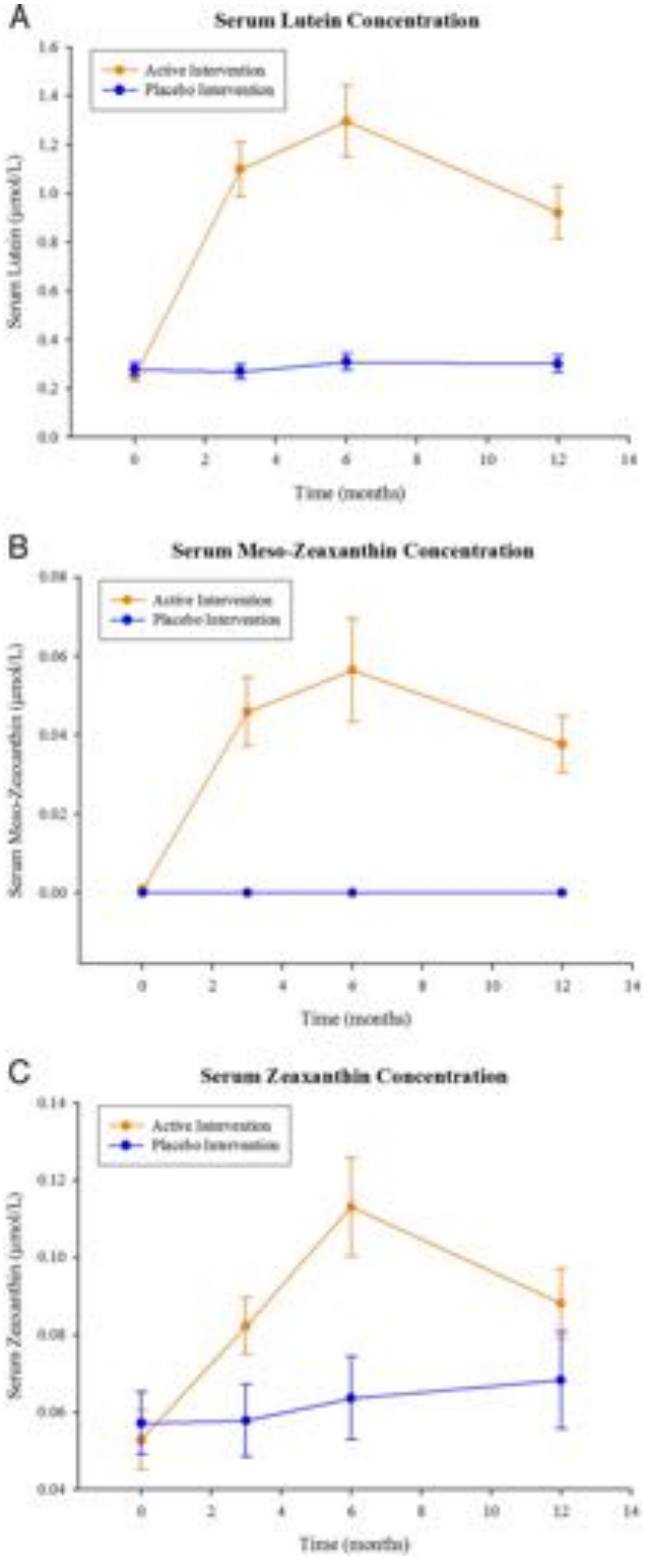

Figure 4. (**A**) Serum L response for the active and placebo groups over the study period. (**B**) Serum MZ response for the active and placebo groups over the study period. (**C**) Serum Z response for the active and placebo groups over the study period.

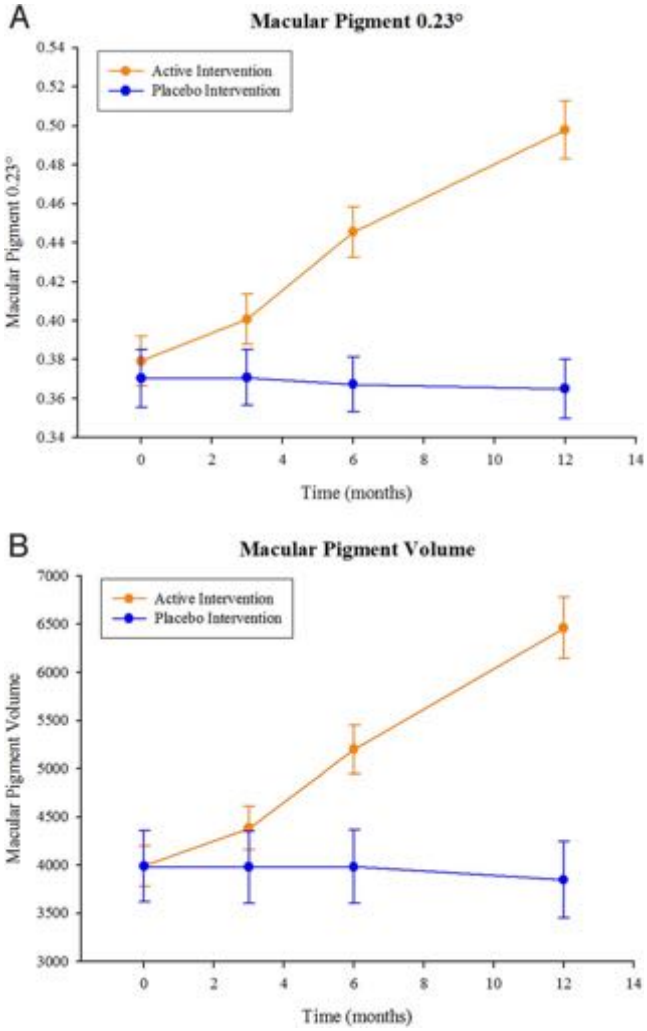

Figure 5. (**A**) Macular pigment response for the active and placebo groups at 0.23° of eccentricity over the study period. (**B**) Macular pigment volume response for the active and placebo groups over the study period.

being solely attributable to the optical impact of augmented MP.

This brings us to the second, and seemingly more plausible mechanism for our observations. The seminal work of Kuffler,[46] and several subsequent investigations,[47] have characterized the anatomic and neurophysiologic basis for CS lateral inhibition. In short, lateral inhibition is the result of retinal circuitry that is wired in such a way as to produce many thousands of overlapping, roughly concentric, subtractive regions called receptive fields.[48] Light differentially affects the center versus surround regions of the receptive field and, ultimately, the perceived difference between the two yields the visual system's ability to detect edges (i.e., contrast). The arrangement of the receptive fields is such that a difference in CS is a function of spatial frequency, and this phenomenon is known as the contrast sensitivity function (CSF), and, when tested with sinusoidal gratings, its peak generally is found to be approximately 4 cycles/deg (although the function is fairly broadly tuned).[49] Based on our results, in some manner, increased macular carotenoid concentration probably enhances lateral inhibitory processes that yield performance increases near the peak of the CSF. There is a plausible mechanism for this effect. For example, it could be that increased MP simply

leads to increased efficiency in the visual cycle. This idea is consistent with our findings, and, given the macular carotenoids' exceptional antioxidant properties,[9] is also consistent with the effect of visual cycle inhibition/disruption by oxidative stress.[50] It has been shown that the retinal carotenoids serve to strongly inhibit the activity of A2E, itself the product of oxidative stress and a potent visual cycle inhibitor.[51] At the level of perception, a more efficient visual cycle is likely to manifest as increased CS, especially for those neural networks that are under the greatest metabolic stress (i.e., near the peak of the CSF). In consideration of our finding of enhanced CS following enrichment of MP in the active group, this idea was first introduced by Stringham et al.,[52] who found a relationship between MPOD and CS for a slightly higher spatial frequency (10 cycles/deg). The idea was subsequently expanded upon,[53] and further supported by the suggestion of a plausible molecular mechanism involving the interplay of retinal carotenoids and nitric oxide,[54] whereby increased macular carotenoids facilitate the ability of nitric oxide to increase the signal-to-noise ratio of horizontal cells that serve center-surround receptive fields.[55]

An important distinction between the findings of our study and those of previous investigators is that we observed improvements in CS that were commensurate with MP augmentation, which suggests that the observed benefits are, indeed, attributable to observed increases in MP over the study period (and not attributable to interindividual variability in factor[s] related to MP). In other words, our findings are not simply associative, and inference of causality is justified.

In terms of everyday meaningfulness of improved CS following supplementation with the macular carotenoids, several practical and clinical benefits can be expected by the individual. Most obvious would be a general improvement in visual discrimination for objects in real-world scenes, such as resolving individual leaves on a tree, whereas perhaps before improvement, leaves would tend to blend together. Indeed, it has been found that the human CSF very closely follows the image characteristics of natural scenes, reflective of the evolution of spatial vision.[56] In an automobile driving situation, increased CS would allow for earlier and more accurate detection of objects.[57,58] Given that automobile safety often is the result of a split-second reaction to rapidly changing environmental conditions, this kind of improvement, no matter how small, would improve outcomes.[59] Indeed, some countries in Europe recently have added measures of CS (rather than performing measures of visual acuity alone) for assessing eligibility criteria to drive. In the United Kingdom, for example, the visual standard to hold a driver's license requires that the applicant achieves a visual acuity of 6/12 (20/40) or better (measured indoors) and demonstrates the ability to read a car number plate (measured [outdoors] at a specified distance),[60] in keeping with a European Union directive on driving licensure.[61] However, and given that subjects with reduced CS have greater difficulty outdoors,[62,63] it is unsurprising that visual acuity is not predictive of the ability to read a number plate in those with poor CS.[57] In other words, poor CS creates a disconnect between the ability to read a car number plate and visual acuity, thereby negating the value of acuity readings for the purpose of assessing a subject's eligibility to drive. Of note, CS also is important for train drivers (in Europe), as European Union (EU) legislation now specifies the need for good CS for those seeking certification to operate locomotives and trains on the EU railway system.[64]

Lastly, general quality of life would likely be improved by enhancements in CS (e.g., enjoying a scenic view, and so forth), and even small improvements in CS for those spatial frequencies near the peak of the CSF could have meaningful effects, for example, making printed text easier to process; thus, easing eye strain and fatigue over the course of a day. Moreover, those engaged in vision-dependent activities for the military (e.g., sniper units, aviators, and so forth) and sports (e.g., baseball players, tennis players, and so forth) could expect improvements in performance.

In conclusion, we found that in subjects free of retinal disease and with low MP, supplementation with a formulation containing all three macular carotenoids resulted in measurable improvements in vision, reflected in enhanced CS at 6 and 1.2 cpd. These findings may have important implications for those endeavoring to maximize their visual performance and experience, whether for professional or leisure activities.

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References

1. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci*. 1993;34:2033–2040.
2. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res*. 1997;64:211–218.
3. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys*. 2001;385:28–40.
4. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retin Eye Res*. 2016;50:34–66.
5. Chew E. The AREDS2 Study Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309:2005–2015.
6. Chew EY, Clemons TE, SanGiovanni JP, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA Ophthalmol*. 2013;132:142–149.
7. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902–912.
8. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802–1811.
9. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010;504:56–60.
10. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.

11. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000;45:115–134.
12. Loughman J, Davison PA, Nolan JM, Akkali MC, Beatty S. Macular pigment and its contribution to visual performance and experience. *J Optom*. 2010;3:74–90.
13. Schultze M. *Ueber den gelben Fleck der Retina. Seinen Einfluss auf normales Sehen und auf Farbenblindheit*. Bonn: Cohen & Sohn; 1866.
14. Walls GL, Judd HD. The intra-ocular colour-filters of vertebrates. *Br J Ophthalmol*. 1933;17:705–725.
15. Nussbaum JJ, Pruett RC, Delori FC. Historic perspectives. Macular yellow pigment. The first 200 years. *Retina*. 1981;1: 296–310.
16. Werner JS, Donnelly SK, Kliegl R. Aging and human macular pigment density. Appended with translations from the work of Max Schultze and Ewald Hering. *Vision Res*. 1987;27:275–268.
17. Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Prog Retin Eye Res*. 2002;21:225–240.
18. McLellan JS, Marcos S, Prieto PM, Burns SA. Imperfect optics may be the eye's defence against chromatic blur. *Nature*. 2002;417:174–176.
19. Williams DR, Coletta NJ. Cone spacing and the visual resolution limit. *J Opt Soc Am A*. 1987;4:1514–1523.
20. Stringham JM, Hammond BR. Macular pigment and visual performance under glare conditions. *Optom Vis Sci*. 2008;85: 82–88.
21. Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. *Invest Ophthalmol Vis Sci*. 2013;54:1781–1788.
22. Hammond BR Jr, Wooten BR, Smollon B. Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optom Vis Sci*. 2005;82:387–404.
23. Bartlett HE, Eperjesi F. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. *Eur J Clin Nutr*. 2007; 61:1121–1127.
24. Rodriguez-Carmona M, Kvansakul J, Harlow JA, Kopcke W, Schalch W, Barbur JL. The effects of supplementation with lutein and/or zeaxanthin on human macular pigment density and colour vision. *Ophthalmic Physiol Opt*. 2006;26:137–147.
25. Yao Y, Qiu QH, Wu XW, Cai ZY, Xu S, Liang XQ. Lutein supplementation improves visual performance in Chinese drivers: 1-year randomized, double-blind, placebo-controlled study. *Nutrition*. 2013;29:958–964.
26. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci*. 1988;29:843–849.
27. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111–123.
28. Thurnham DI, Nolan JM, Howard AN, Beatty S. Macular response to supplementation with differing xanthophyll formulations in subjects with and without age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2015;253: 1231–1243.
29. Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res*. 2012;101:9–15.
30. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871–7880.
31. Sabour-Pickett S, Beatty S, Connolly E, et al. Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina*. 2014;34:1757–1766.
32. Friedman LM, Furberg CD, Demets DL. *Fundamentals of Clinical Trials*. 4th ed. New York: Springer; 2010.
33. Nolan JM, Loskutova E, Howard A, et al. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. *J Alzheimers Dis*. 2015;44:1157–1169.
34. Hohberger B, Laemmer R, Adler W, Juenemann AG, Horn FK. Measuring contrast sensitivity in normal subjects with OPTEC 6500: influence of age and glare. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1805–1814.
35. Nolan JM, Loughman J, Akkali MC, et al. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vision Res*. 2011;51:459–469.
36. Dennison JL, Stack J, Beatty S, Nolan JM. Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-wavelength autofluorescence, and single-wavelength reflectance. *Exp Eye Res*. 2013;116:190–198.
37. Nolan JM, Loskutova E, Howard AN, et al. Macular pigment, visual function, and macular disease among subjects with Alzheimer's disease: an exploratory study. *J Alzheimers Dis*. 2014;42:1191–1202.
38. Hartridge H. Visual acuity and the resolving power of the eye. *J Physiol*. 1922;57:52–67.
39. Marmor MF, Martin LJ. 100 years of the visual cycle. *Surv Ophthalmol*. 1978;22:279–285.
40. Land EH. Recent advances in retinex theory and some implications for cortical computations: color vision and the natural image. *Proc Natl Acad Sci U S A*. 1983;80:5163–5169.
41. Richman J, Lorenzana LL, Lankaranian D, et al. Importance of visual acuity and contrast sensitivity in patients with glaucoma. *Arch Ophthalmol*. 2010;128:1576–1582.
42. Trieschmann M, Beatty S, Nolan JM, et al. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res*. 2007;84:718–728.
43. Nolan JM, Meagher K, Kashani S, Beatty S. What is meso-zeaxanthin, and where does it come from? *Eye (Lond)*. 2013; 27:899–905.
44. Gorusupudi AF, Shyam RF, Li BF, et al. Developmentally regulated production of meso-zeaxanthin in chicken retinal pigment epithelium/choroid and retina. *Invest Ophthalmol Vis Sci*. 2016;57:1853–1861.
45. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292:497–523.
46. Kuffler SW. Discharge patterns and functional organization of mammalian retina. *J Neurophysiol*. 1953;16:37–68.
47. Wandell BA. The retinal representation. In: *Foundations of Vision*. Chapter 5. Sunderland, MA: Sinauer Associates, Inc.; 1995. Available at: https://foundationsofvision.stanford.edu/chapter-5-the-retinal-representation.
48. Gouras P. The effects of light-adaptation on rod and cone receptive field organization of monkey ganglion cells. *J Physiol*. 1967;192:747–760.
49. Campbell FW, Robson JG. Application of Fourier analysis to the visibility of gratings. *J Physiol*. 1968;197:551–566.
50. Moiseyev G, Nikolaeva O, Chen Y, Farjo K, Takahashi Y, Ma JX. Inhibition of the visual cycle by A2E through direct interaction with RPE65 and implications in Stargardt disease. *Proc Natl Acad Sci U S A*. 2010;107:17551–17556.

Investigative Ophthalmology & Visual Science

51. Bhosale P, Serban B, Bernstein PS. Retinal carotenoids can attenuate formation of A2E in the retinal pigment epithelium. *Arch Biochem Biophys*. 2009;483:175–181.
52. Stringham JM, Garcia PV, Smith PA, McLin LN, Foutch BK. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. *Invest Ophthalmol Vis Sci*. 2011;52:7406–7415.
53. Stringham JM, Garcia PV, Smith PA, et al. Macular pigment and visual performance in low-light conditions. *Invest Ophthalmol Vis Sci*. 2015;56:2459–2468.
54. Stringham NT, Stringham JM. Temporal visual mechanisms may mediate compensation for macular pigment. *Perception*. 2015;44:1400–1415.
55. Vielma A, Delgado L, Elgueta C, Osorio R, Palacios AG, Schmachtenberg O. Nitric oxide amplifies the rat electroretinogram. *Exp Eye Res*. 2010;91:700–709.
56. Torralba A, Oliva A. Statistics of natural image categories. *Network*. 2003;14:391–412.
57. Rae S, Latham K, Katsou MF. Meeting the UK driving vision standards with reduced contrast sensitivity. *Eye (Lond)*. 2016; 30:89–94.
58. Marottoli RA, Richardson EDFAU, Stowe MHFAU, et al. Development of a test battery to identify older drivers at risk for self-reported adverse driving events. *J Am Geriatr Soc*. 1998:46:562–568.
59. Green M. "How long does it take to stop?" Methodological analysis of driver perception-brake times. 2000;2:195–216.
60. Driver and Vehicle Licensing Agency. At a glance guide to the current medical standards of fitness to drive. 2014. Available at: www.dft.gov.uk/dvla/medical/ataglance.aspx.
61. European Parliament. Commission Directive 2009/113/EC of 25 August 2009 on amending Directive 2006/126/EC of the European Parliament and of the Council on driving licences. 2009. 24-2-2016. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:223:0031:0035:EN:PDF.
62. van den Berg TJ. Importance of pathological intraocular light scatter for visual disability. *Doc Ophthalmol*. 1986;61:327–333.
63. Abrahamsson M, Sjostrand J. Impairment of contrast sensitivity function (CSF) as a measure of disability glare. *Invest Ophthalmol Vis Sci*. 1986;27:1131–1136.
64. Directive 2007/59/EC of the European Parliament and of the council of the 23 October 2007 on the certification of train drivers operating locomotives and trains on the railway system in the Community. 2007. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02007L0059-20140715.

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Lutein, Zeaxanthin and Meso-zeaxanthin Supplementation Associated with Macular Pigment Optical Density

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Abstract: The purpose of this study was to evaluate the effects of lutein, zeaxanthin and meso-zeaxanthin on macular pigment optical density (MPOD) in randomized controlled trials (RCTs) among patients with age-related macular degeneration (AMD) and healthy subjects. Medline, Embase, Web of Science and Cochrane Library databases was searched through May 2016. Meta-analysis was conducted to obtain adjusted weighted mean differences (WMD) for intervention-versus-placebo group about the change of MPOD between baseline and terminal point. Pearson correlation analysis was used to determine the relationship between the changes in MPOD and blood xanthophyll carotenoids or baseline MPOD levels. Twenty RCTs involving 938 AMD patients and 826 healthy subjects were identified. Xanthophyll carotenoids supplementation was associated with significant increase in MPOD in AMD patients (WMD, 0.07; 95% CI, 0.03 to 0.11) and healthy subjects (WMD, 0.09; 95% CI, 0.05 to 0.14). Stratified analysis showed a greater increase in MPOD among trials supplemented and combined with meso-zeaxanthin. Additionally, the changes in MPOD were related with baseline MPOD levels ($r_{AMD} = -0.43$, $p = 0.06$; $r_{healthy\ subjects} = -0.71$, $p < 0.001$) and blood xanthophyll carotenoids concentration ($r_{AMD} = 0.40$, $p = 0.07$; $r_{healthy\ subjects} = 0.33$, $p = 0.05$). This meta-analysis revealed that lutein, zeaxanthin and meso-zeaxanthin supplementation improved MPOD both in AMD patients and healthy subjects with a dose-response relationship.

Keywords: lutein; zeaxanthin; meso-zeaxanthin; macular pigment optical density

1. Introduction

The macula is a specialized part in the posterior pole of retina, since it mediates central vision, provides the sharpest visual acuity and facilitates the best color discrimination [1]. As the major functional component in the macular region, macular pigment (MP) was uniquely concentrated in the inner and central layers and mainly composed of xanthophyll carotenoids, including lutein, zeaxanthin and meso-zeaxanthin [2–8]. The concentration of these carotenoids in the macular region is about

1000 times greater than that in the blood [8]. The exquisite degree of biological selectivity in the retina indicated that these carotenoids played a pivotal role in maintaining the normal morphology and function of the macula [9]. Furthermore, lutein, zeaxanthin and meso-zeaxanthin are believed to play a major role in protecting retina and retinal pigment epithelium from light-initiated oxidative damage by scavenging reactive oxygen species and filtering blue light, which was involved in the putative pathogenesis of many age-related eye diseases [10–15]. Thus, elevated MP affords protection against the development of many retinal diseases, especially for age-related macular degeneration (AMD); contrarily, low MP enhanced the risk of these diseases [4,6,12,13].

Data from epidemiologic studies suggested that dietary lutein and zeaxanthin intake were inversely associated with the risk of AMD [16–18]. In addition, our previous studies also found that supplementation with these macular carotenoids partially reversed the loss of visual function in patients with early AMD by elevating macular pigment optical density (MPOD), suggesting a causative role of MPOD for the maintenance of normal visual function [19]. Although some intervention studies have showed that lutein, zeaxanthin and meso-zeaxanthin supplementation resulted in significant morphologic changes in macular pigment, the response was variable among different studies and even a few studies failed to find such an increase in MPOD [20–22]. Populations with specific genetic backgrounds or nutritional status may potentially affect the transport and deposition processes of these carotenoids from blood to macula during supplementation [13,17]. The efficacy of supplementation for the different study populations and supplement dose remained uncertain. Furthermore, total zeaxanthin increases with decreasing eccentricity in the macula, and tends to be the dominant carotenoid at the central fovea [23]. These specific distribution patterns suggest that zeaxanthin may play a crucial role in the center of the retina. In addition, It was hypothesized that meso-zeaxanthin, a geometrical isomer of zeaxanthin, was able to protect against age-related eye damage by the special antioxidant properties and light filtering properties [5,24,25]. However, whether zeaxanthin and meso-zeaxanthin should be added in combination with lutein remained to be confirmed. Besides, MPOD depends on the stimuli that are used for its measurement [19,21]. Thus, the influence of different methods used in included studies should be explored.

Therefore, we performed a meta-analysis of randomized controlled trials (RCTs) to determine the effect of lutein, zeaxanthin and meso-zeaxanthin supplementation on MPOD in AMD patients and healthy subjects.

2. Materials and Methods

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26].

2.1. Data Sources and Search Strategy

A comprehensive search was performed to identify all relevant articles in Medline, Embase, Web of Science, and Cochrane Library database up to May 2016, using the search terms lutein, zeaxanthin, meso-zeaxanthin, xanthophyll or carotenoids in conjunction with each of the following words: macular pigment optical density, macular pigment density, macular pigment, MPOD and MP, as well as combinations of these terms. References from retrieved articles were also reviewed for pertinent studies. No language restriction was applied for searching and study inclusion. Experts in the field were content in terms of additional information or potential unpublished studies in the case of missing data.

2.2. Study Selection

The titles and abstracts of potentially eligible studies were identified by the search strategy. Then, the full text articles were reviewed to determine whether they met the inclusion criteria. Studies were included in the meta-analysis if they fulfilled the following criteria: (1) eligible studies were limited to randomized controlled trials (RCTs); (2) subjects were randomized to receive lutein,

zeaxanthin or/and meso-zeaxanthin supplement or placebo; (3) the outcome of interest was MPOD; (4) studies reported the change of MPOD between baseline and at the end of study in the intervention and placebo group. When studies were conducted in healthy subjects, these subjects should be free of retinal disease. If multiple articles were published from the same study, only the most updated data was selected for analysis. Three investigators (Rong Liu, Jun Hui Du and Tao Liu) independently reviewed all identified publications for inclusion using predetermined criteria, with discrepancies resolved by consensus.

2.3. Data Extraction and Study Quality Assessment

For each included study, study characteristics and demographics was recorded as follows: first author, publication year, sample size, population characteristics (age, sex and country), interventions (dose of lutein/zeaxanthin/meso-zeaxanthin and duration of follow-up), change in the mean with standard deviation (SD) for MPOD, numbers enrolled and lost to follow-up. This needs to be clear in the manuscript. When several means and standard deviations were present in a single study, the data was pooled by combining groups into a single group according to the Cochrane recommendation. Where final SDs were not available from trials, they were calculated from confidence intervals (CI) or standard errors reported in study. If the information of blood lutein and zeaxanthin concentration was showed in studies, it was also extracted for further relevant analysis.

Methodological quality of each study was evaluated by the Jadad score, a 5-point study quality assessment instrument. This scale consists of three aspects: the method of randomization, the adequacy of blinding, and the description of withdrawals and dropouts. Studies that scored three or more were considered to be categorized as high quality. Data extraction and quality assessment was conducted independently and in duplicate by three investigators (Rong Liu, Jun Hui Du and Tao Liu), and any disagreement was adjudicated by a fourth author (Le Ma).

2.4. Statistical Analysis

The weighted mean differences (WMD) and corresponding 95% CIs were used as the primary summary measure of the effect of lutein/zeaxanthin/meso-zeaxanthin supplement on MPOD. Statistical heterogeneity among studies was evaluated by Q tests and the degree of heterogeneity was assessed by I^2 statistics. WMD for MPOD were pooled using inverse-variance weighting with the fixed effects or random-effects models. To explore the potential sources of between-study heterogeneity, meta-regression analyses were conducted stratified by health status (AMD patients vs. healthy participants), dose of lutein, zeaxanthin or meso-zeaxanthin supplementation (>10 mg vs. $\leqslant$10 mg), duration of intervention ($\geqslant$12 month vs. <12 month), mean age of subjects (>70 years vs. $\leqslant$70 years), zeaxanthin (with zeaxanthin vs. without zeaxanthin), meso-zeaxanthin (with meso-zeaxanthin vs. without meso-zeaxanthin), other antioxidants use (with other antioxidants vs. without other antioxidants) and geographic area (Europe vs. Asia vs. North America), measurement method of MPOD (objective (fundus autofluorescence, spectral fundus reflectance and VISUCAM NM/FA) vs. psychophysical (heterochromatic flicker photometry and macular assessment profile)) [27]. In pooling dose-response analysis, the relationship between the dose of lutein/zeaxanthin/meso-zeaxanthin supplement and the change in MPOD in each study was examined by linear regression model. The association between the increase in MPOD and blood xanthophyll carotenoids concentration was investigated using Pearson correlation analysis. Sensitivity analyses to examine the influence of each individual study were performed by iteratively excluding each study from this meta-analysis and comparing the point estimates without and with one study at a time. Publication bias was assessed by the Egger regression asymmetry test and the Begg adjusted rank correlation test [28,29]. All statistical analyses were conducted by Stata software, version 10.0 (Stata Corp, College Station, TX, USA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Literature Search

A total of 2456 potentially relevant publications were retrieved during our initial search. After duplicate publications detection and abstract review, full-text versions of the remaining 133 articles were then retrieved for detailed evaluation. Of these, 114 retrieved trials were not eligible due to duplicate publications, lack of a control group, outcomes not suitable for the meta-analysis, means or SDs of pretest and posttest data not included in the publication and not provided by the authors on request. Finally, the remaining 20 articles were eligible for inclusion in our analysis [17,20–22,30–45].

3.2. Study Characteristics

The characteristics of the included studies are presented in Table 1. In these trials, 12 were performed in Europe, 6 in USA and 2 in China. The number of participants in each study ranged from 19 to 172, comprising a total of 1764. Most studies included both men and women, except for 2 in which only men or women were selected. 8 trials supplemented with lutein vs. placebo, 2 treated with zeaxanthin vs. placebo, 8 intervened by combining lutein and zeaxanthin vs. placebo, and 8 had multiple arms (lutein, zeaxanthin or/and meso-zeaxanthin combined with other antioxidants, vs. placebo). The dosage of lutein, zeaxanthin or/and meso-zeaxanthin in the intervention groups among trials varied from 0 mg/day to 20 mg/day. The duration of intervention and follow-up ranged from 8 weeks to 2 years. MPOD was measured by the objective methods in 7 studies, and psychophysical methods in 13 trials. All included studies had a Jadad score of 3 or more, indicating generally high methodological quality.

3.3. The Effect of Lutein, Zeaxanthin or/and Meso-zeaxanthin Supplementation on MPOD in Patients with AMD

Nine RCTs evaluated the efficacy of these carotenoids supplement on the changes in MPOD for AMD patients (Figure 1). The I^2 test for heterogeneity was 99.2% ($p < 0.001$); and the results from random-effects models suggested that combing trials produced a MPOD increase by 0.07 ODU (95% CI, 0.03 to 0.11) in favor of supplementation vs. placebo. In the stratified analysis, a longer supplementation time had a marginally greater effect in comparison with the shorter time (0.17 vs. 0.05; between-group difference, 0.12; $p = 0.05$; Table 2). Trials measured MPOD with objective methods showed a larger increase in MPOD compared with those by psychophysical methods, although the difference did not reach statistical significance (0.09 vs. 0.05; between-group difference, 0.04; $p = 0.37$). The dose-response meta-analysis estimate showed a 0.005 ODU improvement in MPOD for a 1 mg/day increase in these carotenoids supplement. In sensitivity analysis, exclusion of any single trial from the analysis did not alter the overall findings of the effect of supplementation on MPOD. No evidence of publication bias was detected in this study by either Begg ($p = 0.68$) or Egger test ($p = 0.83$).

Table 1. Characteristics of the eligible randomized clinical trials.

Authors (Year)	Study Participants	Trial Duration	No. of Groups	Lnterventions	Measurement Method for MPOD	Follow-Up Rates (%)	Quality Score *
Trieschmann et al. (2007) [20]	130 AMD patients aged (71.4 ± 7.6) years in Germany	6 months	2	12 mg lutein and 1 mg zeaxanthin combined with other antioxidants; placebo	Fundus autofluorescence	94.6	3
Richer et al. (2007) [21]	90 AMD patients aged (74.1 ± 7.5) years in the USA	12 months	3	10 mg lutein; 10 mg lutein combined with other antioxidants; placebo	HFP	84.4	5
Weigert et al. (2011) [30]	126 AMD patients aged (71.6 ± 8.6) years in Austria	6 months	2	20 mg lutein daily in months 1 to 3 and 10 mg lutein daily in months 4 to 6; placebo	Spectral fundus reflectance	87.3	3
Arnold C et al. (2013) [31]	20 AMD patients aged (66.0 ± 8.0) years in Germany	10 weeks	2	10 mg lutein plus 3 mg zeaxanthin; placebo	VISUCAM NM/FA	100.0	5
García-Layana et al. (2013) [32]	44 AMD patients aged (68.5 ± 8.5) years in Spain	12 months	2	12 mg lutein plus 0.6 mg zeaxanthin combined with other antioxidants; placebo	HFP	NR	3
Dawczynski et al. (2013) [33]	172 AMD patients aged (70.0 ± 10.0) years in Germany	12 months	3	10 mg lutein, 1 mg zeaxanthin combined with other antioxidants; 20 mg lutein, 2 mg zeaxanthin combined with other antioxidants; placebo	VISUCAM NM/FA	84.3	3
Murray et al. (2013) [34]	72 AMD patients aged (70.5 ± 8.7) years in UK	12 months	2	10 mg lutein daily; placebo	HFP	86.9	5
Arnold C et al. (2013) [35]	172 AMD patients aged (69.0 ± 10.0) years in Germany	12 months	3	10 mg lutein plus 1 mg zeaxanthin combined with other antioxidants; 20 mg lutein plus 2 mg zeaxanthin combined with other antioxidants; placebo	VISUCAM NM/FA	84.3	5
Huang et al. (2015) [36]	112 AMD patients aged (69.1 ± 7.4) years in China	24 months	4	10 mg lutein; 20 mg lutein; 10 mg lutein plus 10 mg zeaxanthin; placebo	Fundus autofluorescence	96.4	5
Kvansakul et al. (2005) [37]	92 healthy men in UK	12 months	4	10 mg lutein; 10 mg zeaxanthin; 10 mg lutein plus 10 mg zeaxanthin in months 1 to 6 and 20 mg lutein; 20 mg zeaxanthin; 10 mg lutein plus 10 mg zeaxanthin in months 7 to 12; placebo	MAP	79.3	4
Bone et al. (2007) [38]	19 healthy subjects in the USA	120 days	2	14.9 mg of meso-zeaxanthin, 5.5 mg of lutein, and 1.4 mg of zeaxanthin; placebo	HFP	NR	3
Johnson et al. (2008) [39]	57 healthy women in the USA	4 months	3	12 mg lutein plus 0.5 mg zeaxanthin;12 mg lutein plus 800 mg DHA; placebo	HFP	86.0	4
Bone et al. (2010) [40]	100 healthy subjects in the USA	140 days	4	5 mg lutein; 10 mg lutein; 20 mg lutein; placebo	HFP	87.0	4
Connolly et al. (2011) [17]	44 healthy subjects in Ireland	6 months	2	10.6 mg meso-zeaxanthin, 5.9 mg lutein, and 1.2 mg zeaxanthin; placebo	HFP	79.5	5
Nolan et al. (2011) [41]	121 healthy subjects in Ireland	12 months	2	12 mg lutein, 1 mg zeaxanthin combined with other antioxidants; placebo	HFP	62.8	4
Landrum et al. (2012) [42]	30 healthy subjects in the USA	24 weeks	3	20 mg lutein diacetate; 20 mg lutein; placebo	HFP	NR	3
Loughman et al. (2012) [22]	36 healthy subjects in Ireland	6 months	3	20 mg lutein plus 2 mg zeaxanthin; 10 mg meso-zeaxanthin, 10 mg lutein plus 2 mg zeaxanthin; placebo	HFP	88.9	5
Yao et al. (2013) [43]	120 healthy subjects in China	12 months	2	20 mg lutein; placebo	HFP	82.5	4
Bovier et al. (2015) [44]	102 healthy subjects in the USA	4 months	3	20 mg zeaxanthin; 8 mg lutein plus 26 mg zeaxanthin combined with other antioxidants; placebo	HFP	67.6	4
Nolan et al. (2016) [45]	105 healthy subjects in Ireland	12 months	2	10 mg lutein, 2 mg zeaxanthin, and 10 mg meso-zeaxanthin; placebo	Autofluorescence	80.0	5

Abbreviations: AMD, age-related macular degeneration; HFP, heterochromatic flicker photometry; MPOD, macular pigment optical density; NR, not report. * Study quality was judged based on the Jadad scale.

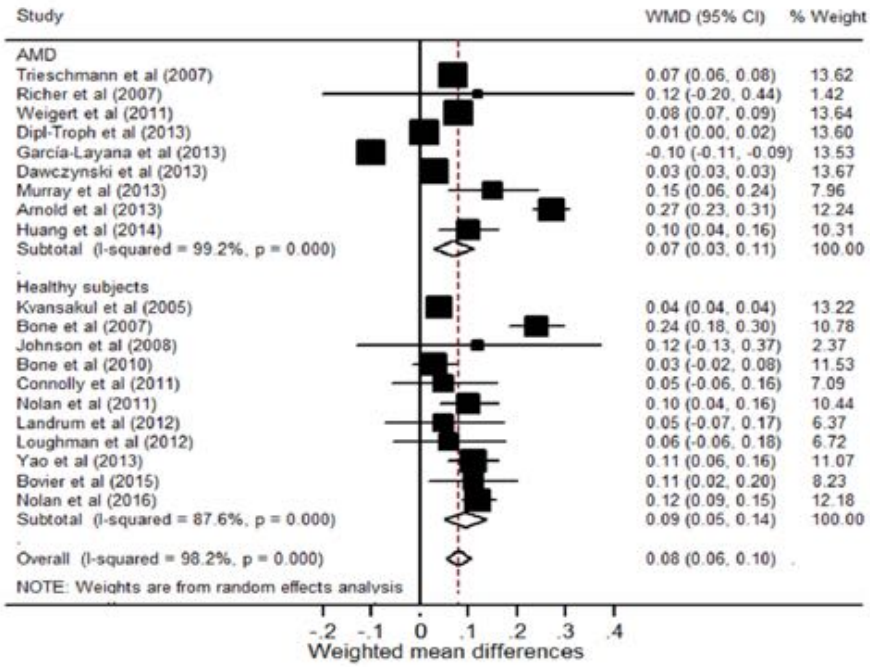

Figure 1. Forest plot showing the efficacy of lutein, zeaxanthin and meso-zeaxanthin supplementation on macular pigment optical density for patients with AMD and healthy subjects. Error bars indicate 95% CIs of the WMDs. The sizes of the squares correspond to the study weight in the random-effects meta-analysis. Diamonds represent the meta-analysis summary effect estimate. AMD, age-related macular degeneration; CI, confidence interval; WMD, weighted mean differences.

Table 2. Stratified analysis for the lutein or/and zeaxanthin or/and meso-zeaxanthin supplements effect on macular pigment optical density (MPOD) across the assessed randomized controlled trials (RCTs).

Subgroup	AMD Patients					Healthy Populations				
	N	WMD	95% CI	P_z	P_h	*N*	WMD	95% CI	P_z	P_h
Dose of supplement										
>10 mg	10	0.07	0.04, 0.12	<0.001	0.93	15	0.12	0.09, 0.15	<0.001	0.01
⩽10 mg	4	0.09	−0.07, 0.19	0.40		4	0.05	0.03, 0.07	0.02	
Duration of intervention										
⩾12 months	11	0.17	0.09, 0.24	<0.001	0.05	6	0.07	0.04, 0.10	<0.001	0.83
<12 months	3	0.05	0.01, 0.09	<0.001		13	0.08	0.03, 0.13	<0.001	
Mean age										
>70 years	7	0.06	0.03, 0.09	<0.001	0.85					
⩽70 years	7	0.11	0.02, 0.19	<0.001						
Zeaxanthin										
With	9	0.07	0.04, 0.11	<0.001	0.60	11	0.09	0.06, 0.13	<0.001	0.21
Without	5	0.08	0.07, 0.09	0.41		8	0.08	0.03, 0.08	0.03	
Meso-zeaxanthin										
With						4	0.13	0.05, 0.22	0.001	0.02
Without						15	0.06	0.03, 0.08	<0.001	
Other antioxidants										
With	7	0.08	0.04, 0.13	<0.001	0.97	3	0.10	0.05, 0.15	0.99	0.55
Without	7	0.08	0.04, 0.13	<0.001		16	0.07	0.05, 0.10	<0.001	
Geographic area										
Europe	9	0.08	0.04, 0.11	<0.001	0.80	8	0.06	0.03, 0.09	<0.001	0.50
Asia	3	0.10	0.05, 0.15	0.27		1	0.11	0.06, 0.16	-	
USA	2	0.12	−0.15, 0.38	0.97		10	0.09	0.02, 0.15	<0.001	
Methods										
Objective	10	0.09	0.07, 0.12	<0.001	0.37					
Psychophysical	4	0.05	−0.15, 0.24	<0.001						

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; MPOD, macular pigment optical density; P_h, P for between-study heterogeneity; P_z, P for Z test; RCTs: randomized controlled trials; WMD, weighted mean differences.

3.4. The Effect of Lutein, Zeaxanthin or/and Meso-zeaxanthin Supplementation on MPOD in Healthy Subjects

The changes in MPOD with these carotenoids supplement for healthy subjects were assessed in 11 RCTs (Figure 1). When all these studies were pooled into the meta-analysis, the intervention group evidently exhibited an augmentation in MPOD by 0.09 ODU compared with placebo (95% CI, 0.05 to 0.14). For subgroup analysis, trials that intervened exceeding 10 mg macular carotenoids per day produced a higher WMD of 0.12 (95% CI, 0.09 to 0.15) than a WMD of 0.05 (95% CI, 0.03 to 0.07) in trials that only supplemented with less than 10 mg (between-group difference, 0.07; $p = 0.01$). Moreover, a greater increase in MPOD was observed in trials supplemented combined with meso-zeaxanthin in comparison with those without meso-zeaxanthin (WMD, 0.13 vs. 0.07; between-group difference, 0.06; $p = 0.02$; Table 2). Additionally, participants receiving additional zeaxanthin supplement did not have a more response in MPOD compared with those who taking only lutein supplement. In the dose-response meta-analysis, each additional 1 mg of these carotenoids supplementation was associated with a 0.004 ODU increase in MPOD. The sensitivity analysis by excluding each of the studies also did not appreciably influence the pooled WMD. No publication bias was found for Begg's rank correlation test ($p = 0.54$) or Egger's linear regression test ($p = 0.05$).

3.5. The Relationship between Baseline MPOD Levels and the Change in MPOD

Correlation analysis was used to investigate the association between baseline MPOD levels and the change in MPOD during treatment (Figure 2). For healthy subjects, the changes in MPOD during supplementation were significantly related with baseline levels ($r = -0.71$, $p < 0.001$). Moreover, the increase in MPOD for AMD patients also marginally exhibited a negative correlation with baseline MPOD ($r = -0.43$, $p = 0.06$).

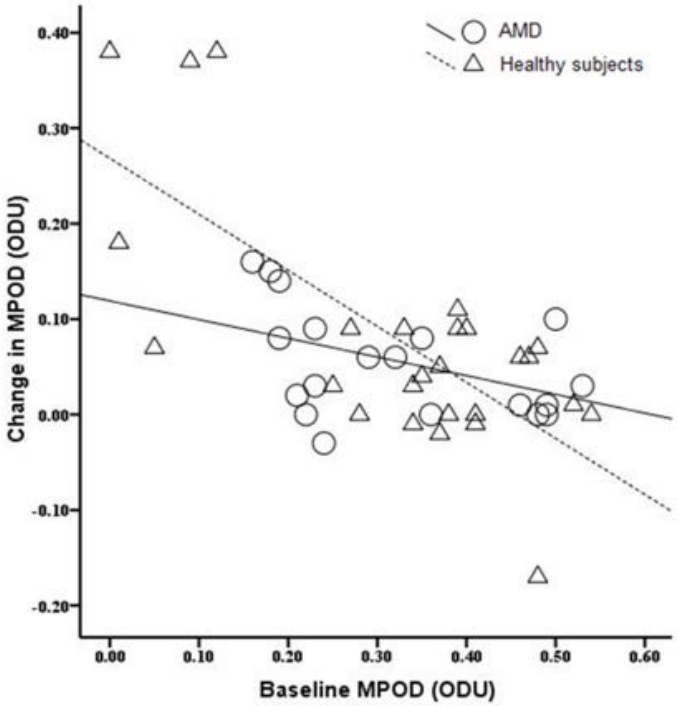

Figure 2. Scatterplot showing the relationship between baseline MPOD levels and the change in MPOD from baseline. MPOD, macular pigment optical density; ODU, optical density unit.

3.6. The Relationship between Blood Xanthophyll Carotenoids Concentration and the Change in MPOD

We subsequently evaluated the relationship between the change in serum carotenoids concentration and the change in MPOD (Figure 3). The results showed that MPOD was improved with

the postintervention increase in blood concentrations both in AMD patients (r = 0.40, p = 0.07) and the healthy populations (r = 0.33, p = 0.05).

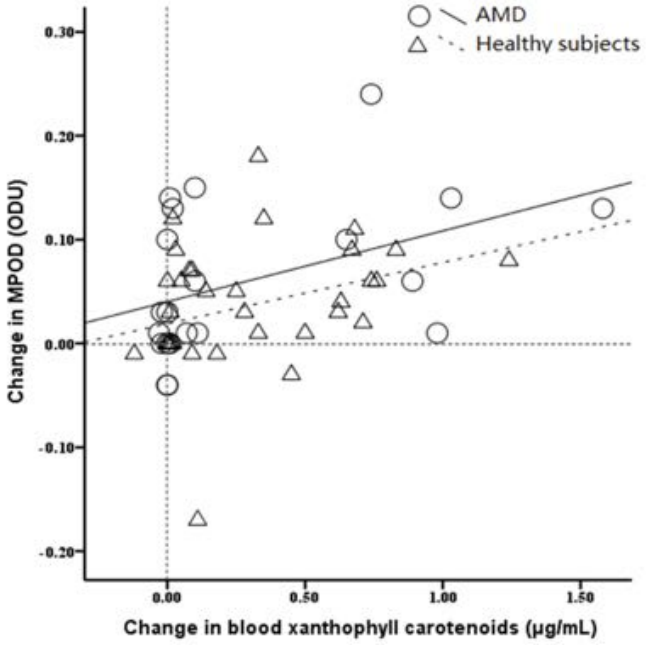

Figure 3. Scatterplot showing the relationship between blood xanthophyll carotenoids concentration and the change in MPOD during supplementation. MPOD, macular pigment optical density; ODU, optical density unit.

4. Discussion

In the current study, we evaluated the effects of lutein, zeaxanthin and meso-zeaxanthin supplementation on MOPD based on the data from the RCTs. Our results showed that the carotenoids supplementation significantly increased the level of MPOD and the inclusion of meso-zeaxanthin resulted in a greater increase in macular pigment compared to supplements lacking this central carotenoid. The increment in MPOD was positively correlated with changes in blood xanthophyll carotenoids concentration. Furthermore, supplementation with these carotenoids for longer than 12 months, a higher dose and the three carotenoids in combination were more effective on MPOD augmentation.

Previous studies have found that the decrease in MP was related with the functional abnormalities of the macula, which eventually led to some age-related degenerative eye diseases [46,47]. Neuringer et al. reported that monkeys fed with the xanthophyll-free diets were found to have no detectable MP in the retina and adipose tissue [47]. As the main constituents of the yellow pigment, lutein, zeaxanthin and meso-zeaxanthin are uniquely concentrated in the macula [12,48,49]. It is hypothesized that these carotenoids could protect the photoreceptor outer segments and the retinal pigment epithelium by screening these susceptible retinal structures from actinic blue light and quenching reactive oxygen species [50]. Barker et al. demonstrated that lutein and zeaxanthin supplementation of xanthophyll-free monkeys and the resulting accumulation of MP provided significant foveal protection against short-wavelength photochemical damage [11]. Their results were in agreement with those reported by Thomson et al., in which quails supplemented with 6-month xanthophyll carotenoids significantly decreased number of dying photoreceptors in retina [51]. Moreover, these carotenoids have also been suggested to offer protection to reduce the lipofuscin accumulation and enhance in lysosomal stability and viability [52]. Thus, lutein, zeaxanthin and meso-zeaxanthin may have a possible specific function in the maintenance of human retinal structures [7,17,48].

Some reports revealed that the donor eyes with AMD showed a drastic decline of MP levels as compared to eyes without AMD [53]. According to previous studies, a lower MPOD appeared to be associated with an increased risk of progression to AMD [54,55]. Our previous intervention study has demonstrated a significant benefit of lutein and zeaxanthin supplementation on the increase of MPOD for patients with early AMD [19]. Consistent with these findings, the results of the present study showed that supplementation with these carotenoids significantly increased the level of MPOD not only in AMD patients but also in healthy subjects. Moreover, the change in MPOD was accompanied by the improvement of these xanthophyll carotenoids statuses. These suggested that supplementation with lutein, zeaxanthin and meso-zeaxanthin lead to the improvements in MPOD as a consequence of maintaining the normal morphology of retina by elevating blood levels [54]. In addition, our results also showed that participants receiving with higher doses supplement were associated with a greater increase in MPOD, especially for the healthy subjects. Previous studies suggested that a consumption of lutein and zeaxanthin above 6-14 mg daily was considered to reduce the risk of eye diseases such as AMD as well as in alleviating the symptoms if present [56,57]. However, epidemiological studies indicated that the combined daily dietary intake of these carotenoids was only approximately 2 mg per day in western countries [58]. Therefore, the additional consumption of these carotenoids supplements should be warranted.

Although zeaxanthin is deposited throughout the human retina, it is preferentially accumulated at the fovea region of macula [59]. Such a specific distribution pattern of these carotenoids within the human macula indicated that combined zeaxanthin and lutein might result in greater improvements in MPOD than lutein alone; however, absence of significantly greater response was noted with combination treatment in the present study. This finding may be partly attributed to the fact that zeaxanthin deposition at the fovea during supplementation may be limited [60,61]. Due to the high chemical similarity of lutein and zeaxanthin, tissue-specific xanthophyll binding proteins may mediate lutein and zeaxanthin capture by competition for the same absorption mediator [61]. Once these protein receptors are saturated, they could not capture more macular xanthophylls, which may limit the amount of zeaxanthin being additionally accumulated [62]. Meanwhile, the relatively higher levels of zeaxanthin naturally present at the central fovea may also limit deposition of zeaxanthin in this area [63]. This hypothesis was also supported by our results that a significant negative association was detected between the changes in MPOD and the baseline levels. Thus, the populations with lower MP may benefit more from the additionally supplementation of xanthophyll carotenoids. Furthermore, meso-zeaxanthin is a different molecular to lutein and zeaxanthin which resides directly over the central of the macula. Although trace amount of meso-zeaxanthin existed in some kind of fish, it could not be found in raw fruits and vegetables, or detected in blood serum [64]. It has the ability to protect against chronic and cumulative eye damage through its capacity to filter the most energetic and potentially damaging wavelengths of visible light and to neutralize free radicals produced by oxidative stress [65]. It has been shown that 1:1:1 mixture of lutein, zeaxanthin and meso-zeaxanthin could quench singlet oxygen more efficiently than any of the three individually. The reason could be explained that three carotenoids may form specific aggregates, which could enhance their ability to quench singlet oxygen [7,17]. Loughman et al. reported the observed change in MPOD was not statistically significant among subjects receiving lutein and zeaxanthin supplementation for 6 months, as the supplement did not contain meso-zeaxanthin [22]. The results of this meta-analysis also indicated that having meso-zeaxanthin in the supplement offers a greater increase in MPOD than supplements lacking this carotenoid, which was in accordance with previous study. In addition, Thurnham and Xu demonstrated that meso-zeaxanthin supplementation caused no noticeable toxicological effects on rats [5,25]. Therefore, additional meso-zeaxanthin supplementation should be encouraged.

Several potential limitations should be taken into account. First, these included studies selected different methods for MPOD measurement. Although the results of the stratified analysis revealed that this factor did not significantly alter the effect of lutein, zeaxanthin or/and meso-zeaxanthin supplementation on MPOD, the potential influence from this factor could not be ruled out completely.

As the stimuli that are used for MPOD measurement, such as peak wavelength, width of the measuring and reference lights, stimulus size, varied across studies, our results might also be affected by these potential confounding factors. Second, majority of the studies intervened less than 2 years, and it is unclear whether a higher dosing strategy over time may be associated with greater benefit. Fortunately, the Central Retinal Enrichment Supplementation Trials (CREST) will illustrate the role of longer-term nutritional supplementation in maintaining the levels of xanthophyll carotenoids in blood and macula, and clarify the effects of lutein, zeaxanthin and meso-zeaxanthin on visual function in normal subjects and in subjects with early AMD [66]. Third, the relatively small sample sizes of the included RCTs in this meta-analysis would reduce the statistical power to assess the association between supplementation with the macular carotenoids and MPOD. However, all of the included studies were considered of high quality, which might enhance the reliability of results. Fourth, other variables, like glare disability and dietary supplementation with carotenoid rich foods, are not included in present study. Thus, further research is needed to study the association between different responses and dietary supplementation with carotenoids-rich foods. Finally, although no significant publication bias was detected, the potential bias could not be ruled out.

5. Conclusions

The present meta-analysis demonstrated significant benefits of lutein, zeaxanthin and meso-zeaxanthin supplementation on MPOD augmentation both in AMD patients and healthy subjects with a dose-response relationship. Moreover, such improvement was positively associated with the increase in blood xanthophyll carotenoids level. As most of the studies involved less than 12 months of follow-up, which limits the evaluation of extended effect of these carotenoids, further larger-scale and longer-term RCTs are required to examine the effects of xanthophyll carotenoids on protecting the morphological integrity of the retina and preventing the progression of AMD.

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References

1. Trevino, R.; Kynn, M.G. Macular function surveillance revisited. *Optometry* **2008**, *79*, 397–403. [CrossRef] [PubMed]
2. Koo, E.; Neuringer, M.; SanGiovanni, J.P. Macular xanthophylls lipoprotein-related genes and age-related macular degeneration. *Am. J. Clin. Nutr.* **2014**, *100*, 336S–346S. [CrossRef] [PubMed]
3. Bone, R.A.; Landrum, J.T.; Fernandez, L.; Tarsis, S.L. Analysis of the macular pigment by HPLC: Retinal distribution and age study. *Invest. Ophthalmol. Vis. Sci.* **1988**, *29*, 843–849. [PubMed]
4. Meagher, K.A.; Thurnham, D.I.; Beatty, S.; Howard, A.N.; Connolly, E.; Cummins, W.; Nolan, J.M. Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br. J. Nutr.* **2013**, *110*, 289–300. [CrossRef] [PubMed]
5. Li, B.; Ahmed, F.; Bernstein, P.S. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch. Biochem. Biophys.* **2010**, *504*, 56–60. [CrossRef] [PubMed]
6. Thurnham, D.I.; Nolan, J.M.; Howard, A.N.; Beatty, S. Macular response to supplementation with differing xanthophyll formulations in subjects with and without age-related macular degeneration. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2015**, *253*, 1231–1243. [CrossRef] [PubMed]

7. Connolly, E.E.; Beatty, S.; Thurnham, D.I.; Loughman, J.; Howard, A.N.; Stack, J.; Nolan, J.M. Augmentation of macular pigment following supplementation with all three macular carotenoids: An exploratory study. *Curr. Eye Res.* **2010**, *35*, 335–351. [CrossRef] [PubMed]
8. Bone, R.A.; Landrum, J.T.; Dixon, Z.; Chen, Y.; Llerena, C.M. Lutein and Zeaxanthin in the Eyes, Serum and Diet of Human Subjects. *Exp. Eye Res.* **2000**, *71*, 239–245. [CrossRef] [PubMed]
9. Bartlett, H.; Howells, O.; Eperjesi, F. The role of macular pigment assessment in clinical practice: A review. *Clin. Exp. Optom.* **2010**, *93*, 300–308. [CrossRef] [PubMed]
10. Sabour-Pickett, S.; Beatty, S.; Connolly, E.; Loughman, J.; Stack, J.; Howard, A.; Klein, R.; Klein, B.E.; Meuer, S.M.; Myers, C.; et al. Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina* **2014**, *34*, 1757–1766. [CrossRef] [PubMed]
11. Barker, F.M.; Snodderly, D.M.; Johnson, E.J.; Schalch, W.; Koepcke, W.; Gerss, J.; Neuringer, M. Nutritional manipulation of primate retinas, V: Effects of lutein, zeaxanthin, and *n*-3 fatty acids on retinal sensitivity to blue-light-induced damage. *Invest. Ophthalmol. Vis. Sci.* **2011**, *52*, 3934–3942. [CrossRef] [PubMed]
12. Nolan, J.M.; Stack, J.; O'Donovan, O.; Loane, E.; Beatty, S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp. Eye Res.* **2007**, *84*, 61–74. [CrossRef] [PubMed]
13. Nolan, J.M.; Akkali, M.C.; Loughman, J.; Howard, A.N.; Beatty, S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp. Eye Res.* **2012**, *101*, 9–15. [CrossRef] [PubMed]
14. Hammond, B.R.; Johnson, E.J.; Russell, R.M.; Krinsky, N.I.; Yeum, K.J.; Edwards, R.B.; Snodderly, D.M. Dietary modification of human macular pigment density. *Invest. Ophthalmol. Vis. Sci.* **1997**, *38*, 1795–1801. [PubMed]
15. Berrow, E.J.; Bartlett, H.E.; Eperjesi, F.; Gibson, J.M. The effects of a lutein-based supplement on objective and subjective measures of retinal and visual function in eyes with age-related maculopathy-a randomised controlled trial. *Br. J. Nutr.* **2013**, *109*, 2008–2014. [CrossRef] [PubMed]
16. Ho, L.; van Leeuwen, R.; Witteman, J.C.; van Duijn, C.M.; Uitterlinden, A.G.; Hofman, A.; de Jong, P.T.; Vingerling, J.R.; Klaver, C.C. Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and ω-3 fatty acids: The Rotterdam study. *Arch. Ophthalmol.* **2011**, *129*, 758–766. [CrossRef] [PubMed]
17. Connolly, E.E.; Beatty, S.; Loughman, J.; Howard, A.N.; Louw, M.S.; Nolan, J.M. Supplementation with all three macular carotenoids: Response, stability, and safety. *Invest. Ophthalmol. Vis. Sci.* **2011**, *52*, 9207–9217. [CrossRef] [PubMed]
18. Joachim, N.; Mitchell, P.; Rochtchina, E.; Tan, A.G.; Wang, J.J. Incidence and progression of reticular drusen in age-related macular degeneration: Findings from an older Australian cohort. *Ophthalmology* **2014**, *121*, 917–925. [CrossRef] [PubMed]
19. Ma, L.; Yan, S.F.; Huang, Y.M.; Lu, X.R.; Qian, F.; Pang, H.L.; Xu, X.R.; Zou, Z.Y.; Dong, P.C.; Xiao, X.; et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology* **2012**, *119*, 2290–2297. [CrossRef] [PubMed]
20. Trieschmann, M.; Beatty, S.; Nolan, J.M.; Hense, H.W.; Heimes, B.; Austermann, U.; Fobker, M.; Pauleikhoff, D. Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: The LUNA study. *Exp. Eye Res.* **2007**, *84*, 718–728. [CrossRef] [PubMed]
21. Richer, S.; Devenport, J.; Lang, J.C. LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age-related macular degeneration to dietary supplementation with xanthophylls. *Optometry* **2007**, *78*, 213–219. [CrossRef] [PubMed]
22. Loughman, J.; Nolan, J.M.; Howard, A.N.; Connolly, E.; Meagher, K.; Beatty, S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest. Ophthalmol. Vis. Sci.* **2012**, *53*, 7871–7880. [CrossRef] [PubMed]
23. Yonova-Doing, E.; Hysi, P.G.; Venturini, C.; Williams, K.M.; Nag, A.; Beatty, S.; Liew, S.H.; Gilbert, C.E.; Hammond, C.J. Candidate gene study of macular response to supplemental lutein and zeaxanthin. *Exp. Eye Res.* **2013**, *115*, 172–177. [CrossRef] [PubMed]
24. Trieschmann, M.; van Kuijk, F.J.; Alexander, R.; Hermans, P.; Luthert, P.; Bird, A.C.; Pauleikhoff, D. Macular pigment in the human retina: Histological evaluation of localization and distribution. *Eye (Lond.)* **2008**, *22*, 132–137. [CrossRef] [PubMed]

25. Thurnham, D.I.; Howard, A.N. Studies on meso-zeaxanthin for potential toxicity and mutagenicity. *Food Chem. Toxicol.* **2013**, *59*, 455–463. [CrossRef] [PubMed]
26. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; PRISMA group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Int. J. Surg.* **2010**, *8*, 336–341. [CrossRef] [PubMed]
27. Howells, O.; Eperjesi, F.; Bartlett, H. Measuring macular pigment optical density in vivo: A review of techniques. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2011**, *249*, 315–347. [CrossRef] [PubMed]
28. Egger, M.; Davey Smith, G.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **1997**, *315*, 629–634. [CrossRef] [PubMed]
29. Begg, C.B.; Mazumdar, M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* **1994**, *50*, 1088–1101. [CrossRef] [PubMed]
30. Weigert, G.; Kaya, S.; Pemp, B.; Sacu, S.; Lasta, M.; Werkmeister, R.M.; Dragostinoff, N.; Simader, C.; Garhöfer, G.; Schmidt-Erfurth, U.; et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **2011**, *52*, 8174–8178. [CrossRef] [PubMed]
31. Arnold, C.; Jentsch, S.; Dawczynski, J.; Böhm, V. Age-related macular degeneration: Effects of a short-term intervention with an oleaginous kale extract-a pilot study. *Nutrition* **2013**, *29*, 1412–1417. [CrossRef] [PubMed]
32. García-Layana, A.; Recalde, S.; Alamán, A.S.; Robredo, P.F. Effects of lutein and docosahexaenoic acid supplementation on macular pigment optical density in a randomized controlled trial. *Nutrients* **2013**, *5*, 543–551. [CrossRef] [PubMed]
33. Dawczynski, J.; Jentsch, S.; Schweitzer, D.; Hammer, M.; Lang, G.E.; Strobel, J. Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: The LUTEGA study. *Graefe's Arch. Clin. Exp. Ophthalmol.* **2013**, *251*, 2711–2723. [CrossRef] [PubMed]
34. Murray, I.J.; Makridaki, M.; van der Veen, R.L.; Carden, D.; Parry, N.R.; Berendschot, T.T. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: The CLEAR study. *Invest. Ophthalmol. Vis. Sci.* **2013**, *54*, 1781–1788. [CrossRef] [PubMed]
35. Arnold, C.; Winter, L.; Fröhlich, K.; Jentsch, S.; Dawczynski, J.; Jahreis, G.; Böhm, V. Macular xanthophylls and ω-3 long-chain polyunsaturated fatty acids in age-related macular degeneration: A randomized trial. *JAMA Ophthalmol.* **2013**, *131*, 564–572.
36. Huang, Y.M.; Dou, H.L.; Huang, F.F.; Xu, X.R.; Zou, Z.Y.; Lu, X.R.; Lin, X.M. Changes following supplementation with lutein and zeaxanthin in retinal function in eyes with early age-related macular degeneration: A randomised, double-blind, placebo-controlled trial. *Br. J. Ophthalmol.* **2015**, *99*, 371–375. [CrossRef] [PubMed]
37. Kvansakul, J.; Rodriguez-Carmona, M.; Edgar, D.F.; Barker, F.M.; Köpcke, W.; Schalch, W.; Barbur, J.L. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. *Ophthalmic. Physiol. Opt.* **2006**, *26*, 362–371. [CrossRef] [PubMed]
38. Bone, R.A.; Landrum, J.T.; Cao, Y.; Howard, A.N.; Alvarez-Calderon, F. Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutr. Metab. (Lond.)* **2007**, *4*, 12. [CrossRef] [PubMed]
39. Johnson, E.J.; Chung, H.Y.; Caldarella, S.M.; Snodderly, D.M. The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. *Am. J. Clin. Nutr.* **2008**, *87*, 1521–1529. [PubMed]
40. Bone, R.A.; Landrum, J.T. Dose-dependent response of serum lutein and macular pigment optical density to supplementation with lutein esters. *Arch. Biochem. Biophys.* **2010**, *504*, 50–55. [CrossRef] [PubMed]
41. Nolan, J.M.; Loughman, J.; Akkali, M.C.; Stack, J.; Scanlon, G.; Davison, P.; Beatty, S. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vis. Res.* **2011**, *51*, 459–469. [CrossRef] [PubMed]
42. Landrum, J.; Bone, R.; Mendez, V.; Valenciaga, A.; Babino, D. Comparison of dietary supplementation with lutein diacetate and lutein: A pilot study of the effects on serum and macular pigment. *Acta Biochim. Pol.* **2012**, *59*, 167–169. [PubMed]
43. Yao, Y.; Qiu, Q.H.; Wu, X.W.; Cai, Z.Y.; Xu, S.; Liang, X.Q. Lutein supplementation improves visual performance in Chinese drivers: 1-year randomized, double-blind, placebo-controlled study. *Nutrition* **2013**, *29*, 958–964. [CrossRef] [PubMed]

44. Bovier, E.R.; Hammond, B.R. A randomized placebo-controlled study on the effects of lutein and zeaxanthin on visual processing speed in young healthy subjects. *Arch. Biochem. Biophys.* **2015**, *572*, 54–57. [CrossRef] [PubMed]
45. Nolan, J.M.; Power, R.; Stringham, J.; Dennison, J.; Stack, J.; Kelly, D.; Moran, R.; Akuffo, K.; Corcoran, L.; Beatty, S. Enrichment of Macular Pigment Enhances Contrast Sensitivity in Subjects Free of Retinal Disease: Central Retinal Enrichment Supplementation Trials—Report 1. *Invest. Ophthalmol. Vis. Sci.* **2016**, *57*, 3429–3439. [CrossRef] [PubMed]
46. Beatty, S.; Murray, I.J.; Henson, D.B.; Carden, D.; Koh, H.H.; Boulton, M.E. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 439–446. [PubMed]
47. Neuringer, M.; Sandstrom, M.M.; Johnson, E.J.; Snodderly, D.M. Nutritional manipulation of primate retinas, I: Effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest. Ophthalmol. Vis. Sci.* **2004**, *45*, 3234–3243. [CrossRef] [PubMed]
48. Hammond, B.R.; Fletcher, L.M.; Roos, F.; Wittwer, J.; Schalch, W. A Double-Blind, Placebo-Controlled Study on the Effects of Lutein and Zeaxanthin on Photostress Recovery, Glare Disability, and Chromatic Contrast. *Invest. Ophthalmol. Vis. Sci.* **2014**, *55*, 8583–8589. [CrossRef] [PubMed]
49. Lien, E.L.; Hammond, B.R. Nutritional influences on visual development and function. *Prog. Retin. Eye Res.* **2011**, *30*, 188–203. [CrossRef] [PubMed]
50. Kumar, N.; Mrejen, S.; Fung, A.T.; Marsiglia, M.; Loh, B.K.; Spaide, R.F. Retinal pigment epithelial cell loss assessed by fundus autofluorescence imaging in neovascular age-related macular degeneration. *Ophthalmology* **2013**, *120*, 334–341. [CrossRef] [PubMed]
51. Thomson, L.R.; Toyoda, Y.; Delori, F.C.; Garnett, K.M.; Wong, Z.Y.; Nichols, C.R.; Cheng, K.M.; Craft, N.E.; Dorey, C.K. Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. *Exp. Eye Res.* **2002**, *75*, 529–542. [CrossRef] [PubMed]
52. Yu, C.C.; Nandrot, E.F.; Dun, Y.; Finnemann, S.C. Dietary antioxidants prevent age-related retinal pigment epithelium actin damage and blindness in mice lacking $\alpha v\beta 5$ integrin. *Free Radic. Biol. Med.* **2012**, *52*, 660–670. [CrossRef] [PubMed]
53. Bone, R.A.; Landrum, J.T.; Mayne, S.T.; Gomez, C.M.; Tibor, S.E.; Twaroska, E.E. Macular pigment in donor eyes with and without AMD: A case-control study. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 235–240. [PubMed]
54. Biesalski, H.K.; Aggett, P.J.; Anton, R.; Bernstein, P.S.; Blumberg, J.; Heaney, R.P.; Henry, J.; Nolan, J.M.; Richardson, D.P.; van Ommen, B.; et al. 26th Hohenheim Consensus Conference, September 11, 2010 Scientific substantiation of health claims: Evidence-based nutrition. *Nutrition* **2011**, *27*, S1–S20. [CrossRef] [PubMed]
55. Puell, M.C.; Palomo-Alvarez, C.; Barrio, A.R.; Gómez-Sanz, F.J.; Pérez-Carrasco, M.J. Relationship between macular pigment and visual acuity in eyes with early age-related macular degeneration. *Acta. Ophthalmol.* **2013**, *91*, e298–e303. [CrossRef] [PubMed]
56. Johnson, E.J.; Maras, J.E.; Rasmussen, H.M.; Tucker, K.L. Intake of lutein and zeaxanthin differ with age, sex and ethnicity. *J. Am. Diet. Assoc.* **2010**, *110*, 1357–1362. [CrossRef] [PubMed]
57. Trumbo, P.R.; Ellwood, K.C. Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: An evaluation using the Food and Drug Administration's evidence-based review system for health claims. *Am. J. Clin. Nutr.* **2006**, *84*, 971–974. [PubMed]
58. Granado, F.; Blázquez, S.; Olmedilla, B. Changes in carotenoid intake from fruit and vegetables in the Spanish population over the period 1964–2004. *Public Health Nutr.* **2007**, *10*, 1018–1023. [CrossRef] [PubMed]
59. Wisniewska, A.; Subczynski, W.K. Distribution of macular xanthophylls between domains in a model of photoreceptor outer segment membranes. *Free Radic. Biol. Med.* **2006**, *41*, 1257–1265. [CrossRef] [PubMed]
60. Vachali, P.P.; Besch, B.M.; Gonzalez-Fernandez, F.; Bernstein, P.S. Carotenoids as possible interphotoreceptor retinoid-binding protein (IRBP) ligands: A surface plasmon resonance (SPR) based study. *Arch. Biochem. Biophys.* **2013**, *539*, 181–186. [CrossRef] [PubMed]
61. Bhosale, P.; Li, B.; Sharifzadeh, M.; Gellermann, W.; Frederick, J.M.; Tsuchida, K.; Bernstein, P.S. Purification and partial characterization of a lutein-binding protein from human retina. *Biochemistry* **2009**, *48*, 4798–4807. [CrossRef] [PubMed]

62. Schalch, W.; Cohn, W.; Barker, F.M.; Köpcke, W.; Mellerio, J.; Bird, A.C.; Robson, A.G.; Fitzke, F.F.; van Kuijk, F.J. Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin-the LUXEA (LUtein Xanthophyll Eye Accumulation) study. *Arch. Biochem. Biophys.* **2007**, *458*, 128–135. [CrossRef] [PubMed]
63. Bhosale, P.; Bernstein, P.S. Vertebrate and invertebrate carotenoid-binding proteins. *Arch. Biochem. Biophys.* **2007**, *458*, 121–127. [CrossRef] [PubMed]
64. Nolan, J.M.; Beatty, S.; Meagher, K.A.; Howard, A.N.; Kelly, D.; Thurnham, D.I. Verification of Meso-Zeaxanthin in Fish. *J. Food Process Technol.* **2014**, *5*, 335. [CrossRef] [PubMed]
65. Beaty, S.; Boulton, M.; Henson, D.; Koh, H.H.; Murray, I.J. Macular pigment and age related macular degeneration. *Br. J. Ophthalmol.* **1999**, *83*, 867–877. [CrossRef]
66. Akuffo, K.O.; Beatty, S.; Stack, J.; Dennison, J.; O'Regan, S.; Meagher, K.A.; Peto, T.; Nolan, J. Central Retinal Enrichment Supplementation Trials (CREST): Design and methodology of the CREST randomized controlled trials. *Ophthalmic. Epidemiol.* **2014**, *21*, 111–123. [CrossRef] [PubMed]

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CLINICAL STUDY

Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration

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Abstract

Purpose **To compare the impact of sustained supplementation using different macular carotenoid formulations on macular pigment (MP) and visual function in early age-related macular degeneration (AMD).**

Patients and methods **Sixty-seven subjects with early AMD were randomly assigned to: Group 1 (20 mg per day lutein (L), 0.86 mg per day zeaxanthin (Z); Ultra Lutein), Group 2 (10 mg per day *meso*-zeaxanthin (MZ), 10 mg per day L, 2 mg per day Z; Macushield; Macuhealth), Group 3 (17 mg per day MZ, 3 mg per day L, 2 mg per day Z). MP was measured using customised heterochromatic flicker photometry and visual function was assessed by measuring contrast sensitivity (CS) and best-corrected visual acuity (BCVA). AMD was graded using the Wisconsin Age-Related Maculopathy Grading System (AREDS 11-step severity scale).**

Results **At 3 years, a significant increase in MP from baseline was observed in all groups at each eccentricity ($P<0.05$), except at 1.75° in Group 1 ($P=0.160$). Between 24 and 36 months, significant increases in MP at each eccentricity were seen in Group 3 ($P<0.05$ for all), and at 0.50° in Group 2 ($P<0.05$), whereas no significant increases were seen in Group 1 ($P>0.05$ for all). At 36 months, compared with baseline, the following significant improvements ($P<0.05$) in CS were observed: Group 2—1.2, 6, and 9.6 cycles per degree (c.p.d.); Group 1—15.15 c.p.d.; and Group 3—6, 9.6, and 15.15 c.p.d. No significant changes in BCVA, or progression to advanced AMD, were observed.**

Conclusion **In early AMD, MP can be augmented with a variety of supplements, although the inclusion of MZ may confer benefits in terms of panprofile augmentation and in terms of CS enhancement.**

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Introduction

Age-related macular degeneration (AMD) is characterised by a spectrum of degenerative changes at the macula, which include drusen and/or hyper-/hypopigmentary changes (known as early AMD), atrophic changes (geographic atrophy, GA, a form of advanced AMD), and choroidal neovascularisation (neovascular or 'wet AMD', another form of advanced AMD).[1]

Macular pigment (MP) is a yellow pigment located in the macular region of the human retina, and is composed of lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ).[2] MP filters short-wavelength blue light (and therefore limits photooxidative damage passively) and its constituent carotenoids act as antioxidants by neutralizing free radicals.[3,4]

In the current study, known as the Meso-zeaxanthin Ocular Supplementation Trial (MOST) AMD study, we compared the effect of sustained supplementation with some or all of MP's constituent carotenoids on visual function, and evaluated the impact of such supplementation on vision and disease progression. Observations that MZ, the dominant carotenoid in the epicentre of the MP's spatial profile, may offer advantages in terms of MP augmentation across its spatial profile[5] and

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in terms of enhancement of visual function[6] prompted this investigation. The 8-week[7] and 12-month[8] reports of the MOST AMD study have been published. In the current study, we present new data on a 3-year follow-up of subjects in the MOST AMD study. Of note, this is the first study to monitor MP, visual function, and AMD status in response to supplementation with all three macular carotenoids in patients with early AMD, over a 36-month period.

Materials and methods

The design and methodology of the MOST AMD study has been reported previously.[8] In brief, MOST AMD is a single-blind, randomised controlled clinical trial. Clinical assessments were carried out at the Institute of Eye Surgery (http://www.ioes.ie/), Waterford, Ireland. Before study enrolment, an eligibility screening visit was conducted by an ophthalmologist with a special interest in retinal disease (SB). The eligibility criteria included early AMD (one to eight on AREDS 11-step severity scale[9] in at least one eye (the study eye), confirmed by the Ocular Epidemiology Reading Center at the University of Wisconsin, Madison, WI, USA); best-corrected visual acuity (BCVA) $\geq$6/12 in the study eye; and no other ocular pathology.

Subjects were randomly assigned to one of three parallel groups: Group 1—20 mg L, 0.86 mg Z (Ultra Lutein supplied by Natural Organics, Inc., Melville, NY, USA); Group 2—10 mg MZ, 10 mg L, 2 mg Z (Macushield (Macuvision Europe Limited, Solihull, UK)/Macuhealth LMZ3 (MacuHealth LLC, Birmingham, MI, USA)); Group 3—17 mg MZ, 3 mg L, 2 mg Z (supplied by Industrial Organica, Monterrey, Mexico (not commercially available)). The above treatment groups (formulations) were selected to be comparable total concentrations of macular carotenoids (ie 22 mg). Of note, however, discrepancies between label claim and measured values of the supplements used in this trial have been reported previously, and in particular, the finding that the Group 1 supplement contained small amounts of MZ (0.30 mg).[10,11] This has implications for the findings presented below.

The supplements were prepared in a soft gel capsule. Subjects were instructed to take one capsule daily with a meal. All study supplements were indistinguishable in terms of external appearance, and were packaged in identical containers. Study visits were conducted at baseline, 12 months, 24 months, and 36 months.

Ethics

Ethics approval was granted by the Waterford Regional Hospital Ethics Committee. Written and informed consent was obtained from each subject before study enrolment. The tenets of the Declaration of Helsinki were adhered to in all study procedures.

Outcome measures

The primary outcome measure was change in MP as measured by customized heterochromatic flicker photometry (cHFP) at 36 months. Secondary outcome measures included BCVA, letter contrast sensitivity (CS), serum concentrations of macular carotenoids, and grade of AMD.

Study procedures

MP measurement MP was measured using the Macular Densitometer (Macular Metrics, Corp., Providence, RI, USA) at 0.25°, 0.5°, 1.0°, and 1.75° retinal eccentricity, with a reference point at 7°.[12]

Serum L, Z, and MZ analysis Serum L, Z, and MZ were quantified by high-performance liquid chromatography using methodology described previously.[7,13]

Visual acuity BCVA was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (LogMAR) chart (Test Chart 2000 PRO; Thomson Software Solutions, Hatfield, Hertfordshire, UK) viewed at 4 m.

Letter CS Letter CS was assessed using the LogMAR ETDRS (Test Chart 2000 PRO; Thomson Software Solutions) chart at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, and 15.15 c.p.d., respectively) viewed at 4 m.

Retinal photography and AMD grading

Following prior pupillary dilation (0.5% proxymetacaine hydrochloride, 2.5% phenylephrine hydrochloride, and 1% tropicamide), 45° stereoscopic color fundus photographs were taken in three retinal photographic fields (optic disc, macula, temporal to macula) using a Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany). Photographs were transferred to the Ocular Epidemiology Reading Center at the University of Wisconsin via an encrypted system. Photographs were graded in a masked manner using a modified version of the Wisconsin Age-Related Maculopathy Grading System[14,15] and adhered to the AREDS 11-step severity scale.[9]

Statistical analysis

One eye (the study eye) of each subject comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM, Armonk, NY, USA). To compare the effects of the three supplements (on each outcome measure, over time), we used repeated-measures analysis of variance, and contingency table analysis, as appropriate. Cognisant that this exploratory study would likely have insufficient power for such analyses, however, we did some additional analyses. In fact, and beyond the previously reported 12-month data,[8] we decided upon two strands of analysis: (a) between supplement group analysis over time: despite the small sample sizes, supplement groups were compared with each other, for changes in each outcome variable over the 3 years of the study. For interval outcome variables (MP, serum carotenoids, BCVA, CS), the method of analysis was repeated-measures analysis of variance, with time as a within-subjects factor and supplement as a between-subjects factor; we used the Greenhouse–Geisser correction for lack of sphericity. *Post hoc* analysis, with Bonferroni adjustment for multiple testing, was used where appropriate. For categorical outcome variables (AMD grade), we used contingency table analysis to compare supplements; (b) within-supplement group changes in each outcome variable, over the 3 years of the study. We used paired *t*-tests analysis here.

Tests of significance, for all *t*-test analyses, were two-tailed, and the 5% level of significance was used throughout. With the exception of *post hoc* analyses for the repeated-measures analysis of variance, we did not correct for multiple tests.

Results

Sixty-seven subjects were enrolled at baseline, with 47 subjects completing the final study visit at 36 months. Only those subjects who completed each study visit were included in analysis. Therefore, if a subject attended his/her 12- or 24-month visit, but did not complete the 36-month visit, he/she was not included in the analysis. Where a subject did complete a study visit, but where a variable was not measured or recorded, that subject was also excluded from all analyses relating to that variable. Exclusions occurred only in the MP and CS analysis because data were not available at all study visits (MP analysis: 5 subjects; CS analysis: 6 subjects). We have also included the sample size in all tables for clarity.

Baseline characteristics (eg age, gender, smoking status, education) of participants in intervention groups have been described previously, and the intervention groups were statistically comparable in terms of these variables.[8]

MP and its constituent carotenoids in serum

Macular pigment

(a) Comparing supplement groups In the repeated-measures analysis of change in MP (at 0.25°, 0.5°, 1.0°, and 1.75°), the within-subjects Time × Supplement interaction effect was not significant ($P = 0.759$, 0.726, 0.703, 0.110, respectively, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect (on MP levels) over time, at any eccentricity, does not differ significantly between supplement groups. The boxplots in Figure 1 graphically illustrate these findings.

(b) Within-supplement group analyses of MP are given in Table 1.

Serum concentrations of lutein

(a) Comparing supplement groups In the repeated-measures analysis of change in serum L, the within-subjects Time × Supplement interaction effect was significant ($P = 0.029$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect (on serum L levels) over time differs significantly between the supplements used. *Post hoc* analysis indicates that increases in serum L over time in groups 1 and 2 are comparable ($P = 1$, after Bonferroni adjustment for multiple testing), and each of these groups exhibit significantly greater increases than group 3 ($P = 0.029$ and $P = 0.004$, respectively, after Bonferroni adjustment for multiple testing). The boxplots in Figure 2a graphically illustrate these findings.

(b) Within-supplement group analyses of serum L are given in Table 2.

Serum concentrations of MZ

(a) Comparing supplement groups In the repeated-measures analysis of change in serum MZ, the within-subjects Time × Supplement interaction effect was significant ($P = 0.011$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect over time (on serum levels of MZ) differs significantly between the supplement groups. *Post hoc* analysis indicates that increases in MZ over time in Groups 2 and 3 are comparable ($P = 1$, after Bonferroni adjustment for multiple testing), and each of these groups exhibits significantly greater increases than Group 1 ($P = 0.001$ for both, after Bonferroni adjustment for multiple testing). The boxplots in Figure 2b graphically illustrate these findings.

(b) Within-supplement group analyses of serum MZ are given in Table 2.

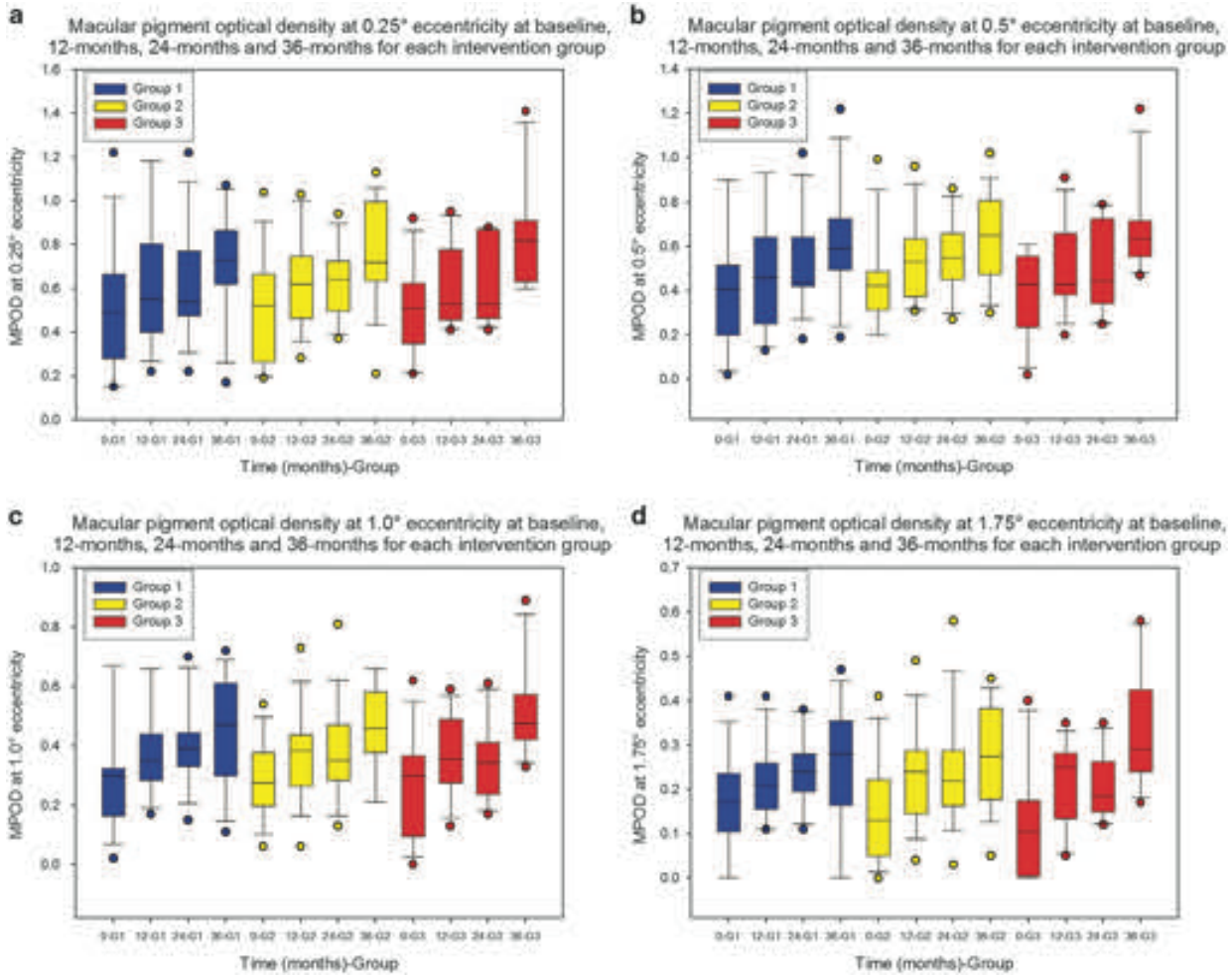

Figure 1 Macular pigment response at different retinal eccentricities over the course of the MOST AMD study. Boxplots representing macular pigment optical density at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group: Group 1—20 mg L and 0.86 mg Z; Group 2—10 mg MZ, 10 mg L, and 2 mg Z; Group 3—17 mg MZ, 3 mg L, and 2 mg Z Macular pigment was measured at 0.25° (a), 0.5° (b), 1.0° (c), and 1.75° (d) eccentricity using cHFP. 0-G1, Baseline Group 1; 12-G1, 12 months Group 1; 24-G1, 24 months Group 1; 36-G1, 36 months Group 1; 0-G2, Baseline Group 2; 12-G2, 12 months Group 2; 24-G2, 24 months Group 2; 36-G2, 36 months Group 2; 0-G3, Baseline Group 3; 12-G3, 12 months Group 3; 24-G3, 24 months Group 3; 36-G3, 36 months Group 3. MPOD, macular pigment optical density.

Serum concentrations of zeaxanthin

(a) Comparing supplement groups In the repeated-measures analysis of change in serum Z, the within-subjects Time × Supplement interaction effect was not significant ($P = 0.081$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect over time does not differ significantly between the supplements. The boxplots in Figure 2c graphically illustrate these findings.

(b) Within-supplement group analyses of serum Z are given in Table 2.

Changes in visual function

(a) Comparing supplement groups There were no significant Time × Supplement interaction effects for any vision-related outcome measures (BCVA, letter CS at any spatial frequency), indicating that the observed effects over time in terms of these variables (see below) did not differ between intervention groups.

Best-corrected visual acuity

Within-supplement group analysis There were no significant within-supplement changes in BCVA ($P > 0.05$, for all), with the exception of a statistically significant improvement in Group 3 between 12 and 24 months.

Contrast sensitivity

Within-supplement group analysis of CS are given in Table 3. At 36 months, compared with baseline, the following significant improvements ($P < 0.05$) in CS were observed: Group 2—1.2, 6, and 9.6 c.p.d.; Group 1—15.15 c.p.d.; Group 3—6, 9.6, and 15.15 c.p.d.

Table 1 Within-supplement group analysis of macular pigment by intervention groups

Intervention	*N*	*Baseline, mean ± SD*	*36 Months, mean ± SD*	*%Δ*	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	*%Δ*	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	*%Δ*	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*
MP at 0.25																	
Group 1	13	0.51 ± 0.29	0.72 ± 0.24	41	0.004	0.51 ± 0.29	0.61 ± 0.30	20	0.039	0.61 ± 0.30	0.61 ± 0.25	0	0.896	0.61 ± 0.25	0.72 ± 0.24	18	0.134
Group 2	16	0.50 ± 0.24	0.76 ± 0.23	52	0.001	0.50 ± 0.24	0.63 ± 0.21	26	0.001	0.63 ± 0.21	0.64 ± 0.17	2	0.802	0.64 ± 0.17	0.76 ± 0.23	19	0.095
Group 3	12	0.51 ± 0.20	0.85 ± 0.25	67	0.000	0.51 ± 0.20	0.62 ± 0.19	22	0.021	0.62 ± 0.19	0.62 ± 0.19	0	0.924	0.62 ± 0.19	0.85 ± 0.25	37	0.003
MP at 0.5																	
Group 1	13	0.41 ± 0.28	0.62 ± 0.26	51	0.000	0.41 ± 0.28	0.47 ± 0.27	15	0.194	0.47 ± 0.26	0.53 ± 0.21	13	0.092	0.53 ± 0.21	0.62 ± 0.26	16	0.087
Group 2	16	0.45 ± 0.21	0.64 ± 0.20	42	0.000	0.45 ± 0.21	0.54 ± 0.18	20	0.011	0.54 ± 0.18	0.55 ± 0.16	2	0.343	0.55 ± 0.16	0.64 ± 0.20	16	0.034
Group 3	12	0.39 ± 0.19	0.68 ± 0.20	74	0.000	0.39 ± 0.19	0.50 ± 0.20	22	0.016	0.50 ± 0.20	0.50 ± 0.20	0	0.879	0.50 ± 0.20	0.68 ± 0.20	36	0.011
MP at 1.0																	
Group 1	13	0.30 ± 0.19	0.45 ± 0.19	50	0.006	0.30 ± 0.19	0.38 ± 0.15	27	0.053	0.38 ± 0.15	0.40 ± 0.14	5	0.339	0.40 ± 0.14	0.45 ± 0.18	13	0.298
Group 2	16	0.29 ± 0.13	0.46 ± 0.15	59	0.000	0.29 ± 0.13	0.37 ± 0.16	28	0.010	0.37 ± 0.16	0.38 ± 0.16	3	0.730	0.38 ± 0.16	0.46 ± 0.15	21	0.071
Group 3	12	0.26 ± 0.17	0.52 ± 0.16	100	0.000	0.26 ± 0.17	0.37 ± 0.14	42	0.010	0.37 ± 0.14	0.35 ± 0.13	−6	0.473	0.35 ± 0.13	0.52 ± 0.16	49	0.011
MP at 1.75																	
Group 1	13	0.17 ± 0.11	0.23 ± 0.19	35	0.160	0.17 ± 0.11	0.22 ± 0.09	29	0.055	0.22 ± 0.09	0.24 ± 0.08	9	0.256	0.24 ± 0.08	0.23 ± 0.19	−4	0.870
Group 2	16	0.15 ± 0.12	0.28 ± 0.11	87	0.000	0.15 ± 0.12	0.24 ± 0.11	60	0.007	0.24 ± 0.11	0.24 ± 0.13	0	0.793	0.24 ± 0.13	0.28 ± 0.11	17	0.383
Group 3	12	0.12 ± 0.13	0.34 ± 0.14	183	0.000	0.12 ± 0.13	0.21 ± 0.09	75	0.006	0.21 ± 0.09	0.21 ± 0.07	0	0.899	0.21 ± 0.07	0.34 ± 0.14	62	0.003

Abbreviations: MP, macular pigment; N, number of subjects; SD, standard deviation; Sig., significance; %Δ, percentage change.
Macular pigment was measured at 0.25°, 0.5°, 1.0°, and 1.75° eccentricity using customized heterochromatic flicker photometry. Statistical significance was tested using paired *t*-test. Level of significance set at $P < 0.05$. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).
Group 1, 20 mg lutein and 0.86 mg zeaxanthin; Group 2, 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3, 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

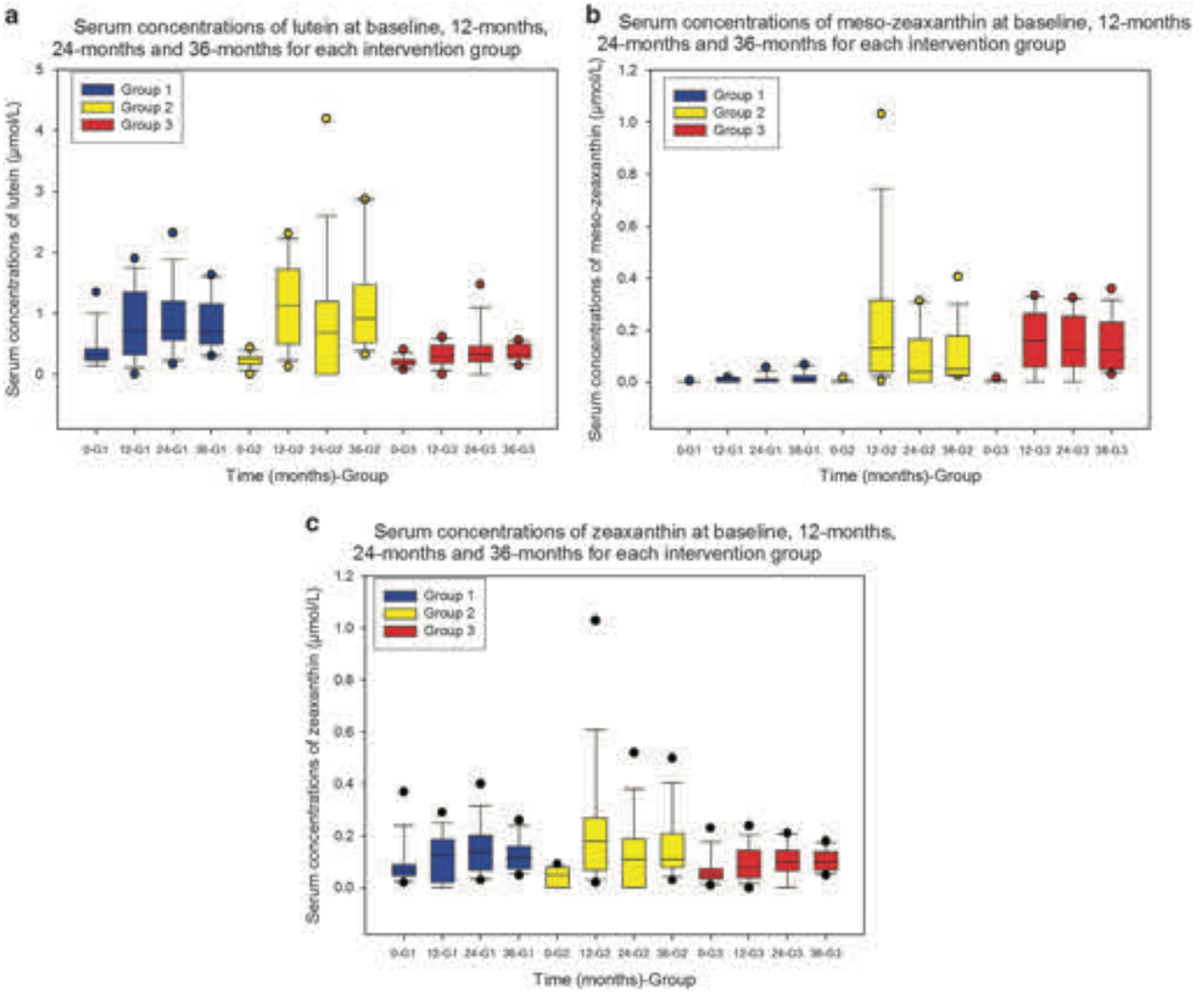

Figure 2 Serum response of L, MZ, and Z over the course of the MOST AMD study. Boxplots representing serum concentrations of L (a), MZ (b), and zeaxanthin (c) at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group: Group 1—20 mg L and 0.86 mg Z; Group 2—10 mg MZ, 10 mg L, and 2 mg Z; Group 3—17 mg MZ, 3 mg L, and 2 mg Z. Serum macular carotenoids were analysed by HPLC and expressed as µmol/L; 0-G1, Baseline Group 1; 12-G1, 12 months Group 1; 24-G1, 24 months Group 1; 36-G1, 36 months Group 1; 0-G2, Baseline Group 2; 12-G2, 12 months Group 2; 24-G2, 24 months Group 2; 36-G2, 36 months Group 2; 0-G3, Baseline Group 3; 12-G3, 12 months Group 3; 24-G3, 24 months Group 3; 36-G3, 36 months Group 3.

Changes in grade of AMD

Because of the limited number of subjects in this study, we collapsed adjacent grades of AMD, as follows: AREDS grades 1–3 (representing eyes at low risk of progression to advanced AMD), and AREDS grades 4–8 (representing eyes at high risk of progression to advanced AMD). In terms of this collapsed and simplified classification, intervention groups were statistically similar in terms of baseline findings ($P = 0.44$, χ^2 test). Using this simplified and modified system, no study eye in any intervention group progressed from low risk to high risk of progression to advanced AMD over the course of the study period, and no study eye regressed from high risk to low risk of progression to advanced AMD in any intervention group, and finally, no subject progressed to advanced AMD (AREDS grades 9–11) over the study period. Given that findings were identical for all three intervention groups, there was no need for statistical investigation of differences between intervention groups in terms of changes in risk for progression to advanced AMD.

We also investigated clinically meaningful change in AMD grade along the AREDS 11-step scale, defined as a change of at least two steps along this scale. Thus, an increase of two steps between baseline and final visit at 36 months was considered clinically meaningful disease progression and a decrease of two steps was considered a clinically meaningful disease regression. On this basis, there was no clinically meaningful change in AMD grade

Table 2 Within-supplement group analysis of serum macular carotenoids by intervention groups

Intervention	N	*Baseline, mean ± SD*	*36 Months, mean ± SD*	*%Δ*	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	*%Δ*	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	*%Δ*	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*
Lutein																	
Group 1	14	0.39 ± 0.31	0.81 ± 0.44	108	0.006	0.39 ± 0.31	0.81 ± 0.58	108	0.014	0.81 ± 0.58	0.90 ± 0.57	11	0.616	0.90 ± 0.57	0.81 ± 0.44	−10	0.412
Group 2	15	0.24 ± 0.11	1.14 ± 0.83	375	0.001	0.24 ± 0.11	1.11 ± 0.67	363	0.000	1.11 ± 0.67	0.85 ± 1.05	−23	0.336	0.85 ± 1.05	1.14 ± 0.83	34	0.250
Group 3	13	0.20 ± 0.08	0.36 ± 0.13	80	0.001	0.20 ± 0.08	0.31 ± 0.17	55	0.021	0.31 ± 0.17	0.39 ± 0.36	26	0.367	0.39 ± 0.36	0.36 ± 0.13	−8	0.694
Zeaxanthin																	
Group 1	14	0.09 ± 0.09	0.13 ± 0.06	44	0.124	0.09 ± 0.09	0.12 ± 0.09	33	0.314	0.12 ± 0.09	0.15 ± 0.09	25	0.251	0.15 ± 0.09	0.13 ± 0.06	−13	0.202
Group 2	15	0.04 ± 0.03	0.16 ± 0.12	300	0.001	0.04 ± 0.03	0.22 ± 0.24	450	0.012	0.22 ± 0.24	0.13 ± 0.14	−41	0.221	0.13 ± 0.14	0.16 ± 0.12	23	0.298
Group 3	13	0.06 ± 0.05	0.11 ± 0.04	83	0.005	0.06 ± 0.05	0.09 ± 0.06	50	0.031	0.09 ± 0.06	0.10 ± 0.07	11	0.653	0.10 ± 0.07	0.11 ± 0.04	10	0.799
Meso-zeaxanthin																	
Group 1	14	0.00 ± 0.00	0.02 ± 0.02		0.010	0.00 ± 0.00	0.01 ± 0.01		0.008	0.01 ± 0.01	0.01 ± 0.02	0	0.393	0.01 ± 0.02	0.02 ± 0.02	100	0.371
Group 2	15	0.00 ± 0.00	0.11 ± 0.11		0.001	0.00 ± 0.00	0.22 ± 0.27		0.007	0.22 ± 0.27	0.09 ± 0.11	−59	0.083	0.09 ± 0.11	0.11 ± 0.11	22	0.314
Group 3	13	0.00 ± 0.00	0.14 ± 0.10		0.000	0.00 ± 0.00	0.16 ± 0.11		0.000	0.16 ± 0.11	0.15 ± 0.11	−6	0.911	0.15 ± 0.11	0.14 ± 0.10	−7	0.743

Abbreviations: N, number of subjects; SD, standard deviation; Sig., significance; %Δ, percentage change.

Serum Lutein, Zeaxanthin and Meso-zeaxanthin were analysed by HPLC and expressed as μmol/L. Statistical significance tested using paired *t*-test. Level of significance set at $P < 0.05$. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).

Group 1, 20 mg lutein and 0.86 mg zeaxanthin; Group 2, 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3, 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

Table 3 Within-supplement group analysis of letter contrast sensitivity by intervention groups

Intervention	N	*Baseline, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	*% Δ*	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	*%Δ*	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*
Letter CS 1.2cpd																	
Group 1	12	1.87 ± 0.25	1.89 ± 0.16	1	0.817	1.87 ± 0.25	1.96 ± 0.23	5	0.222	1.96 ± 0.23	1.76 ± 0.22	−10	0.000	1.76 ± 0.22	1.89 ± 0.16	7	0.059
Group 2	15	1.71 ± 0.24	1.86 ± 0.18	9	0.012	1.71 ± 0.24	1.93 ± 0.29	13	0.004	1.93 ± 0.29	1.85 ± 0.25	−4	0.207	1.85 ± 0.25	1.86 ± 0.18	1	0.861
Group 3	13	1.75 ± 0.31	1.82 ± 0.20	4	0.432	1.75 ± 0.31	1.89 ± 0.27	8	0.069	1.89 ± 0.27	1.86 ± 0.24	−2	0.602	1.86 ± 0.24	1.82 ± 0.20	−2	0.494
Letter CS 2.4cpd																	
Group 1	12	1.76 ± 0.30	1.87 ± 0.17	6	0.227	1.76 ± 0.30	1.89 ± 0.33	7	0.077	1.89 ± 0.33	1.70 ± 0.25	−10	0.011	1.70 ± 0.25	1.87 ± 0.17	10	0.065
Group 2	15	1.68 ± 0.31	1.81 ± 0.21	8	0.087	1.68 ± 0.31	1.86 ± 0.31	11	0.000	1.86 ± 0.31	1.78 ± 0.26	−4	0.221	1.78 ± 0.26	1.81 ± 0.21	2	0.657
Group 3	13	1.63 ± 0.31	1.78 ± 0.21	9	0.083	1.63 ± 0.31	1.85 ± 0.29	13	0.005	1.85 ± 0.29	1.77 ± 0.22	−4	0.225	1.77 ± 0.22	1.78 ± 0.21	1	0.947
Letter CS 6cpd																	
Group 1	12	1.42 ± 0.30	1.60 ± 0.15	13	0.112	1.42 ± 0.30	1.49 ± 0.41	5	0.224	1.49 ± 0.41	1.39 ± 0.26	−7	0.200	1.39 ± 0.26	1.60 ± 0.15	15	0.037
Group 2	15	1.37 ± 0.24	1.52 ± 0.25	11	0.040	1.37 ± 0.24	1.44 ± 0.30	5	0.079	1.44 ± 0.30	1.39 ± 0.34	−3	0.357	1.39 ± 0.34	1.52 ± 0.25	9	0.164
Group 3	13	1.23 ± 0.44	1.52 ± 0.27	24	0.034	1.23 ± 0.44	1.55 ± 0.29	26	0.005	1.55 ± 0.29	1.48 ± 0.20	−5	0.357	1.48 ± 0.20	1.52 ± 0.27	3	0.547
Letter CS 9.6cpd																	
Group 1	12	1.14 ± 0.31	1.35 ± 0.16	18	0.043	1.14 ± 0.31	1.14 ± 0.32	0	0.959	1.14 ± 0.32	1.14 ± 0.28	0	1.000	1.14 ± 0.28	1.35 ± 0.16	18	0.031
Group 2	15	1.06 ± 0.27	1.27 ± 0.34	20	0.024	1.06 ± 0.27	1.17 ± 0.39	10	0.072	1.17 ± 0.39	1.06 ± 0.37	−9	0.115	1.06 ± 0.37	1.27 ± 0.34	20	0.025
Group 3	13	0.94 ± 0.48	1.30 ± 0.22	38	0.020	0.94 ± 0.48	1.17 ± 0.44	24	0.031	1.17 ± 0.44	1.23 ± 0.27	5	0.503	1.23 ± 0.27	1.30 ± 0.22	6	0.201
Letter CS 15.15cpd																	
Group 1	12	0.75 ± 0.32	1.02 ± 0.23	36	0.033	0.75 ± 0.32	0.83 ± 0.31	11	0.055	0.83 ± 0.31	0.79 ± 0.29	−5	0.509	0.79 ± 0.29	1.02 ± 0.23	29	0.011
Group 2	15	0.70 ± 0.37	0.91 ± 0.38	30	0.083	0.70 ± 0.37	0.78 ± 0.44	11	0.278	0.78 ± 0.44	0.60 ± 0.47	−23	0.013	0.60 ± 0.47	0.91 ± 0.38	52	0.029
Group 3	13	0.61 ± 0.48	0.97 ± 0.25	59	0.019	0.61 ± 0.48	0.81 ± 0.38	33	0.028	0.81 ± 0.38	0.93 ± 0.35	15	0.169	0.93 ± 0.35	0.97 ± 0.25	4	0.555

Abbreviations: CS, contrast sensitivity; cpd, cycles per degree; N, number of subjects; SD, standard deviation; Sig., level of significance set at $P<0.05$; %Δ, percentage change.

Letter contrast sensitivity was assessed using Thompson Test Chart PRO and recorded in the logarithm of contrast sensitivity (Log CS) units. Statistical significance was tested using paired *t*-test. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).

Group 1: 20 mg lutein and 0.86 mg zeaxanthin; Group 2: 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3: 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

in 43 (93%) study eyes, whereas 3 (7%) study eyes (one subject in Group 1 and two subjects in Group 3) exhibited a clinically meaningful progression along the AREDS 11-step scale, and these observed changes were not statistically different between intervention groups ($P = 0.29$, Fisher's exact test).

Discussion

The present study reports on the impact of sustained supplementation with different carotenoid formulations on serum concentrations of MP's constituent carotenoids, MP, visual function (BCVA and letter CS), and disease progression in subjects with early AMD.

The strengths of this study include: (1) it is a randomized clinical trial comparing three different formulations containing some or all of MP's constituent carotenoids, with a follow-up of 3 years; (2) MP was measured using a validated technique at regular intervals throughout the study period; (3) assessment of visual function was not restricted to BCVA, and included CS; (4) assessment of AMD morphology was performed by an accredited reading centre in a masked manner.

Serum response to supplementation reflected the carotenoid content of the supplement used. For example, serum L exhibited an increase in all three supplementation groups, but to a greater extent in Groups 1 and 2, where intake of L was at least three times the typical dietary intake of this carotenoid.[16,17] Similarly, a significant rise in serum Z was noted following supplementation, but that was comparable across supplement groups, reflecting similar concentrations of this carotenoid in each of the three formulations tested. Finally, serum MZ response is noteworthy for several reasons. First, MZ was detected in the serum of patients supplemented with a formulation with no declared MZ content. However, we have shown that MZ is indeed present in commercially available formulations containing L, including Ultra Lutein, the Group 1 supplement used in this study.[10] Finally, it is also worth noting that serum L and serum Z responses were unaffected by the presence of substantial concentrations of MZ (10 mg or more) in the formulation used, thereby allaying previously expressed concerns that the inclusion of MZ in a supplement may adversely impact upon the circulating bioavailability of the other two macular carotenoids.

MP increased significantly in all groups at each eccentricity (with the exception of Group 1 at 1.75°) at 3 years. It is surprising to see that MP did not increase at 1.75° in Group 1, given that L is the dominant carotenoid at this locus, and this seemingly counterintuitive observation might be because subjects in Group 1 were bioconverting L to MZ at the macula.[18,19] Consistent with this hypothesis, only groups that received supplemental MZ exhibited significant augmentation of MP across the spatial profile of this pigment.

In terms of MP increase over the course of the study, it was observed that MP continues to increase further and significantly in the third year of supplementation (but only in groups supplemented with meaningful concentrations of MZ) following a relative plateau in the second year of supplementation. Indeed, MP did not increase significantly between 12 and 24 months in any intervention group, at any eccentricity. Although the exact mechanism of macular carotenoid uptake has not been fully elucidated, it is plausible that there are several mediators (eg binding proteins, enzymes) that influence the capture, accumulation, and stabilisation of these carotenoids at the macula,[20] but further research is needed to understand these mechanisms.

There was no significant change in BCVA over the course of the present study, other than a transient improvement between 12 and 24 months in Group 3. Murray *et al*[21] reported the impact of supplemental L on MP and visual acuity in patients with early AMD in a randomised, double-blind, placebo-controlled, multicentre 12-month trial. At the end of their study, there was no change in BCVA in the L group, whereas BCVA in the placebo group had deteriorated significantly.[21] In the present study, there was a nonsignificant increase in BCVA in all intervention groups, consistent with the view that BCVA stabilised over the 3-year period of the study in this cohort of patients with early AMD. The CARMA trial, a randomised controlled trial of L, Z, and coantioxidants *vs* placebo, reported no significant change in BCVA at 1 year, although there was a demonstrable benefit in terms of differential BCVA between intervention and placebo groups at 3 years.[22,23] Of note, visual acuity, which is a measure of the spatial resolving power of the visual system and remains the most commonly used measure of vision in clinical practice,[24] is probably not sensitive enough to detect subtle but important changes in visual function experienced when monitoring subjects with early AMD.[25]

CS measures the threshold between visible and invisible at a given spatial frequency, and could be loosely described as 'faintness appreciation'[26] and is a better tool than BCVA for assessing visual function in early AMD.[25] In Group 2 (a supplement with a formulation containing all three of MP's constituent carotenoids), there was a statistically significant improvement in CS at the lowest spatial frequency (2.4 c.p.d.), whereas this was not observed for Groups 1 and 3. At the highest spatial frequency (15.15 c.p.d.), letter CS improved in Groups 1 and 3 at 36 months, but not in Group 2. At intermediate spatial frequencies (6 and 9.6 c.p.d.), however, only supplementation with formulations containing

appreciable amounts of MZ (Groups 2 and 3) resulted in a significant improvement in letter CS. Although some, but not all, previous studies have reported improvements in CS following supplementation with macular carotenoids in subjects with early AMD, our results suggest that those studies that failed to report an improvement in CS may be explained, at least in part, by a lack of MZ in the supplement formulation used.[23,27] Finally, an important and novel finding of the current study rests on the observation that further and significant improvements in CS are experienced beyond 24 months of supplementation with MP's constituent carotenoids, suggesting that sustained supplementation is indeed necessary to exert a beneficial effect on visual function.

With respect to AMD, only three study eyes exhibited clinically meaningful disease progression (1 subject from Group 1 and 2 subjects from Group 3), and no study eye progressed to advanced AMD over the 3-year study period. This study is not adequately powered or designed to make meaningful comment on AMD progression.

The current study compared the impact of supplementation with different carotenoid formulations on visual function, and our findings suggest that a formulation containing MZ yields benefits in terms of MP augmentation and in terms of CS enhancement. Further, sustained supplementation appears necessary, for at least 3 years, if MP is to be augmented maximally and CS is to be optimised over that period of time. Of note, modest visual benefits were observed in the current study. Future clinical trials should examine the impact of supplementation with formulations containing MZ and Z at similar doses. The Central Retinal Enrichment Supplementation Trial (CREST), currently underway, will also add to our understanding of the role of the macular carotenoids, including MZ, on vision in healthy and diseased eyes.[28]

Limitations of the MOST AMD study include its small numbers and the fact that it is a single blind clinical trial with no placebo arm. With respect to the use of placebo in the current study, we believe that the findings arising from the secondary analysis of the AREDS2 may render the use of placebo in patients with early (including intermediate) AMD ethically questionable.[29,30] Of note, the term early AMD in this study includes patients with intermediate AMD (as defined by AREDS). However, the absence of placebo may render it difficult to demonstrate clinical efficacy of the different carotenoid formulations used in this study and our results should be interpreted with full appreciation of this limitation. We used the single-blind design because the current study was the first clinical trial to compare the impact of supplementation with three different carotenoid formulations (including MZ) on visual function in subjects with early AMD and therefore we wanted to monitor more closely the effects of the three carotenoid formulations in terms of response among these subjects. Statistically, this exploratory study was underpowered for a direct comparison of the three supplements. Differences in effects between supplements were, in general, likely to be small, meaning that impractically large numbers of subjects would have been required to obtain statistically significant results.

In conclusion, we report that the inclusion of MZ in a supplement formulation seems to confer benefits in terms of MP augmentation and in terms of enhanced CS in subjects with early AMD. An important and novel finding rests on the observation that sustained supplementation with the macular carotenoids seems necessary to maximally augment MP and to optimise CS over a 3-year period in patients with early AMD.

Summary

What was known before

- MP augmentation can be achieved with a variety of supplements.
- The inclusion of MZ in a formulation appears to confer greater benefits in terms of visual function and augmentation of MP in subjects with early AMD at 12 months.

What this study adds

- Sustained supplementation in subjects with early AMD results in further augmentation of MP following 2 years of continuous supplementation, and confers visual benefit in these patients in terms of CS.
- The inclusion of MZ in a formulation appears to be important if increases in MP, and consequential improvements in vision, are to be maximised in subjects with early AMD receiving supplements.

Conflict of interest

JMN and SB do consultancy work for nutraceutrical companies in a personal capacity and as directors of Nutrasight Consultancy Limited. ANH is a 'honorary director' of Howard Foundation Holdings Limited and Nutriproducts Limited, which licence and supply nutraceutical ingredients. DIT is a consultant of Howard Foundation Holdings Limited. All other authors declare no conflict of interest.

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References

1 Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD *et al.* An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 1995; **39**: 367–374.
2 Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 2001; **385**: 28–40.
3 Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 1997; **38**: 1802–1811.
4 Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY, Katz NB. Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Exp Eye Res* 2001; **72**: 215–223.
5 Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res* 2012; **101**: 9–15.
6 Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci* 2012; **53**(12): 7871–7880.
7 Nolan JM, Loskutova E, Howard AN, Moran R, Mulcahy R, Stack J *et al.* Macular pigment, visual function, and macular disease among subjects with Alzheimer's disease: an exploratory study. *J Alzheimers Dis* 2014; **42**(4): 1191–1202.
8 Sabour-Pickett S, Beatty S, Connolly E, Loughman J, Stack J, Howard A *et al.* Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina* 2014; **34**(9): 1757–1766.
9 Davis MD, Gangnon RE, Lee LY, Hubbard LD, Klein BE, Klein R *et al.* The age-related eye disease study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol* 2005; **123**: 1484–1498.
10 Meagher KA, Thurnham DI, Beatty S, Howard AN, Connolly E, Cummins W *et al.* Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr* 2013; **110**: 289–300.
11 Nolan JM, Meagher K, Kashani S, Beatty S. What is meso-zeaxanthin, and where does it come from? *Eye (Lond)* 2013; **27**: 899–905.
12 Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci* 1999; **40**: 2481–2489.
13 Thurnham DI, Tremel A, Howard AN. A supplementation study in human subjects with a combination of meso-zeaxanthin, (3*R*,3'*R*)-zeaxanthin and (3*R*,3'*R*,6'*R*)-lutein. *Br J Nutr* 2008; **100**: 1307–1314.
14 Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology* 1991; **98**: 1128–1134.
15 Sparrow JM, Dickinson AJ, Duke AM. The Wisconsin Age-related Macular Degeneration grading system: performance in an independent centre. *Ophthalmic Epidemiol* 1997; **4**: 49–55.
16 O'Neill ME, Carroll Y, Corridan B, Olmedilla B, Granado F, Blanco I *et al.* A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *Br J Nutr* 2001; **85**: 499–507.
17 O'Connell ED, Nolan JM, Stack J, Greenberg D, Kyle J, Maddock L *et al.* Diet and risk factors for age-related maculopathy. *Am J Clin Nutr* 2008; **87**: 712–722.
18 Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci* 2005; **46**: 692–702.
19 Bhosale P, Zhao dY, Serban B, Bernstein PS. Identification of 3-methoxyzeaxanthin as a novel age-related carotenoid metabolite in the human macula. *Invest Ophthalmol Vis Sci* 2007; **48**: 1435–1440.
20 Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S. Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration. *Surv Ophthalmol* 2008; **53**: 68–81.
21 Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. *Invest Ophthalmol Vis Sci* 2013; **54**: 1781–1788.
22 Beatty S, Nolan JM, Muldrew KA, Woodside J, Stevenson MR, Chakravarthy U. Visual outcome after antioxidant supplementation. *Ophthalmology* 2013; **120**: 645.
23 Beatty S, Chakravarthy U, Nolan JM, Muldrew KA, Woodside JV, Denny F *et al.* Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology* 2013; **120**: 600–606.
24 Charalampidou S, Loughman J, Nolan J, Stack J, Cassidy L, Pesudovs K *et al.* Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol* 2011; **129**(9): 1155–1161.
25 Kleiner RC, Enger C, Alexander MF, Fine SL. Contrast sensitivity in age-related macular degeneration. *Arch Ophthalmol* 1988; **106**: 55–57.
26 Pelli DG, Bex P. Measuring contrast sensitivity. *Vision Res* 2013; **90**: 10–14.
27 Garcia-Layana A, Recalde S, Alaman AS, Robredo PF. Effects of lutein and docosahexaenoic acid supplementation on macular pigment optical density in a randomized controlled trial. *Nutrients* 2013; **5**: 543–551.
28 Akuffo KO, Beatty S, Stack J, Dennison J, O'Regan S, Meagher KA *et al.* Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology of the CREST Randomized Controlled Trials. *Ophthalmic Epidemiol* 2014; **21**: 111–123.
29 Age Related Eye Disease Study 2 Research Group. Lutein +zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 2013; **309**: 2005–2015.
30 Chew EY, Clemons TE, SanGiovanni JP, Danis RP, Ferris FL III, Elman MJ *et al.* Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 Report No. 3. *JAMA Ophthalmol* 2014; **132**(2): 142–149.

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Clinical Trials

Dietary Intervention With a Targeted Micronutrient Formulation Reduces the Visual Discomfort Associated With Vitreous Degeneration

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Purpose: To investigate the impact of supplementation with a targeted micronutrient formulation on the visual discomfort associated with vitreous degeneration.

Methods: In this clinical trial, 61 patients with symptomatic vitreous floaters were randomized to consume daily, the active supplement consisting of 125 mg L-lysine, 40 mg vitamin C, 26.3 mg *Vitis vinifera* extract, 5 mg zinc, and 100 mg *Citrus aurantium* or placebo for 6 months. Change in visual discomfort from floaters, assessed with the Floater Disturbance Questionnaire, was the primary outcome measure. Secondary outcome measures included best-corrected visual acuity, letter contrast sensitivity, photopic functional contrast sensitivity with positive and negative contrast polarity, and quantitative vitreous opacity areas.

Results: After supplementation, the active group reported a significant decrease in their visual discomfort from floaters ($P < 0.001$), whereas the placebo group had no significant change in their visual discomfort ($P = 0.416$). At 6 months, there was a significant decrease in vitreous opacity areas in the active group ($P < 0.001$) and an insignificant increase in vitreous opacity areas in the placebo group ($P = 0.081$). Also, there was a significant improvement in photopic functional contrast sensitivity with positive contrast polarity in the active group after supplementation ($P = 0.047$).

Conclusions: The findings of this study indicate improvements in vision-related quality of life and visual function of patients suffering from vitreous floaters after supplementation with a formulation of antioxidative and antiglycation micronutrients. Notably, these improvements were confirmed by the decrease in vitreous opacity areas in the active group.

Translational Relevance: This targeted dietary intervention should be considered to support patients with symptomatic vitreous degeneration.

Read the full study

https://tvst.arvojournals.org/article.aspx?articleid=2777982

SUMMARY OF THE **FLOATER INTERVENTION STUDY** (FLIES)

Gold Standard of Evidence: Randomized, Double-Blind, placebo-Controlled Study

DESIGN:

- 61 patients randomized to receive micronutrient formulation or placebo for 6 months
- Primary outcome: change in visual discomfort from floaters, assessed via questionnaire
- Secondary outcomes: BCVA, Letter Contrast Sensitivity, Photopic Contrast Sensitivity and Size of Vitreous Opacity Area

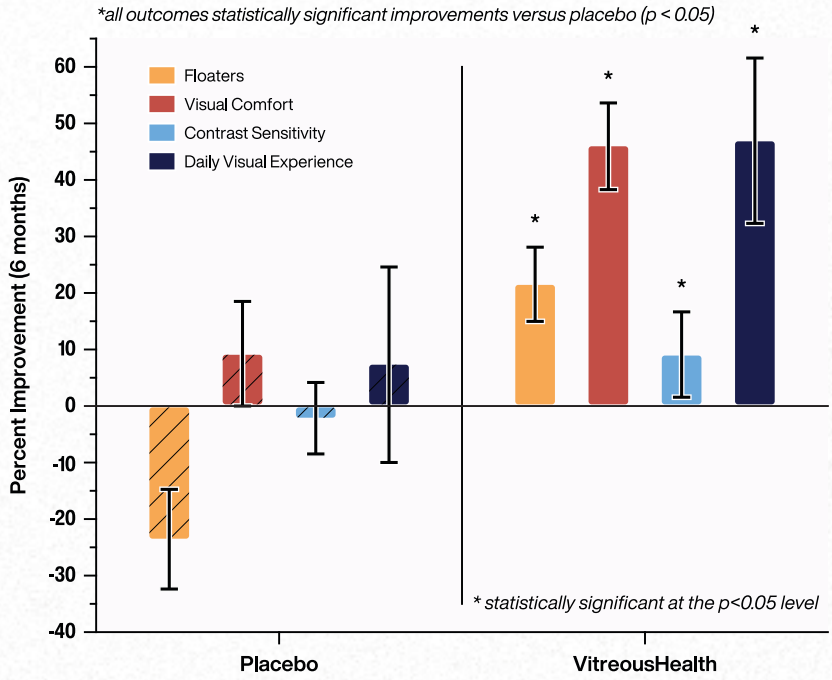
RESULTS:

Participants in the micronutrient formulation group experienced*:

- 46% decrease in visual discomfort from floaters
- 47% improvement in daily visual experience
- 22% reduction in floater area
- 9% improvement in contrast sensitivity

67% OF PARTICIPANTS IN THE MICRONUTRIENT FORMULATION GROUP REPORTED IMPROVEMENT IN VISUAL DISCOMFORT FROM FLOATERS

VitreousHealth vs. Placebo



Another advancement in eye supplementation from **MacuHealth,** the brand eye care professionals have trusted for over a decade.

References: 1. Ankamah et al. 2021. doi.org/10.1167/tvst.10.12.19

Learn more about vitreous floaters at **MacuHealth.com**

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PRODUCT GUIDE

Providing industry-leading, science-certified eye care supplements for every stage of life

Introducing

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FOREWORD BY JIM STRINGHAM, PhD.

Chief Scientific Officer, MacuHealth

Science is the pursuit of truth. As the Chief Scientific Officer for MacuHealth, I help to ensure that this truth forms the foundation of everything we do. Our products are independently tested and verified to be effective in dozens of studies and clinical trials worldwide. We take a great deal of pride knowing that our products are helping people and improving their lives. And we're by no means done. As science evolves, so do we.

I am trained as a vision scientist, and vision plays a vital role in the quality of life. Indeed, of all the senses, eyesight is perhaps the most important; even a single glance reveals the richness of the world and brings significant meaning to life.

Our mission at **MacuHealth** is to create products that resonate with this notion: that improving visual health and performance leads to a significantly enhanced quality of life. It's consistent, of course, with the primary goal of eye care providers — to provide the best care for your patients so that they may experience all the benefits of good vision. Working together, we can achieve these goals.

Sincerely,

Behind The Science

MacuHealth provides science-certified eye care supplements for patients at every stage of life. With a focus on innovation, our company redefined industry standards with our Triple Carotenoid Formula, which contains a patented 10:10:2 mg ratio of Lutein, Meso-Zeaxanthin and Zeaxanthin.

Our products have been independently researched for over a decade and are third-party tested and verified for safety, potency, stability, integrity and bioavailability - also backed by NSF and Informed Sport to be free from banned substances.

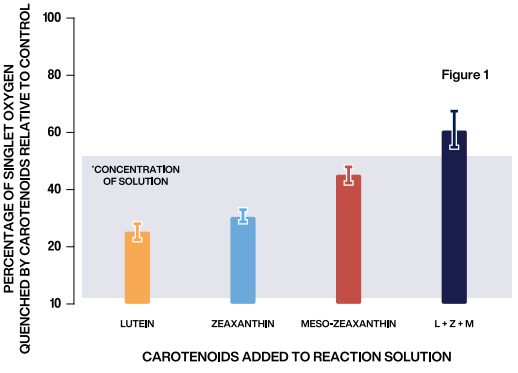
We manufacture our softgels with natural ingredients extracted from marigold flowers grown in Mexico to provide superior product efficacy and protection from degradation, all with no side effects or contraindications.

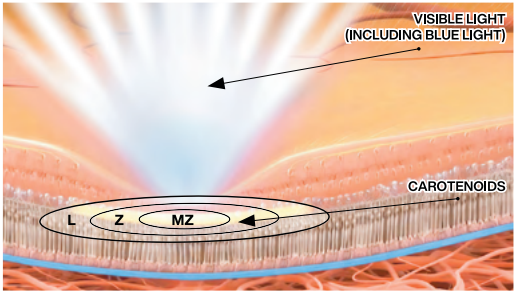
Why Meso-Zeaxanthin?

Meso-Zeaxanthin is concentrated in the central peak of the macular pigment density distribution. It has the highest antioxidant capacity of the three macular carotenoids and clinical studies have shown that supplementing with Meso-Zeaxanthin, in addition to Lutein and Zeaxanthin, provides far superior results than using Lutein and Zeaxanthin alone.[1]

What is Micro-Micelle?

Micro-Micelle™ Technology is an exclusive process that enhances the bioavailability of MacuHealth's patented Triple Carotenoid Formula. Lutein, Meso-Zeaxanthin and Zeaxanthin, in their pure, natural form, tend to crystallize, making the carotenoids difficult for the body to absorb.





View of the macular pigment and respective areas where the three carotenoids help rebuild macular pigment.

By utilizing the micro-micelle formulation, the carotenoids retain their solubility and stability, which optimizes the potential for visual health and performance improvements.

Studies show that when compared to standard macular carotenoid supplements, MacuHealth's micro-micelle formulation produced, on average, a 6x higher serum response and 1.5x retinal response over a six-month supplementation period.[2]

In short, Micro-Micelle™ Technology delivers maximum value and all the benefits that come with it.

1. Li et al. 2010. doi:10.1016/j.abb.2010.07.024
2 Green Gomez et al 2020. Doi:10.3390/antiox9080767

MacuHealth MSRP: 79.99

Scientifically formulated and clinically proven to rebuild the macular pigment, MacuHealth protects eyes from harmful free radicals and oxidative stress with three essential and powerful antioxidants called carotenoids — Lutein, Meso-Zeaxanthin and Zeaxanthin. Our patented formula is for anyone who wants to maximize visual performance or manage the symptoms of age-related macular degeneration (AMD).

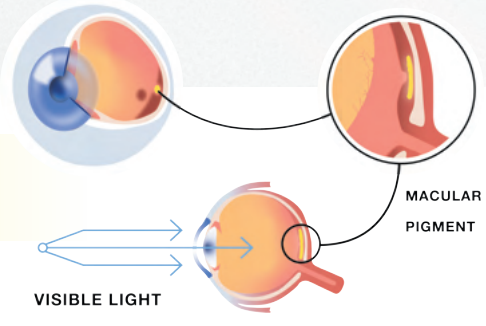
CLINICAL STUDY RESULTS:

- 85% of patients experienced improved contrast sensitivity in 12 months[1]
- 83% of those with early AMD symptoms experienced improved contrast sensitivity after 24 months[2]
- 90% of patients experienced improved glare tolerance after 24 months[2]
- 93% of patients experienced improved vision in low-light conditions[2]

TARGET PATIENTS:

- Complaints of glare (e.g. oncoming headlights at night)
- Pre-and post-cataract surgery
- At risk of or diagnosed with AMD
- General visual complaints despite good refraction

In clinical studies, 100% of participants using our **Triple Carotenoid Formula** experienced improvements in macular pigment optical density. [1,2]



IMPROVE YOUR VISION

Simulation of how MacuHealth helps to improve real-world vision by reducing glare.

SUPPLEMENT FACTS

Serving Size: 1 softgel, Servings Per Container: 90

Amount per serving		% DV
Lutein (L)	10 mg	**
Meso-Zeaxanthin (MZ)	10 mg	**
Zeaxanthin (Z)	2 mg	**

** % Daily Value (DV) not established

Other Ingredients: Sunflower Oil, Gelatin Capsule (Gelatin, Glycerin, Purified Water, Annatto), Marigold Flower Extract, Yellow Beeswax, Tween 80, Soy Lecithin, Ascorbyl Palmitate, d-alpha Tocopherol Acetate). **Contains Soy.**

Directions: Take 1 softgel daily, preferably with a meal.

1. Nolan et al. 2016. doi:10.1167/iovs. 16-19520
2. Akuffo et al. 2017. doi:10.1167/iovs. 16-21192


CLINICALLY TESTED & GLUTEN FREE
MacuHealth
Now with Micro-Micelle™ Technology
Triple Carotenoid Formula
CREATED | TESTED | VALIDATED BY SCIENCE
MACULAR HEALTH
CONTRAST SENSITIVITY
VISUAL PERFORMANCE
90 SOFTGELS | DIETARY SUPPLEMENT

MacuHealth Plus+ MSRP: 91.99

MacuHealth Plus+ is formulated for those already diagnosed with age-related macular degeneration (AMD). It contains the same ingredients as AREDS2® formulas with two important distinctions.

1. **Fortified with 10 mg of Meso-Zeaxanthin**
   Clinical studies show that Meso-Zeaxanthin offers superior eye protection and effectively manages AMD symptoms.

2. **Lower, Safer Levels of Zinc**
   MacuHealth Plus+ contains 25 mg of zinc, which is under the National Institutes of Health's daily tolerable limit of 40 mg and consistent with providing patients the maximum benefit in reducing the progression of AMD.

a. The AREDS2 study published in 2013 found that a formulation with 25 mg of zinc provided the same protection against developing advanced AMD as one with 80 mg of zinc.

MacuHealth Plus+ is AREDS2 but better.

Figure 1: AMD vision is simulated next to normal vision. MacuHealth Plus+ may reduce risk of AMD progression.

Figure 2: A woman covering her eye focuses on an Amsler grid for symptoms of AMD.

NORMAL VISION **AMD**

Figure 1

Figure 2

SUPPLEMENT FACTS

Serving Size: 4 softgels, Servings Per Container: 90

Amount per serving			% DV
Calories		15	
Total Fat		1 g	1%**
Vitamin C	(as Ascorbic Acid)	500 mg	556%
Vitamin E	(as d-alpha Tocopherol)	268 mg	1787%
Zinc	(as Zinc Oxide)	25 mg	227%
Copper	(as Cupric Oxide)	1.2 mg	133%
Lutein (L)		10 mg	†
Meso-Zeaxanthin (MZ)		10 mg	†
Zeaxanthin (Z)		2 mg	†

** Percent Daily Values (DV) are established on a 2,000 calorie diet
† Daily Value (DV) not established

Other Ingredients: Sunflower Oil, Gelatin Capsule (Gelatin, Glycerin, Purified Water, Caramel Color), Marigold Flower Extract, Yellow Beeswax, Tween 80, Soy Lecithin, Ascorbyl Palmitate, d-alpha Tocopherol Acetate. **Contains Soy.**

Directions: Take 4 softgels daily, preferably with a meal.


CLINICALLY TESTED & GLUTEN FREE
MacuHealth PLUS+
Now with Micro-Micelle™ Technology
Triple Carotenoid Formula
CREATED | TESTED | VALIDATED BY SCIENCE
BASED ON THE AREDS2 STUDY*
FORTIFIED WITH MESO-ZEAXANTHIN*
CONTAINS 25 MG OF ZINC*
Formulated for those diagnosed with Age-Related Macular Degeneration*
360 SOFTGELS | DIETARY SUPPLEMENT

VitreousHealth MSRP: 64.95

In a recent survey, 76% of respondents reported seeing floaters.[2] Now there's VitreousHealth, the first and only scientifically proven supplement for eye floaters.

Based on the double-blind, placebo-controlled Floater Intervention Study (FLIES)[1], this patented blend of antioxidants gives the eye what it needs to reduce the severity of floaters.

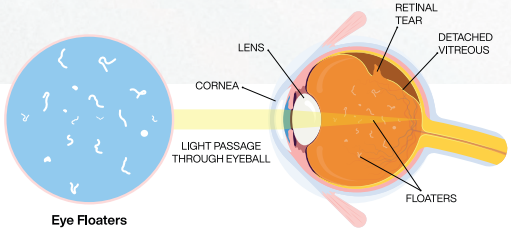
CLINICAL STUDY RESULTS:

67% of patients recognized an improvement in their symptoms within six months. The formulation is proven to deliver:

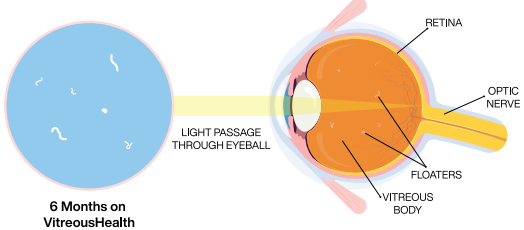
- **46%** decrease in visual discomfort from floaters
- **47%** improvement in daily visual experience
- **9%** improvement in contrast sensitivity

FOR PATIENTS WITH:

- Floaters
  (all stages of Vitreous Degeneration)
- Pre-and post-surgery
  (Vitrectomy, Laser Vitreolysis, Cataracts)



Eye Floaters



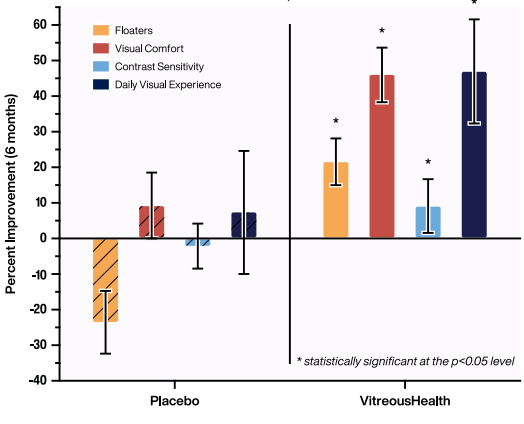
6 Months on VitreousHealth

VISUAL OPACITY TESTING

Patient on VitreousHealth:
Vitreous opacity **decreased by 70%** with supplementation
(Average decrease of 22%)

A PROVEN FORMULA

VitreousHealth vs. Placebo Group



SUPPLEMENT FACTS

Serving Size: 1 capsule

Amount per serving		% DV
Zinc	5 mg	45%
Vitamin C	40 mg	45%
Grape Seed Extract	26.3 mg	**
of which Proanthocyanidins	25 mg	**
Citrus Fruit Extract	100 mg	**
of which Bioflavonoids as Hesperidin	60 mg	**
L-lysine	125 mg	**

** % Daily Value (DV) not established

Other Ingredients: HPMC, MCC, Silica, Magnesium Stearate

Directions: Take 1 capsule daily, preferably with a meal.

1. Ankamah et al. 2021. doi.org/10.1167/tvst.10.12.19
2. Webb, Blake F et al. "Prevalence of vitreous floaters in a community sample of smartphone users." International journal of ophthalmology vol. 6,3 402-5. 18 Jun. 2013, doi:10.3980/j.issn.2222-3959.2013.03.27

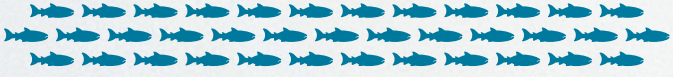

MacuHealth
VitreousHealth
Eye Floaters Formula
CREATED | TESTED | VALIDATED BY SCIENCE
MANAGE EYE FLOATERS*
REDUCE VISUAL DISCOMFORT*
ALL-NATURAL INGREDIENTS
90 CAPSULES | DIETARY SUPPLEMENT

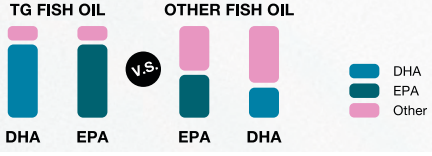
TG Omega-3 Fish Oil MSRP: 44.95

MacuHealth presents TG Omega-3, the best re-esterified triglyceride fish oil on the market today. With high levels of EPA and DHA, TG Omega-3 offers unparalleled benefits to the eye, heart, immune system and brain.

- rTG formula for increased stability, bioavailability and significantly better absorption[1]
- 2,450 mg total omega-3s with high EPA and DHA
- Distilled up to 5 times to eliminate toxins and fishy taste
- Customized dosing options for patient-specific needs

Taking 3 softgels of TG Omega-3s each day for a week is equivalent to eating 37 cans of tuna without elevating mercury levels.





CUSTOMIZED DOSING OPTIONS:

Patient	Age	Dosage
Dry Eyes		4 per day
Male	14 & Over	3 per day
Female	14 & Over	2 per day
Children	9–13	2 per day
Pre/Postnatal		3 per day

TG Omega-3: High purity and high concentrations of EPA and DHA ensure ocular benefits.

Friend of the Sea is the only sustainable fisheries certification process recognized and supervised globally by a National Accreditation Body.

SCAN FOR VIDEO
To learn about the small catch, cold open water fish sourced from Peru for TG Omega-3

SUPPLEMENT FACTS

Serving Size: 4 softgels, Servings Per Container: 30

Amount per serving		% DV**
Calories	35	
Calories from Fat	30	
Total Fat	3.5 g	5%
Cholesterol	< 5 mg	1%
Protein	1 g	2%
Total Omega-3	2,450 mg	†
EPA	1,100 mg	†
DHA	1,100 mg	†
Other Omega-3	250 mg	†

** % Daily Value (DV) based on a 2,000 calorie diet
† Daily Value not established

Other Ingredients: Fish Oil, Gelatin, Glycerin, Purified Water, Natural Lemon Flavor, Antioxidant (Rosemary Extract, Sunflower Oil, Natural Tocopherols, Ascorbyl Palmitate).
Contains: Fish (Anchovy, Sardine, Mackerel)

Dry Eye Management: 4 softgels

Overall Health: 3 softgels | **Pre/Postnatal: 3 softgels**

1. Ahmmed et al. 2020. doi:10.1111/1541-4337.12510


MacuHealth
TG OMEGA-3
FISH OIL
PREMIUM rTG FORMULATION
EYE, HEART, BRAIN HEALTH
SCIENTIFICALLY TESTED
120 SOFTGELS | DIETARY SUPPLEMENT

Vizion Edge ECP

MSRP: 79.99

Vizion Edge ECP is for those who want to improve their athletic performance, have visually demanding careers or are concerned about the effects of harmful blue light. Now in a new 90-count bottle with an enhanced formula using Micro-Micelle™ Technology, Vizion Edge ECP protects eyes, improves visual performance and reduces eye strain and fatigue.

CLINICAL STUDY RESULTS:

Patients experienced the following after 6 months of continuous supplementation[1]:

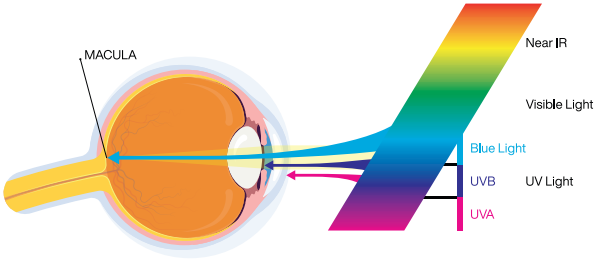
- 44% decrease in disability glare
- 34% reduction in frequency of headaches per week
- 27% reduction in eye fatigue

TARGET PATIENTS:

- Concerns about harmful blue light and excessive screen time
- Complaints of eye strain
- General visual complaints
- Athletes who want to boost visual performance

PROTECT VISION AND IMPROVE VISUAL PERFORMANCE

Prolonged blue light exposure from digital screens and the sun can cause eye strain, headaches, eye fatigue and poor sleep. Over time, the damage blue light inflicts on the retina increases and can lead to diseases such as macular degeneration. Vizion Edge ECP increases macular pigment levels in the retina to promote blue light absorption and the reduction of oxidative stress, protecting the retina from harm and leading to improved visual performance, including improved contrast sensitivity and temporal visual processing speed.



Sustained exposure to blue light causes eye strain and can damage the macula, the part of your retina that processes central vision.

SUPPLEMENT FACTS

Serving Size: 1 softgel, Servings Per Container: 90

Amount per serving		% DV
Lutein (L)	10 mg	**
Meso-Zeaxanthin (MZ)	10 mg	**
Zeaxanthin (Z)	2 mg	**

** % Daily Value (DV) not established

Other Ingredients: Sunflower Oil, Gelatin Capsule (Gelatin, Glycerin, Purified Water, Annatto), Marigold Flower Extract, Yellow Beeswax, Tween 80, Soy Lecithin, Ascorbyl Palmitate, d-alpha Tocopherol Acetate). **Contains Soy.**

Directions: Take 1 softgel daily, preferably with a meal.

1. Stringham et al. 2017. doi:10.3390/foods6070047


VE
VIZION EDGE
SPECIALLY FORMULATED TO
PROTECT AGAINST BLUE LIGHT +
IMPROVE VISUAL PERFORMANCE
90 SOFTGELS | DIETARY SUPPLEMENT

What It Means to Become a MacuHealth Provider

You'll be a provider of a safe, scientifically proven solution for those suffering from symptoms related to floaters, dry eye or age-related macular degeneration. MacuHealth has several supplements that nurture the entire eye and offers three solutions to get our products to your patients.

In-Practice Retail

Have MacuHealth products ready in your practice to serve your patient's needs directly to ensure they're adhering to their prescribed treatment. It's the most convenient method for your practice and patients to implement our supplements into your office.

Direct to Patient

Let us be your shipping department! Provide your patients with the best eye supplements available without carrying the inventory. Use our convenient online doctor portal, and we'll bill your practice and send the order directly to the patient.

Doctor Prescription Program

Provide your patients with a unique doctor's Rx code and earn a referral fee for each bottle ordered on our website or through our customer service line. They'll save 10% on their first order when using the code.

What You Receive as a MacuHealth Provider

The Tools You Need

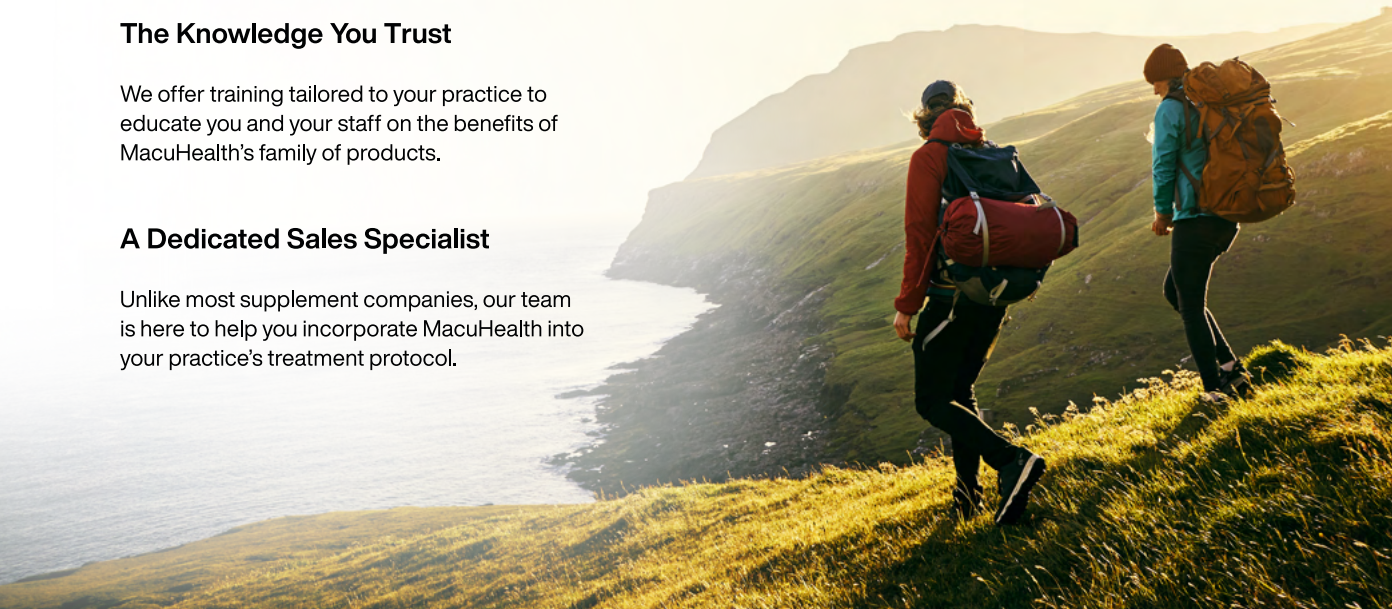
We want you to succeed, and we'll supply your office with all the brochures, flyers and digital assets available to help successfully implement MacuHealth into your practice.

The Knowledge You Trust

We offer training tailored to your practice to educate you and your staff on the benefits of MacuHealth's family of products.

A Dedicated Sales Specialist

Unlike most supplement companies, our team is here to help you incorporate MacuHealth into your practice's treatment protocol.



Here's What Doctors Are Saying

"What I do know is that I spent the first 33 years of my career watching patient after patient go down the tubes with AMD... and I've spent the last nine years joyously watching our patients' eyes outlive them."
- Dr. Gregory B. Clay, Clay-Rhynes Clinic, Durant, OK

"I have always tried to provide my patients with the best recommendations for their vision today and the future. MacuHealth is a proven nutritional supplement, and my patients appreciate that we are helping them with a product that studies have shown is best for their vision. MacuHealth has been very beneficial for our practice as well."
- Ryan Powell, OD, Vision Source Eyecare, Kansas City MO

"I love this product. I've used other macula supplements and [MacuHealth] works best...As a doctor I find it's the one that helps patients the most."
- Dr. Joan Miller

"While stressing the importance of a diet rich in colorful fruits and vegetables, I also frequently prescribe MacuHealth, explaining how this specific triple carotenoid formula, backed by the best research, optimizes macular protection and visual performance. Simply put, these products have been an outstanding success in our practice."
- Mark W. Roark, OD, FAAO Allisonville Eye Care Center, Fishers, Indiana

How MacuHealth Changes Patients' Lives

"I started with AREDS, but then I was reading a bulletin that mentioned the three important ingredients in MacuHealth and decided to buy it. After some time, [my AMD] stabilized, and I started seeing [my doctor] less often."
– Marcia

"My doctor recommended MacuHealth. She said, "Don't buy the cheap stuff either." It has improved my condition! I have had hard drusen in both eyes, and each time she exams me, there are fewer. I have done nothing other than taking MacuHealth daily."
– Sharon

"I felt worried and scared to lose my vision. I would advise taking MacuHealth and taking it early. My doctor diagnosed me early, which has been key [to my recovery]."
– Virginia

"I take MacuHealth regularly. Both my parents and maternal grandmother had AMD, so I understand the importance of eye care. It makes me appreciate my sight even more."
– Mary

Trusted and endorsed by doctors worldwide

Providing industry-leading, science-certified eye care supplements for every stage of life

SUPPLEMENTS FOR EVERY STAGE OF LIFE

MacuHealth®
LEARN MORE AT:
www.MacuHealth.com

2022 NMOA Mid Year Convention

Handouts for Saturday, October 1st Lectures

Dr. Pinakin Davey

A Guide To Glaucoma Management

Diagnostic Benefit Beyond OCT: Cone Contrast Testing

Carotenoids and its Benefits: More Than Meets the Eyes

A Guide to Glaucoma management- New and the yet to come

PINAKIN G. DAVEY OD, PHD, FAAO, FOWNS, FARVO
PROFESSOR COLLEGE OF OPTOMETRY
WESTERNU DIRECTOR OF CLINICAL RESEARCH

Western University of Health Sciences
College of Optometry

1

Disclosures

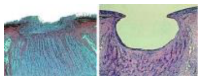
Has a relevant financial relationship with
Sanofi, ZeaVision, Guardion Health and Innova systems as a speaker or research / consultant

The content and format of this course is presented without commercial bias and does not claim superiority and commercial product or service.

2

Glaucoma an epidemic/ endemic

- Glaucoma in USA more than **3 million**;
- Ocular hypertensive 4.5% of population >40 age
- Glaucoma suspects 4 times more than glaucoma
- 64.3 million worldwide; 76 million by 2020 ; 111.8 by 2040



3

Diagnosis

- 50% undiagnosed
- Overdiagnosed in clinics
- How can it be both?

4

What is glaucoma?

- Definition:
  - "Ocular tissue damage at least partially related to intraocular pressure"

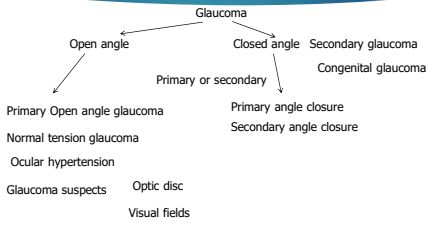
5

Definitions

- Glaucoma- A rose by any name…
- Glaucoma accelerated loss of retinal ganglion cell
- Most common optic neuropathy…but one of many
- Pallor greater than cupping- Other neuropathy
- Cupping greater than pallor- glaucoma
- No limits on the number of diseases a person can have

6

Types of glaucoma



7

Risk factors for glaucoma examined in population based studies

- Demographic
  - Age
  - Gender
  - Race
- Ocular
  - IOP
  - Optic nerve head
  - Myopia
  - Hypermetropia
- Systemic
  - Diabetes
  - Systemic hypertension
- Genetic
  - Family history
- Other
  - Cigarette smoking
  - Alcohol intake
  - Socio economic factors

8

Intraocular pressure

- Diagnosis- not helpful
- Treatment- only proven method
- Progression- very closely associated with IOP
- Risk factor- without a doubt most important risk factor
- In fact, the only alterable risk factor!

9

Intraocular pressure

- Major risk factor
  - Not as fundamental as once thought.
- Prevalence increases with increase in IOP
- Visual field loss slows down with decrease in IOP
- Even if both eyes have IOP lower than 21. The eye with greater IOP will lose field quicker.

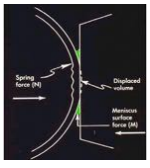
10

Corneal thickness and IOP issues

Modified Imbert-Fick law

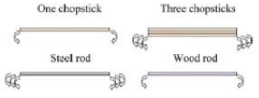
W + S = P x A +B

Where
1. S = Surface tension
2. B = Force required to bend the cornea



11

Understanding biomechanics of cornea



Liu J & Roberts CJ (2005)
Influence of corneal biomechanical properties on intraocular pressure measurement - Quantitative analysis.
J Cataract Refract Surg 31:146-155

12

Ocular Response Analyzer

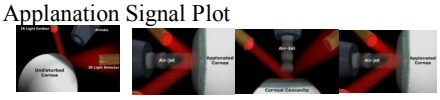
- IOPG - Goldmann Correlated IOP
- IOPCC - Corneal Compensated IOP
- CH - Corneal Hysteresis

13

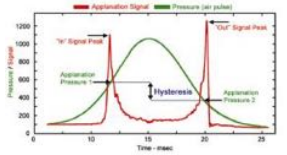
Ocular response analyzer

14

Applanation Signal Plot



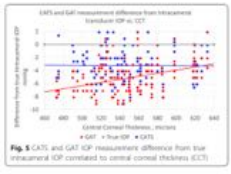
"Dynamic bi-directional applanation"

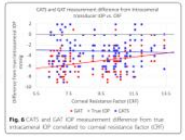


Corneal Phase:Normal Flat Indented Flat Normal

15

CATS™ Tonometer Prism





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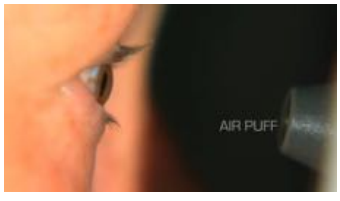
COVID 19- considerations

17



NCT in COVID times?

18

Increased microbial loading in aerosols produced by non-contact air-puff tonometer and relative suggestions for the prevention of coronavirus disease 2019 (COVID-19)

Quantitative Evaluation of Aerosol Generation from Non-contact Tonometry and its Correlation with Tear Film Characteristics

Quantitative High-speed Assessment of Droplet and Aerosol From an Eye After Impact With an Air-puff Amid COVID-19 Scenario

Effect of Intraocular Pressure on Aerosol Density Generated by Noncontact Tonometer Measurement

19

Summary

Author	device	results	Main results
Guo H, PloS one (2020)	Nidek NCT	Aerosols produced	Alcohol and air disinfection UV
Hao W Adv Ther (2021)	-	Aerosols produced	Aerosols coagulate as they spread; 50 cms safe distance <1.0 micron; PPE recommended
Shetty R J of Glaucoma (2020)	Shin-Nippon NCT 200	No droplet in natural setting; Large droplets 100-500 micron considered	When excess tears (artificially induced droplets seen)
Tang Y J of Glaucoma (2020)	Canon NCT	≤ 2.5 micron, <10 micron; Aerosols produced	IOP level can determine aerosolization; next to air jet concentration highest

20

Disposable tips- Goldmann tonometer

- 10% Bleach soak 5-minutes and wash with water and let dry…effective against most microbes
- No disinfectant technique works against prions.

21

Disposable CATS tonometer probe FDA approved

22

Rebound tonometer

23

Advantages

- No anesthetic requirements
- More natural position rather than slitlamp
- Disposable probe
- May have use in screenings
- Best device for pediatric glaucoma

24

Principles of rebound tonometer

- Dynamic electro-mechanical
- A propelling coil and sensing coil surround the probe
- Probe is magnetized
- Temporary current to propelling coil propels probe forward.
- Sensor monitors change

- Probe dampened on contact with cornea
  - Speed reduced drastically if high IOP
  - Speed decreased relatively slowly if IOP is low.

25

Icare® ic200- FDA approved – January 2020

- 200 degrees of positional freedom
  - Sitting, reclined, supine, etc.
- Why is this interesting?

Clinical tip: Measuring seated and supine gives you an idea of potential range of IOP during the day

- Overweight individuals
- Supine IOP is greater than seated and

provides an estimate of range of IOP fluctuation

26

Intraocular pressure –new frontiers

27

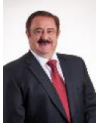
Phase 3

Phase 2

Phase 1 concept development

SCAN ME

Tonometry- LightTouch



- John Maggiano MD

j.maggiano@lighttouch.care

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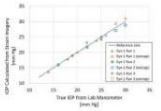
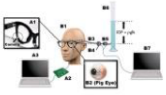
Tonometry- Glasses- Globe Biomedical

Dr. Matt Rickard PhD





mrickard@globebiomedical.com

SCAN ME

- SBIR Phase I: Feasibility of a Wearable Blindness Prevention System
- *Amount: $224,206.00*
- SBIR Phase II: Feasibility of a Wearable Blindness Prevention System
- *Amount: $714,786.00*

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miLens- Smart lens inc.

- IOP measurement
- Still important in glaucoma
- Early testing phase
- Milens has no electronics
- Use smart phone to measure IOP

SCAN ME

Davey Lab presenting at ARVO 2022

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Rules- General about disinfection

- Always contact manufacturer for the cleaning guidelines.
- They are responsible for providing consumer the information.
- Most "easy to use plastic" is cleaned with isopropyl alcohol 70%
- 70% cleans better than 90%...!
- List the cleaning procedures in your SOP

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Serial tonometry- CPT 92110

- Serial tonometry (separate procedure) with multiple measurements of intraocular pressure over an extended time period with interpretation and report, **same day** (e.g., diurnal curve or medical treatment of acute elevation of intraocular pressure)
- When IOP is consistently lower than 21 in glaucoma suspect
- When IOP is greater than 21 in any subject
- Monitoring at risk individuals
- Usually once 12 months unless medically necessary

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Blood pressure and Glaucoma

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Independent risk factors

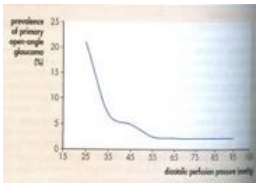
- Low Diastolic blood pressure
- Low Mean Ocular Perfusion Pressure
- Low Diastolic Perfusion Pressure
- Measure Blood pressure after 5 minutes being seated
- Measure BP twice, 5 minutes apart
- If SBP was 10 mmHg or DBP was 5mmHg different measure 3rd time
- Average two measurements that are the closest.

Zheng et al. IOVS 2010

34

Systemic hypertension and glaucoma

- Blood pressure and pathogenesis of glaucoma
  - Hospital based study
- Baltimore Eye Survey examined perfusion pressure
- Diastolic perfusion pressure= DBP-IOP



Tielsch et al Hypertension perfusion pressure and primary open angle glaucoma Arch ophthalmol 1995

35

Mean Ocular perfusion pressure- Elevated IOP

- MOPP = 2/3 [DBP + 1/3(SBP – DBP)] – IOP.

	Blood pressure					
IOP	120/80	120/70	120/60	110/70	110/60	100/60
22	40.2	35.7	31.3	33.5	29.1	26.8
24	38.2	33.7	29.3	31.5	27.1	24.8
26	36.2	31.7	27.3	29.5	25.1	22.8
28	34.2	29.7	25.3	27.5	23.1	20.8

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Mean Ocular perfusion pressure- "Normal IOP"

- MOPP = 2/3 [DBP + 1/3(SBP – DBP)] – IOP.

Blood pressure

IOP	120/80	120/70	120/60	110/70	110/60	100/60
18	44.2	39.7	35.3	37.3	33.3	30.8
16	46.2	41.7	37.3	39.3	35.3	32.8
14	48.2	43.7	39.3	41.3	37.3	34.8
12	50.2	45.7	41.3	43.3	39.3	36.8

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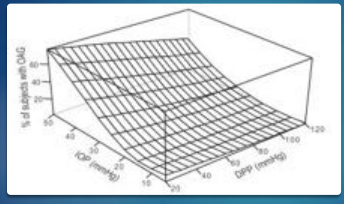
Perfusion pressure and IOP



Zheng et al. IOVS 2010

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Summary perfusion pressure

Greater the IOP lower the blood flow

Elevated IOP with Lower blood pressure – Bad combination

In terms of Perfusion pressure

Clinical tip: Measure BP and Pulse rate on all glaucoma patients
Particularly in suspects that IOP is not high, but nerves appear suspicious

39

Genetic factors

- Positive family history, one of the most heritable of chronic diseases
- Bias:
  - + ve Family history makes a person have frequent check ups
  - Recall bias
    - Sibling with glaucoma odds ratio 3.69
    - Parents with glaucoma odds ratio 2.67
    - Children with glaucoma odds ratio 1.12

40

Summary

- Prevalence of POAG is Caucasians over 40 years of age 2% and in African American and African Caribbean is "four times" that.
- Hispanics greater risk than African American as they grow older
- Overall, quite underdiagnosed- 50% unknown
- Glaucoma suspects- increases need for care dramatically

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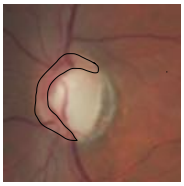
Nerve head evaluation

42

Neuroretinal rim characteristics

- Color of rim- pale rims not good
- Width of rim in all sectors
- ISNT rule
- ISNT rule is accurate about 70% of times



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Nerve head size

- 60 D 1X
- 78 D 1.2 X
- 90 D 1.3 X

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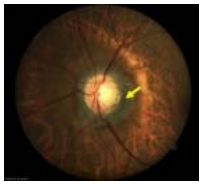
Peripapillary atrophy

- Where
- How large
- *1/8, ¼, ½ , ¾, 1, > 1 DD*
- *Zone alpha and beta*
- *Zone beta to disc area ratio and risk of glaucoma*



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Optic disc hemorrhages

- Transient
- Inferior temporal or superior temporal regions mainly
- Record present or absent
- If present ; which clock hour

46

CD ratio

- Vertical
- Horizontal
- Largest
- CD ratio of imaging devices will not match your findings!

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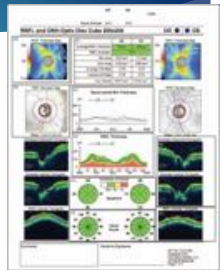
Do we need nerve head analysis now that we have OCT accurately measuring RNFL and ONH?

- Of course, we do
- Red syndrome
- Green syndrome
- OCT is not ready to replace doctor analysis yet



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Goals- managing glaucoma patients

- Document status of optic nerve structure and function
- Target pressure range- so damage is unlikely to happen
- Maintain IOP below target pressure
- Monitor status of the optic nerve and reset target pressure if deterioration occurs.
- Minimize side effects of management and impact on vision and general health and quality of life.
- Educate and engage the patient in management

49

Diagnosis

Give me 7 years and I will tell you if you have glaucoma

50

Glaucoma OCT evaluation

- Anterior chamber evaluation
  - Angle evaluation
  - Corneal thickness
- Macula evaluation
- Retinal Nerve fiber layer
- Optic disc Analysis and photography

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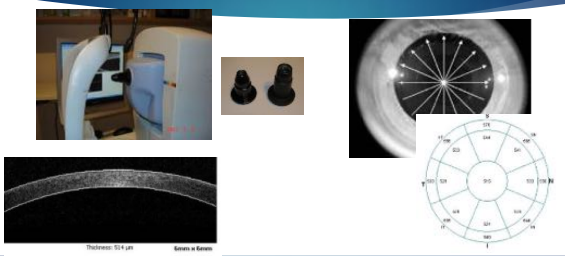
Anterior segment OCT - 92312



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Central corneal thickness

- Measurement error in intraocular pressure values
- Risk factor

53

Pachymetry

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Ultrasound pachymetry is standard

- As central data as possible
- Greater number of measurements increase your reproducibility of data
- Always use lowest data

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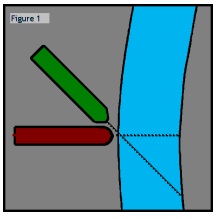
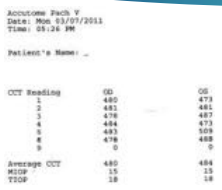
Why lowest data?



- Perpendicular measurements are lowest or smallest in value

56

Why not average the data?

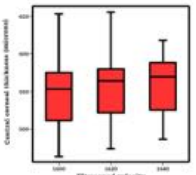


- Average 484 microns
- Lowest 473 microns
- Averaging helps decrease error but does not eliminate it.

A Haun, P Gunvant, M Baskaran, L Vijaya: Central corneal thickness measurement using a pachometer: Mean or lowest values? Invest Ophthalmol Vis Sci, 2004, 45: E-Abstract 137

57

Do all pachymeters give us the same measurements?



- **NO**
- values vary on the basis of
  - Velocity of ultrasound used in algorithm
  - MHz of probe
    - 20 MHz ± 3 microns accuracy
    - 50 MHz ± 1 micron accuracy

The whiskers represent 95% CI of population

MB Taub, FW Chang, **P Gunvant**: Factors affecting measurement of central corneal thickness. ***Optometry and Vision Science***, 2007; 84: E-abstract 075286

58

Difference between optical and ultrasound pachymetry measurements

Author	Difference in OCT and ultrasound values
Kim et al *AJO 2008*	26 microns
Wang et al *J Refract Surg 2008*	38 microns
Gunvant & Darner *Medical Imaging 2011*	13 microns

Kim, H.Y., Budenz, D.L., Lee P.S. et al., " Comparison of central corneal thickness using anterior segment optical coherence tomography vs ultrasonic pachymetry, Am J Ophthalmol.; 145:228-232 (2008).
Wang, J.C., Bunce, C., and Lee, H.M., "Intraoperative corneal thickness measurement using optical coherence pachymetry and cornea-gage plus ultrasound pachymetry J Refract Surg. 24(6):610-4 (2008
P Gunvant, R Darner: Evaluation of corneal thickness measurements obtained using optical coherence tomography and ultrasound technique and determination of specificity in keratoconus screening Medical Imaging 79661 81-88

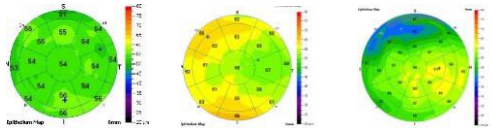
59

Why should you evaluate the cornea and conjunctiva

- About 25% of people continue medications at 2 year period…
- Look at Epithelium
- Pay attention to dry eye and glaucoma –particularly if on topical medications
- Even when patient does not complain they may have sub-clinical dry eyes.

60

Anterior segment OCT- Epithelium



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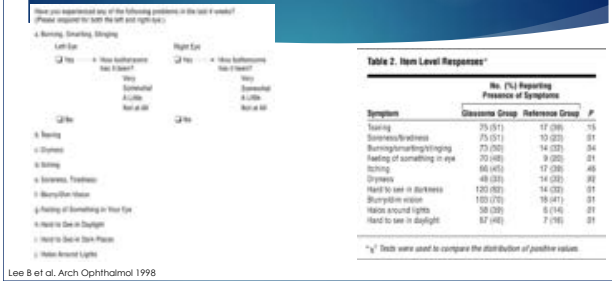
Glaucoma Symptom Scale



Lee B et al. Arch Ophthalmol 1998

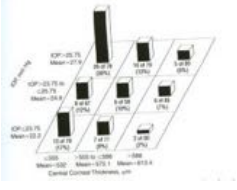
62

If you patient is ocular hypertensive

- Work-up
- Rule out other neuropathies , APD, pallor
- IOP (Range), OCT- RNFL and Ganglion cell complex/analysis , VF (2), Pachy, ORA, fundus photos, Gonio
- Optional /more interesting
- Pattern ERG, Phnr, color contrast, pulsar perimetry

63

How to use CCT data in glaucoma management?



- Error in IOP measurements
- Ocular hypertensive patients
  - Thinner cornea at greater risk of developing glaucoma

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The Scoring Tool for Assessing Risk (S.T.A.R. II) calculator

Probability of conversion in 5- years
<5% observe and monitor
5 to 15% consider treatment
>15% treat

- OHTs and EGPS data
- Intended for use only in untreated OHT patients
- Age (30-80)
- IOP 20-32 mmHg
- CCT 475 to 650 microns
- PSD 0.50 to 3.00 dB
- C/D ratio vertical 0.00 to 0.8

https://ohts.wustl.edu/risk/

65

Gonioscopy- CPT 92020

A = Above Schwalbe line, totally occluded angle.
B = Behind the Schwalbe line, peripheral iris is in contact with TM.
C = Scleral spur Iris root at the level of scleral spur
D = Deep anterior ciliary body seen.
E = extremely deep

Iris insertion

Angle approach

Curvature of periheral iris

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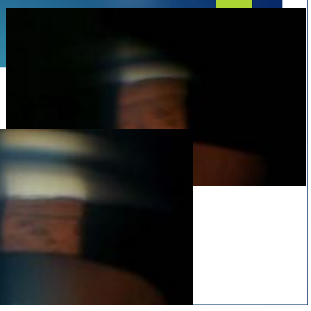
Indentation gonioscopy

- Angle narrow
- Angle appears closed
- Angle structures not visible in a quadrant
- Ruling out synechiae



67

Angle Measurement with Quantification

Clinical tip: Angle 20 degree or less needs further evaluation

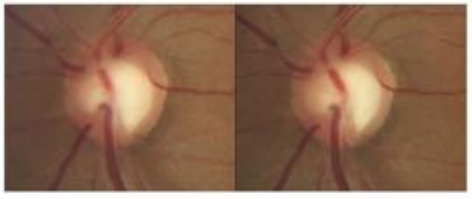
68

Structure and OCT- 92133

69

Gold standard

- Simultaneous stereo photography!
  - Problems?



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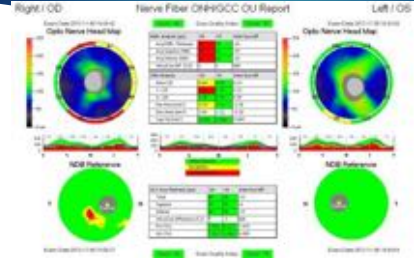
Case



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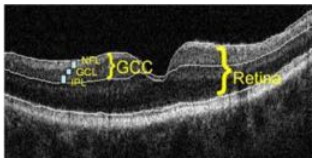
Ganglion Cell Complex (GCC)- 92133



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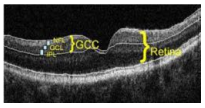
Macula analysis

- Optovue
  - NFL+ GCL + IPL
- Zeiss Ganglion Cell analysis- GCL+ IPL
- Topcon Maestro gives both
  - NFL+ GCL+ IPL
  - GCL+IPL



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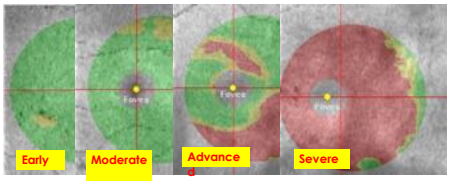
Ganglion Cell Loss. . .

Typically Glaucomatous GCC follows either the inferior or superior arcuate



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Progression and glaucoma

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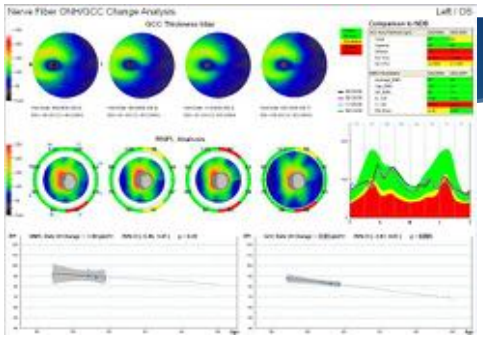
Progression

- Consensus is limited
- Visual fields tend to fluctuate in early glaucoma
- Reliable and repeatable structural measurements is very valuable
  - Fourier domain OCT 5 microns accuracy.

76



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Slow progressors VS fast VS non glaucoma

- Progressing rapidly in month ….no major spike in IOP…possibly not glaucoma or at least some thing more
- Look at nerve carefully signs of pallor… neurological testing, pupils, color
- If definitely glaucoma by criteria discussed before
- Then consider the following…
- 6-OCT scans in 2 years

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Six-Scans in 2 years

- Helps determine if this person is progressing (really glaucoma)
- Helps identify rate of progression (with much greater accuracy)
- Accuracy much more than once or twice a year
- Fast versus slow progressors….
- Determine if your treatment options are correct….

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Macula analysis

80

Is Glaucoma a peripheral disease ?

- Yes
- So why don't we measure visual fields from 30-60 degrees from center ?

81

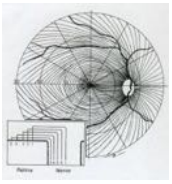
"If damage occurs throughout the retina, how does visual acuity remain stable?"

82

What about other visual tasks?

83

Axonal facts



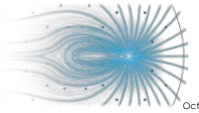
- 700,000 to 1.2 million
- Large variation
- Count of axons increase with increase in area.
- 50% of axons to the macula

84

Is central retina and visual field more protected?

- Yes there is some truth to that
- But not as much as once considered…
- Lots of OCT studies identify macular damage early in glaucoma!

Humphrey field analyzer -24-2



Octopus –G protocol

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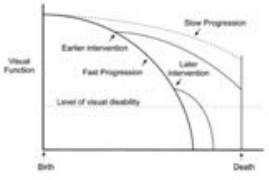
Early detection

86

Early detection and prompt treating saves irreversible vision loss



J Caprioli; Am J of Ophthalmol, Volume 145, Issue 2, February 2008, Pages 343-353

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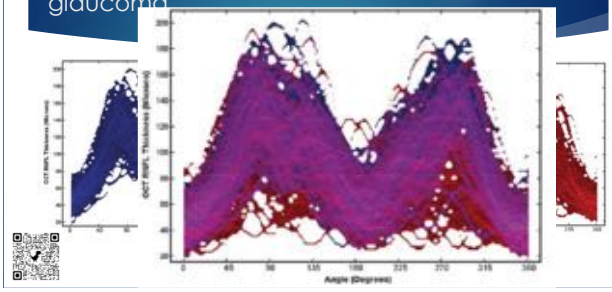
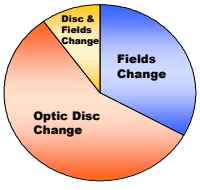
RNFL in a population with and without glaucoma



88

Does structural loss always precede functional loss in glaucoma?

- OHTS reports 55% of subjects reached endpoint (POAG) based on changes in the optic disc only.
- A further 10% of subjects had concurrent optic disc and visual field changes.
- Only 35% of glaucoma was found by visual field changes.



Kass et al., Arch Ophthalmol. 2002;120:701-703

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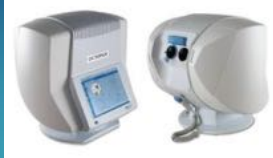


Octopus -600 Pulsar perimetry

90

Why does it help targeting specific ganglion cells?

91

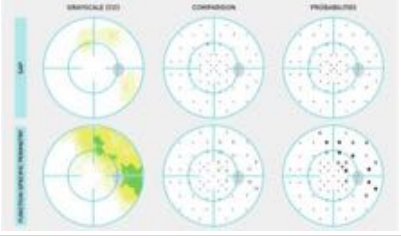
Design of the PULSAR stimulus

92

Sensitivity thresholds with PULSAR perimetry

93

Example of SAP and function-specific perimetry in the same eye



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Other advantages and considerations of Pulsar perimetry

- The measurement technique is more resilient to optical blur due erroneous refractive error
- The outcomes are also more resistant due to cataract related blur
- May be able to identify an early scotoma
- May show exaggerated damage compared to white on white perimetry
- Is designed for "early damage" detection for moderate to advanced loss switch to white-on-white perimetry.

95

Advances in perimetry

96

Opportunities for Improvement in Central 10 Degrees

Glaucomatous damage of the macula

Donald C. Hood, Ali S. Raza, Carlos Gustavo V. de Moraes, Jeffrey M. Liebmann, and Robert Ritch

- glaucomatous damage of the macula is common and can occur early in the disease
- can be missed and/or underestimated with standard 24-2 VF tests that use a 6°grid

The Prevalence and Nature of Early Glaucomatous Defects in the Central 10° of the Visual Field

24-2 and 10-2 VF Examples

Blue cross region on the 24-2 VF = central 10-2 VF.
(A) Both are abnormal.
(B) 24-2 VF normal; 10-2 VF abnormal
(C) 24-2 VF abnormal; 10-2 VF normal

97

Highest Importance Locations Chosen from 10-2 Pattern

Selecting additional test locations to enhance the 24-2 pattern using a scoring system

- Expert group selected specific 10-2 test point locations
- Prevalence and depth of glaucomatous macular defects were systematically evaluated to select optimum test points
- Pattern covers areas known to be susceptible to glaucomatous defects both from structural and functional studies

Selected test locations are shown in red boxes

The expert group: Donald C. Hood, Stuart K. Gardiner, Allison M. McKendrick and William H. Swanson.

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Resulting SITA Faster 24-2C Pattern on HFA3

The 24-2C test pattern combines all 24-2 points + ten selected 10-2 points (shown in OD orientation)

Large Gray	24-2 pattern
Large Orange	Ten additional 24-2C points
Small Gray	10-2 pattern

99

Minimize Time and Maximize Information in VF Testing with HFA3

SITA Faster 24-2
- test in 2 minutes or less
- ~50% faster than SITA Standard; ~30% faster than SITA Fast

SITA Faster 24-2C
- more information in central field
- ~20% faster than SITA Fast 24-2

Add new tests to patient progression
- Mixed SITA GPA
- Includes SITA Faster, Fast, Standard, 24-2, 30-2, and 24-2C in progression analysis

100

24-2C SITA Faster

Flagged points detected centrally in OD

24-2 SITA Standard | 24-2C SITA Faster

101

24-2C SITA Faster

Flagged points detected centrally in OS

24-2 SITA Standard | 24-2C SITA Faster

102

New 24-2C SITA Faster protocol

- Free upgrade if you have HFA III
- Gives more macula points.
- Results comparable to 24-2 SITA FAST
- Thresholds are ± 3 dB
- Gives you some macula information
- You need 10-2 if damage is noticed in macula region

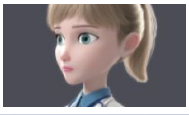
103

Virtual reality perimetry- Are you ready for prime time?

104

VisuALL

- VisuALL is a Virtual Reality Platform (VRP) that is cloud enabled to monitor the eye function
- VisuALL S: Enhanced version for eyecare professionals
- VisuALL H: Simplified model for Home



105

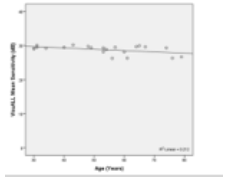
106

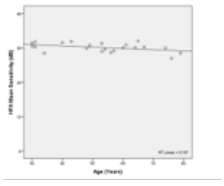
ORIGINAL STUDY

Preliminary Report on a Novel Virtual Reality Perimeter Compared With Standard Automated Perimetry

Reza Razeghinejad, MD, Alberto Gonzalez-Garcia, MD,† Jonathan S. Myers, MD,* and L. Jay Katz, MD**

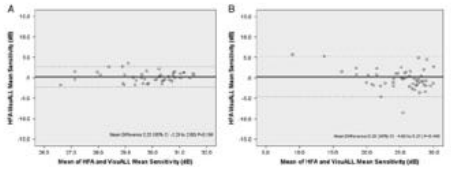
(*J Glaucoma* 2021;30:17–23)





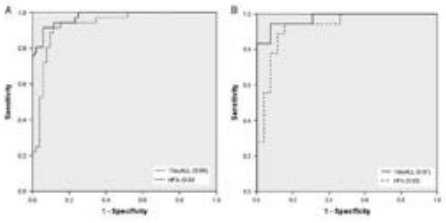
107

Comparison to HFA



108

Sensitivity, specificity and ROC- Accurate diagnosis



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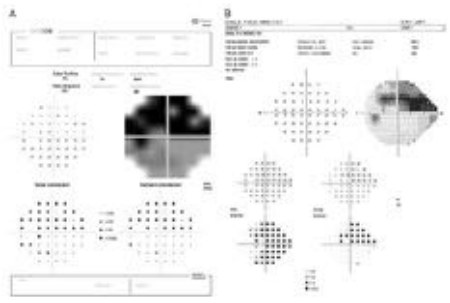


110

VisuALL Tests/Protocols

- Perimetry
  - 24-2 Adults & Pediatrics
  - 10-2 Adults & Pediatrics
  - Supra-threshold Adults & Pediatrics
  - Other
- Visual Acuity Test
  - Landolt C Near
  - Landolt C 20'
- Color Vision Test
  - Isochromatic
  - D-15

111

StrctuoFuction correlations

112

Ocular Structure and Visual Function

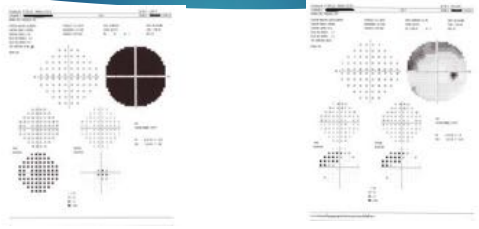
- Structure precedes functional damage
- Function precedes structural damage
- Both damage visible simultaneously

113

How do I correlate structure and function ?

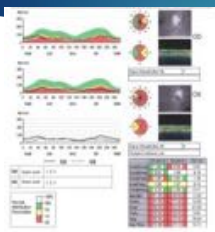
114

Structure and function differences



115

Structure and function differences -2



116

ARVO JOURNALS
From: The Structure and Function Relationship in Glaucoma: Implications for Detection of Progression and Measurement of Rates of Change
Invest. Ophthalmol. Vis. Sci.. 2012;53(11):6939-6946. doi:10.1167/iovs.12-10345

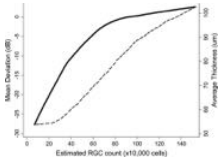
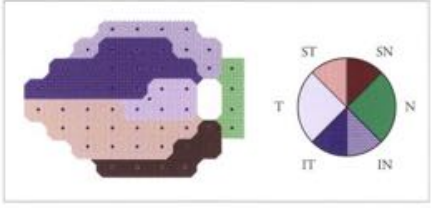


Figure Legend:

Relationship between MD, average RNFL thickness measurements, and estimated RGC counts. At early stages of damage (high RGC counts), changes in estimated RGC counts correspond to relatively smaller changes in MD (continuous line) and relatively larger changes in average RNFL thickness (dashed line). At advanced stages of damage (low RGC counts), changes in estimated RGC counts correspond to relatively large changes in MD, but only small changes in average RNFL thickness.

117

Structure function correlation

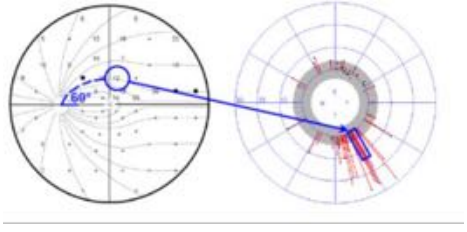


Perimetry Update 1998/1999, pp. 381–389

118

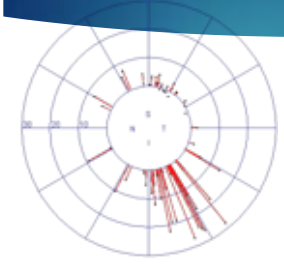
Polar graph

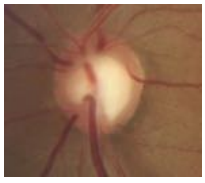
119



119

Bridging structure & function



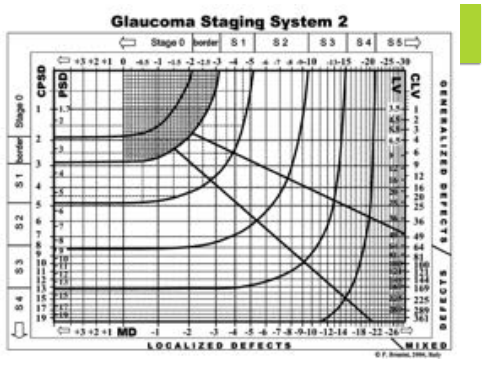


120

Staging glaucoma

- Mean deviation – cataract not clinically significant (clinical judgement in dilated evaluation)
- MD
- Early < -6.00 dB
- Moderate >-6.00 to <-12.00 dB
- Severe ≥ 12.00dB

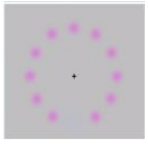
121



122

Points to remember during fields testing

- Same light levels as calibration
- Patch fellow eye well
- Pupil at least 3 mm; consistently do the same thing
- Explanation, explanation, explanation
- Mask will fog lens; tape the mask
- Breaks will save you repeat testing (@2 minutes)
- Ptosis, dermatochalasis –Tape and lubricate
- Lens close to eye but should not touch eyelashes



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Omidenepag Isopropyl 0.002%

NEW DRUG

124

Pharmacologic Characterization Omidenepag Isopropyl 0.002%

- Pro drug hydrolyzed in eye during corneal penetration to Omidenepag (Active form)
- Omidenepag hydrolyzed form of Omidenepag Isopropyl 0.002% lowers IOP
- Highly selective prostanoid EP2 receptor agonist

125

Pharmacologic Characterization Omidenepag Isopropyl 0.002% cont..

- EP2 receptors found in various parts of brain (cerebral cortex, thalamus, hypothalamus), spinal cord and eye
- EP2 which is a G-protein coupled receptor is expressed in cornea, conjunctiva, sclera, trabecular meshwork, lens , iris, ciliary body, choroid and retina
- Decreases IOP via both conventional and unconventional pathways
- Phase III AYAME Study- Non inferior to Latanoprost
- Does not change Iris color*
- Does not change orbital fat*

126

Omidenepag Isopropyl 0.002%

- Approved once daily for glaucoma and OHT Japan 2018
- Approved once daily for OAG and OHT Korea 2019, Taiwan 2020
- Was delayed approval in November 2021
  - Now possibly first or second quarter if 2022

127

Selective laser trabeculopalsty

128

SLT and glaucoma

- Great first or second line option.
- Non-compliant individuals
- Individuals that fluctuation of IOP is a concern
- In theory can be repeated to lower IOP.
- Is the IOP lowering the same second attempt?

129

RESEARCH ARTICLE Open Access

Repeatability of selective laser trabeculoplasty for open-angle glaucoma

Brian A. Francis[*1], Nils Loewen[2†], Bryan Hong[3], Laurie Dustin[4], Kevin Kaplowitz[5], Robert Kinast[6], Jason Bacharach[7], Sunita Radhakrishnan[7], Andrew Iwach[7], Lidiya Rudavska[8], Parul Ichhpujani[9] and L. Jay Katz[10]

- Inclusion criteria prior SLT with diminution of response over time
- N=137

Table 2 *Significance of within treatment change, $p < 0.001$. IOP intraocular pressure, SLT selective laser trabeculoplasty

		SLT1 Mean (standard deviation)	SLT2 Mean (standard deviation)	Paired t-test p-value
IOP	Baseline (n = 137)	20.3 (5.2)	19.4 (5.0)	0.03
	6–12 months (n = 130,119)	16.3 (4.3)	16.3 (4.8)	0.86
	12–15 months (n = 100,99)	16.4 (3.9)	16.7 (4.7)	0.24

Francis et al. BMC Ophthalmology (2016) 16:128
DOI 10.1186/s12886-016-0299-9

130

Primary Selective Laser Trabeculoplasty for Open-Angle Glaucoma and Ocular Hypertension

Clinical Outcomes, Predictors of Success, and Safety from the Laser in Glaucoma and Ocular Hypertension Trial

- A total of 611 eyes (195 OHT and 416 OAG) of 355 patients received SLT, and 622 eyes (185 OHT and 437 OAG) of 362 patients received topical medication at baseline.
- Early absolute IOP-lowering after SLT was no different between OHT and OAG eyes
- No difference was noted in early absolute IOP-lowering between topical medication and primary SLT
- Early absolute IOP-lowering with primary SLT was positively associated with baseline IOP

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Primary Selective Laser Trabeculoplasty for Open-Angle Glaucoma and Ocular Hypertension

Clinical Outcomes, Predictors of Success, and Safety from the Laser in Glaucoma and Ocular Hypertension Trial

- At 36 months, 536 eyes (87.7% of 611 eyes) of 314 patients (88.5% of 355 patients) were available for analysis.
- Some **74.6% of eyes (400 eyes) treated with primary SLT achieved drop-free disease-control at 36 months; 58.2% (312 eyes) after single SLT**.
- Six eyes of 6 patients experienced immediate post-laser IOP spike (>5 mmHg from pretreatment IOP) with 1 eye requiring treatment.

132

Primary Selective Laser Trabeculoplasty for Open-Angle Glaucoma and Ocular Hypertension

Clinical Outcomes, Predictors of Success, and Safety from the Laser in Glaucoma and Ocular Hypertension Trial

- Conclusions: Primary SLT achieved comparable early absolute IOP-lowering in OHT versus OAG eyes.
- Drop-free disease-control was achieved in approximately 75% eyes at 36 months after 1 or 2 SLTs, the majority of these after single SLT.
- These analyses are exploratory but support primary SLT to be effective and safe in treatment-naive OAG and OHT eyes.

133

Narrow angle/acute attack/angle closure glaucoma

134

Van Herrick angle estimation

- 1:1 – Open angle, VH grade 4
- 1:1/2 – Open angle, VH grade 3
- 1:1/4 – Narrow angle, VH grade 2 (Angle Closure Possible)
- 1: <1/4 – Angle closure likely, VH grade 1

135

Treatment for narrow angles

Prophylactic laser iridotomy

Laser iridoplasty- appositionally closed angle

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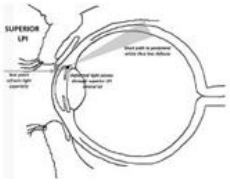
Dysphotopsia following Laser PI

- halos, shadows, crescents and commonly blurred line (sometimes colored)
- Conventionally LPI under the lid or fully covered
- Hypothesis decreases "ghost images"

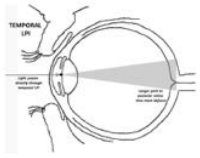


137

Dysphotopsia after Temporal versus Superior Laser Peripheral Iridotomy: A Prospective Randomized Paired Eye Trial

VANESSA VERA, ABDULLA NAQI, GRAHAM W. BELOVAY, DEVESH K. VARMA, AND IQBAL IKE K. AHMED

- Superior LPI 10.7% linear dysphotopsia
- Temporal LPI 2.4%
- Partially or completely covered LPI four times more likely to demonstrate new linear dysphotopsia



Am J Ophthalmol 2014;157:929–935.

138

Does LPI prevent angle closure? ZAP trial

139

Laser peripheral iridotomy for the prevention of angle closure: a single-centre, randomised controlled trial

www.thelancet.com Vol 393 April 20, 2019

Zhongshan Angle Closure Prevention (ZAP) Trial

140

ZAP

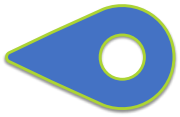
- 11,991 screened
- 889 enrolled
- 889 eyes treated fellow eye 889 not treated
- Primary outcome Angle closure by 72 months
- Incidence of angle closure
  - 4.19 per 1000 treated
  - 7.97 per 1000 untreated
- Small but significant protective effect of treatment
- Widespread prophylactic laser for angle closure suspects not recommended

141

Effectiveness of early lens extraction for the treatment of primary angle-closure glaucoma (EAGLE): a randomised controlled trial

www.thelancet.com Vol 388 October 1, 2016

EAGLE trial



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EAGLE trial

Hypothesis tested –

- clear lens extraction after primary angle closure glaucoma
- leads to **better quality of life, lowering of IOP and less need for glaucoma surgery** than standard care
- Standard care – Laser iridotomy and medications

Interpretation **Clear-lens extraction showed greater efficacy and was more cost-effective than laser peripheral iridotomy, and should be considered as an option for first-line treatment.**

143

Management of acute raise in IOP

144

Medical treatment- Goals

- Lower intraocular pressure
- Alleviate pain
- Clear cornea
- Prevent synechiae

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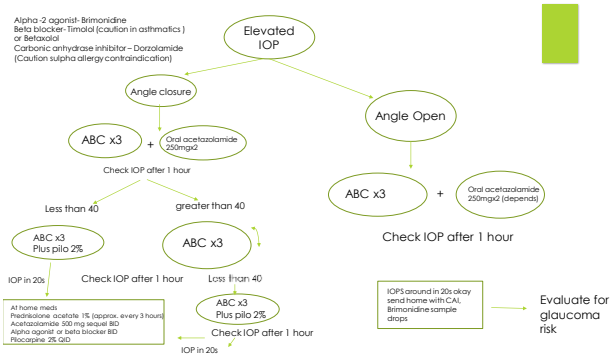
Intravenous medications

- Acetazolamide 500mg intravenous
- Intravenous Mannitol
- Best therapy however is not always available in clinics

146



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OCT Angiography

BRINGS STRUCTURE AND FUNCTION TOGETHER

148

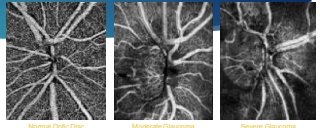
How does OCT angiography work?

- Uses motion contrast to detect blood flow
- Rapidly acquire multiple cross-sectional images from a single location on the retina

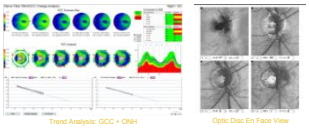
149

Glaucoma

OCT Angiography: Function



OCT: Structure



Previously diagnosed patient. Images courtesy of Michel Puech, MD, FRCS

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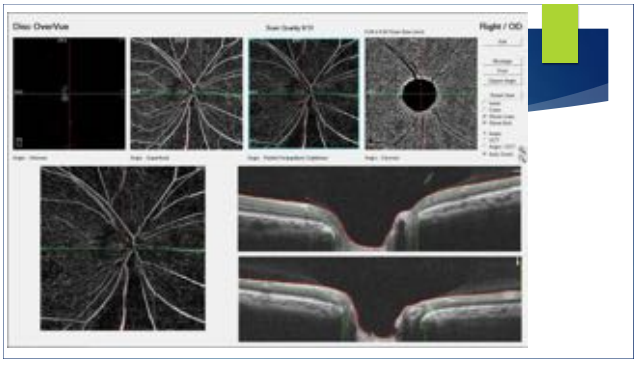

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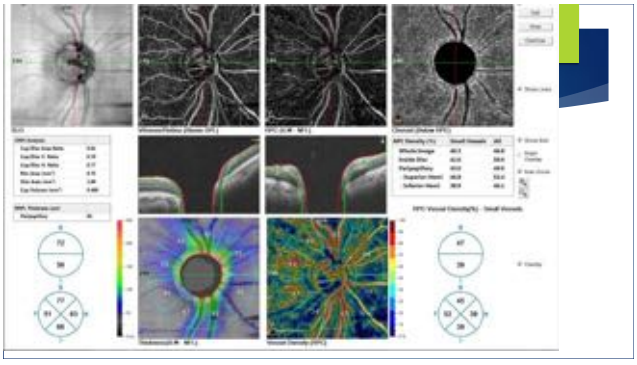

152

"So how and what do I use from OCT angiography data?"

153

Lots of information

- OCT angiography provides a wealth of structure and function information
  - Radial Peripapillary Capillaries
  - Macular region capillaries network
  - Montage
  - GCC, RNFL, ONH,SLO image, Vitreous and choroid
  - Progression

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Goals of glaucoma management

- Document status of optic nerve structure and function
- Target pressure- so damage is unlikely to happen
- Maintain IOP below target pressure
- Monitor status of the optic nerve and reset target pressure if deterioration occurs.
- Minimize side effects of management and impact on vision and general health and quality of life.
- Educate and engage the patient in management

155

Current practice patterns

- Unacceptable high pressures will inevitably destroy optic nerve tissue
- Safe levels of IOP by any means warranted
  - If these don't work or not sufficient
  - drugs like – prostaglandins
  - reduction in inflow – beta blockers
- Maximal medical therapy
- Consider surgery

156

Once established when do I see my patient?

- Once established need regular follow-up
- IOP check 4 times a year
- Two visual fields twice a year
- OCT twice a year
- Fundus photos twice a year
- Gonioscopy once at diagnosis and as needed
- Pattern or PhNR ERG twice a year
- Color contrast sensitivity?

157

Target pressure

- A theoretical value below which visual field and ONH appear stable (not deteriorating).
- Calculated from highest recorded IOP.
- Conventionally 20-30% decrease in IOP.
- 40% or more if severe glaucoma

158

Where should the IOP be?

- No real number
- Start with 20-30% drop (depends on stage and level of IOP)
- Medicare expects at least 15%
- Monitor for progression
- Advanced glaucoma you want IOP to be less than 12
- Pressure should not fluctuate much

159

IOP level- rule of thumb

- Early glaucoma 18 mmHg
- Moderate glaucoma 15 mmHg
- Advanced glaucoma <12 mmHg

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Medications

- First line drugs- prostaglandin analogs, prostonoid
- Second line: Beta blockers, Alpha 2 agonist, Carbonic anhydrase inhibitor, rock inhibitor, Rocklatan
- Combination Rocklatan (PGA+ rock inhibitor)
- Third: Combination with prostaglandin
  - Eg: PGA (Travatan Z) and CAI+ Beta blocker (COSOPT)
  - PGA + brinzolamide/brimonidine (Simbrinza)

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Do we really have the luxury to use them all?

- Stage of disease
  - Visual field status
- Stage of nerve damage
  - Rim tissue remaining
- Type of glaucoma
  - POAG – medical first makes sense
  - Secondary glaucoma
  - Congenital glaucoma
  - Complete angle closure

  (Secondary glaucoma, Congenital glaucoma, Complete angle closure: treated differently)
- Adherence, compliance, persistence issues
- Effect of medications and future outcomes of surgery

162

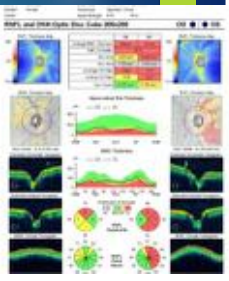
New glaucoma patient untreated and not established

- Work-up
- Rule out other neuropathies , APD, pallor
- IOP (Range), OCT- RNFL and Ganglion cell complex/analysis , VF (2), Pachy, ORA, fundus photos, Gonio
- Optional
- Pattern ERG, Phnr, color contrast

163

OCT analysis

- No real number/guidance
- Color coded for limits of normality
- Signal strength very important
- Errors in segmentation
- Inferior average most diagnostic



164

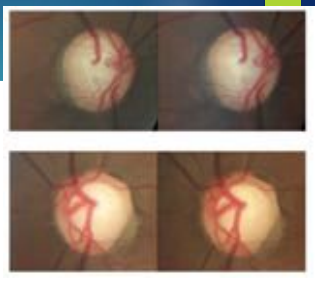
Visual fields

- Need two fields to establish baseline
- Threshold ideal… 24-2 SITA standard, Octopus G protocol,
- fast strategies like SITA-FAST, Faster, Octopus TOP (next best)
- Covering fellow eye crucial
- Mask patients (fog lenses cause spurious results), tape the top part of mask

165

Fundus photos

- Stereo images worth is weight in gold….
- Low- medium flash setting avoid beaching of image and better visualization



166

Neuroprotection and glaucoma

167

Macular Pigment and glaucoma

- We know the advantages of multivitamins and AMD
  - Prevents oxidative damage
  - Quenches any free radical
  - Prevents photoreceptor death
  - Absorbs stray light
- Oxidative damage can also occur in glaucoma
  - Both Anterior and posterior segment

168

Where is the evidence?

- Aqueous humor has lot of vitamin-C
- Macular pigment optical density can be lower in glaucoma patients than individuals without glaucoma

169

Evidence of lower macular pigment optical density in chronic open angle glaucoma

Clinical science

Macular pigment is associated with glare-affected visual function and central visual field loss in glaucoma

We Fong Siah,[1] Colm O'Brien,[1,2] James J Loughman[3,4]

Lower Macular Pigment Optical Density in Foveal-Involved Glaucoma

Ophthalmology 2015;122:2029-2037 © 2015

Wi Fong Siah, MRCPI, FRCOphth,[1] James Loughman, FAOI, PhD,[1,2] Colm O'Brien, FRCOphth, MD[1]

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Glaucoma

Macular Pigment and Visual Function in Patients With Glaucoma: The San Diego Macular Pigment Study

Fábio B. Daga,[1,2] Nara G. Ogata,[1,2] Felipe A. Medeiros,[1,2] Rachel Moran,[3] Jeffrey Morris,[4] Linda M. Zangwill,[1] Robert N. Weinreb,[1] and John M. Nolan[5,6]

[1]Hamilton Glaucoma Center and Shiley Eye Institute and Department of Ophthalmology, University of California San Diego, La Jolla, California, United States
[2]Department of Ophthalmology, Duke Eye Center, Duke University, Durham, North Carolina, United States
[3]Nutrition Research Centre Ireland, School of Health Sciences, Carriganore House, Waterford Institute of Technology, West Campus, Carriganore, Waterford, Ireland
[4]Moran Eye Group, Ivinskas, Vista, California, United States

Clinical Ophthalmology

Dovepress

ORIGINAL RESEARCH

Comparison of Macular Pigment Optical Density in Glaucoma Patients and Healthy Subjects – A Prospective Diagnostic Study

This article was published in the following Dove Press journal:
Clinical Ophthalmology

171

Zeaxanthin and Retinal Ganglion Cell

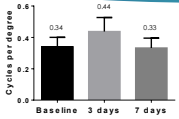
172

Method

OKR Visual Acuity measurement. Visual acuity was measured by immobilizing the mouse head and restraining the mouse with a home-made OKR device. An infrared camera was used to monitor and record pupil movement and videos were analyzed with the Tracker video analysis and modeling tool. A 34.3 cm-diameter grating drum rotated around the mouse illuminated with a 200 lx-white light. The grating frequency was decreased using the stair case method until eye tracking ceased, identifying the smallest grating detected by the mouse's eye.

173

Fundamental issue mouse retina cannot accumulate carotenoids



1 µL of 5 µg/µL zeaxanthin in DMSO was injected intravitreally to mice. Visual acuity was assessed before the injection and 3 and 7 days after the injection in a device that measures optokinetic reflex (OKR). Y-axis indicates the OKR spatial frequency thresholds of the mouse eyes. N = 4.

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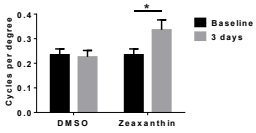
Result

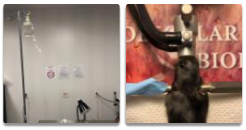


- **Zeaxanthin improves visual acuity in mice.** 1 µL of 5 µg/µL zeaxanthin in DMSO or 1 µL of DMSO was injected intravitreally to mice. Visual acuity was assessed before and 3 days after the injection in a device that measures optokinetic reflex (OKR). Y-axis indicates the OKR spatial frequency thresholds of the mouse eyes. N = 8. * p < 0.05. Two way ANOVA followed by Bonferroni test.

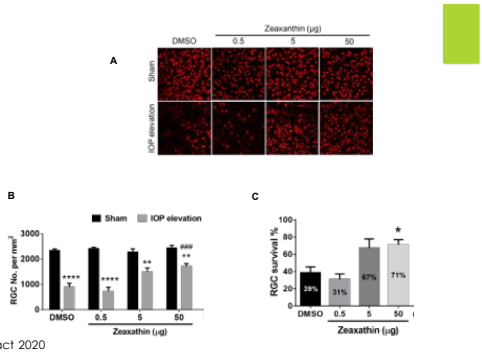
175

IOP SPIKE 1 HOUR 110 MMHG

- Mouse acute glaucoma model. Mice were anesthetized with isoflurane. Pupils were dilated with 1% tropicamide. The anterior chamber of the right eye was cannulated with a 33-gauge infusion needle connected to a normal saline reservoir, which was elevated to maintain an intraocular pressure of 110 mmHg for 60 min. Retinal ischemia was confirmed by whitening of the fundus. A sham procedure performed without elevating the pressure in the contralateral left eye was used as control.



176



IOVS e-abstract 2020

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nutrients

MDPI

Review

Carotenoids in the Management of Glaucoma: A Systematic Review of the Evidence

Drake W. Lem [1], Dennis L. Gierhart [2] and Pinakin Gunvant Davey [1,*]

- We know the advantages of multivitamins and AMD
  1) Prevents oxidative damage, 2) Quenches any free radical 3) Prevents photoreceptor death 4) Absorbs stray light
- Oxidative damage can also occur in glaucoma
  - Both Anterior and posterior segment
  - Animal studies and few human trials suggest carotenoids vitamin therapy exerts synergistic neuroprotective benefits.

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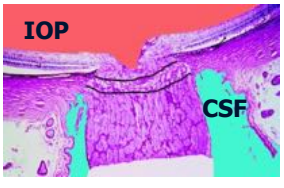
Summary MPOD and Glaucoma

- Measure Macular pigment in glaucoma patients
- Measure Ganglion Cell Complex/ Analysis
- Recommend multivitamin intake with good amount of Lutein and Zeaxanthin- **Dosage matters**!
- Helps age-related diseases and may provide some benefits to glaucoma.

179

Can intracranial pressure be culprit in glaucoma

- IOP may be one of two pressures that are potentially a problems in glaucoma
- Remember lamina cribrosa is thinner in patients with glaucoma



180

Factors and CSF- pressure

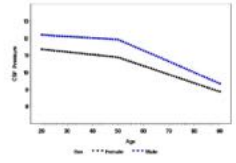


Table 6. Intraocular pressure, cerebrospinal fluid pressure, and translaminar pressure difference by age groups.

Age	IOP AVG	CSFP	N	TLPd	Difference* P value
20-49	14.5	10.1	178	4.4	
50-59	14.5	11.3	106	3.6	0.41% 0.004
60-69	14.7	9.9	75	4.8	1.39% 0.746
70-79	14.7	8.3	65	6.4	1.43% 0.729
>80	15.6	7.6	37	8.2	7.36% 0.193

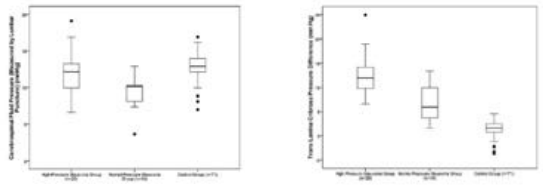
The percentage difference is calculated by mean IOP average of each age group against mean IOP average at age 20-49; significance measured by two-tailed Student's t-test. IOP: intraocular pressure; CSFP: cerebrospinal fluid pressure; TLPd: translaminar pressure difference.

181

Cerebrospinal Fluid Pressure in Glaucoma

A Prospective Study

Ruojin Ren, MD,[1,2] Jost B. Jonas, MD,[1,4] Guogheng Tian, MD,[3] Yi Zhen, MD,[2] Ke Ma, MD,[1] Shuning Li, MD,[2] Hongtao Wang, MD,[2] Bin Li, MD,[1] Xiaojun Zhang, MD,[1] Ningli Wang, MD[2]



Ophthalmology 2010;117:259–266 © 2010

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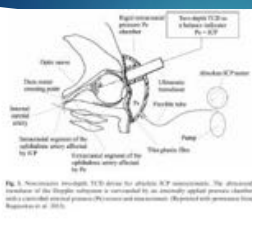
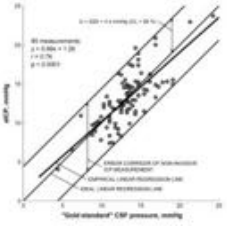
Non-invasive ICP measurements





Update in intracranial pressure evaluation methods and translaminar pressure gradient role in glaucoma
Siaudvytyte et al Acta Ophthalmol. 2015: 93: 9–15

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Equinox

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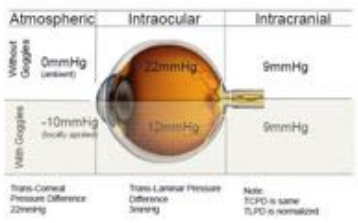
IOP with Equinox



Slide courtesy Justin Schweitzer

185

Minimally invasive glaucoma surgery

186

Is cataract surgery a "Glaucoma treatment" ?

- Lowers IOP – Normals, POAG, OHT, Angle closure
- Opens and deepens Anterior Chamber
- Trabecular meshwork changes dues to phaco energy?
- Trabecular aspiration
- IOP is indeed declined… not as much as one would expect
- IOP high then the drop in pressure is high
- If anatomically narrow or blocked then the phaco indeed helps
- The change is IOP is not permanent …IOP goes back to normal levels in 2 years

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Cardinal features as proposed by Saheb and Ahmed in 2012

- Ab interno, micro-incisional approach (*note: Some use an ab-externo approach.)
- Minimal trauma/disruption to normal anatomy and physiology
- Demonstrable/reliable IOP lowering
- Extremely high safety profile
- Rapid post-op recovery, with minimal need for follow-up

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When and how

- MIGS typically require shorter operation time and allow for more rapid recovery.
- MIGS can be combined with/without cataract extraction for patients with mild to moderate glaucoma and cataracts.
- OAG, or other types like exfoliation and pigment dispersion cases
- MIGS may be less effective in lowering IOP than traditional glaucoma surgeries,

189

When and how

- MIGS do fill a gap in the treatment of patients who would benefit from lower IOP but do not warrant the risk of traditional surgery.
- Decrease medication use
- Combined with cataract
- Narrow angles ? Hence cataract surgery

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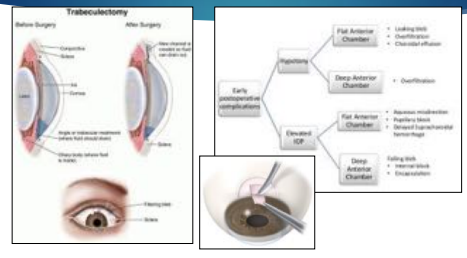
Why MIGS?



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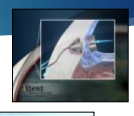
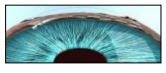
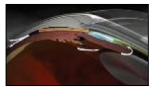
MIGS Treatments

- Increase Trabecular Outflow
  - iStent and iStent inject
  - Trabectome
  - Kahook Blade
  - Hydrus Microstent
- Increase Uveoscleral Outflow
  - Cypass (withdrawn)
  - iStent Supra (not approved)



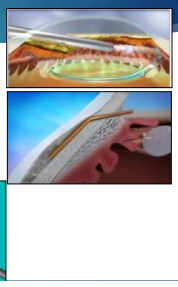




192

MIGS Treatments

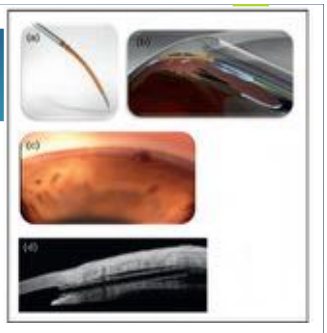
- Reduce Aqueous Production
  - Endoscopic photocoagulation ECP
- Subconjunctival Filtration
  - Xen Gel Stent
- Ab Externo
  - Inn Focus (not approved
  - Ahmed Shunt



193

Cypass stent

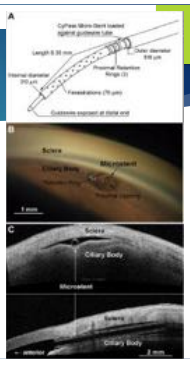
- The CyPass implant is made of polyamide material
- Inserted ab interno into the suprachoroidal space through a manual inserter



194

Two-Year COMPASS Trial Results: Supraciliary Microstenting with Phacoemulsification in Patients with Open-Angle Glaucoma and Cataracts

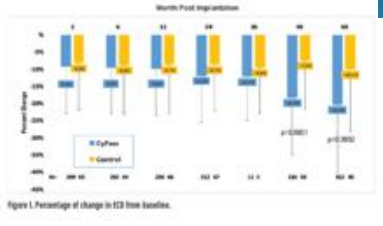
- This RCT demonstrated safe and sustained 2-year reduction in IOP and glaucoma medication use after microinterventional surgical treatment for mild-to-moderate POAG.
- 505 subjects
- 131 were randomized to the control
- 374 were randomized to the microstent group
- Decrease 7.4 mmHg for the microstent group versus 5.4 mmHg in controls (P < 0.001), with 85% of microstent subjects not requiring IOP lowering medications at 24 months



195

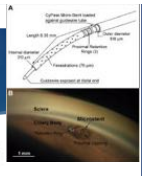
Compass XT results

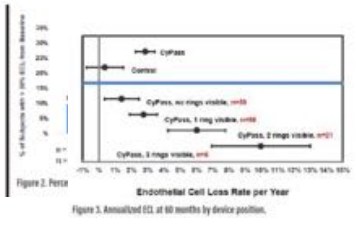
- 5 year extension trial
- Insisted by FDA during approval
- 282 included 253 completed



196

Compass XT results continued





197

Risk assessment in patient with Cypass

- Routine gonioscopy is needed
- Contact with Endothelium must be noted.
- Baseline corneal thickness and endothelial cell count is needed
- Note rings visible
- Look for edema or guttata

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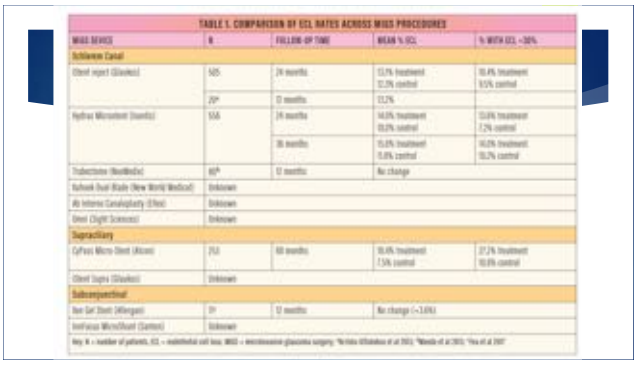

199

MIGS

- Minimal trauma, high efficacy, high safety profile, and rapid recovery.
- There is an increasing interest and availability of MIGS procedures.
- Important to have good science and long-term follow-up data.
- MIGS devices may offer benefits to our patients with glaucoma
  - through IOP reduction
  - reduced need for glaucoma medications
  - high safety profile.
- MIGS are here to stay for the foreseeable future and its role increasing.

200

Sustained release

201

Bimatoprost Sustained-Release Implants for Glaucoma Therapy: 6-Month Results From a Phase I/II Clinical Trial

RICHARD A. LEWIS, WILLIAM C. CHRISTIE, DOUGLAS G. DAY, E. RANDY CRAVEN, THOMAS WALTERS, MARINA BEJANIAN, SUSAN S. LEE, MARGOT L. GOODKIN, JANE ZHANG, SCOTT M. WHITCUP, AND MICHAEL R. ROBINSON, FOR THE BIMATOPROST SR STUDY GROUP

10 µg injected at baseline

DURYSTA™

Utah, Indiana and Kentucky- optometrists can inject Durysta more following!!!

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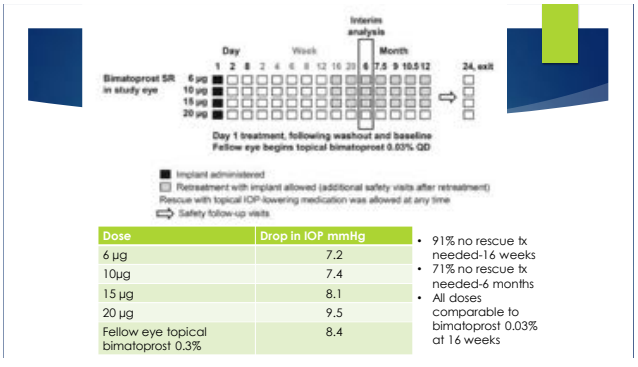

Dose	Drop in IOP mmHg
6 µg	7.2
10µg	7.4
15 µg	8.1
20 µg	9.5
Fellow eye topical bimatoprost 0.3%	8.4

- 91% no rescue tx needed-16 weeks
- 71% no rescue tx needed-6 months
- All doses comparable to bimatoprost 0.03% at 16 weeks

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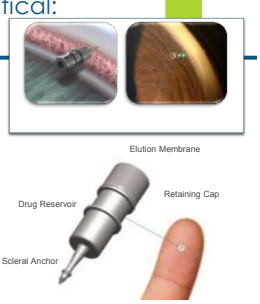
Sustained-Release Pharmaceutical: **iDose TR**

- Alternative to topical medications to address non-adherence & other medication drawbacks
- Titanium implant (1.8 mm x 0.5 mm) designed for continuous drug delivery directly into anterior chamber, membrane designed to elute specially formulated travoprost
- Designed to provide long duration intracameral pharmaceutical; secure, anchored design; facile implantation and exchange upon drug depletion
- AMA approved Cat III codes for implantation, removal and re- implantation of drug delivery system into anterior chamber, effective 7/21



iDose TR is not approved by the FDA and limited by US law to investigational use

204

iDose TR US Multicenter Phase II Trial: Study Design

Key Aspects of Study Design
154-patient, multi-center, randomized, double-blind trial
Evaluated 2 iDose models with two different travoprost elution rates, compared to topical timolol ophthalmic solution, 0.5%
Primary efficacy endpoint of non-inferiority to topical timolol
Subjects diagnosed with mild to moderate OAG or ocular hypertension, on 0 to 3 meds with baseline IOP between 21 mmHg and 36 mmHg
Additional medications were added if IOP was above 18 mmHg

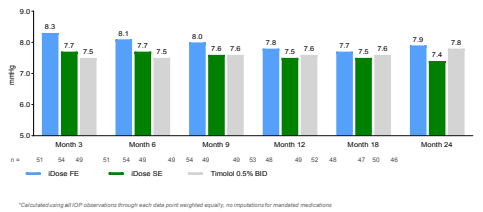
iDose TR is not approved by the FDA and limited by US law to investigational use

205

iDose US Multicenter Phase II Trial: **24 Month Outcomes**

PHASE 2 CLINICAL DATA AT 24 MONTHS*

Average Reduction in IOP



*Calculated using all IOP observations through each data point weighted equally; no imputations for mandated medications

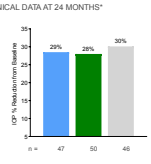
iDose TR is not approved by the FDA and limited by US law to investigational use

206

iDose US Multicenter Phase II Trial: **24 Month Outcomes**

Average IOP % Reduction from Baseline

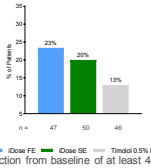
PHASE 2 CLINICAL DATA AT 24 MONTHS*



Average IOP reduction from baseline was 29% and 28% for the fast- and slow-release *iDose TR* arms, respectively, versus 30% for the timolol control arm at 24 months

Proportion of Subjects with ≥ 40% IOP Reduction from Baseline

PHASE 2 CLINICAL DATA AT 24 MONTHS*



IOP reduction from baseline of at least 40% was shown in 23% and 20% of patients for the fast- and slow-release iDose TR arms, respectively, versus 13% of patients for the timolol control arm at 24 months

*Calculated using all IOP observations through each data point weighted equally; no imputations for mandated medications

iDose TR is not approved by the FDA and limited by US law to investigational use

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Thank You!

Pinakin Davey OD, PhD, FAAO, FOWNS, FARVO
Professor
Director of Clinical Research
pdavey@westernu.edu
909-469-8473

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Diagnostic Yield Beyond OCT-Rabin Cone Contrast

Pinakin G. Davey OD, PhD, FAAO, FOWNS, FARVO
Professor and Director of Clinical Research
Western University of Health Sciences

1

Disclosures

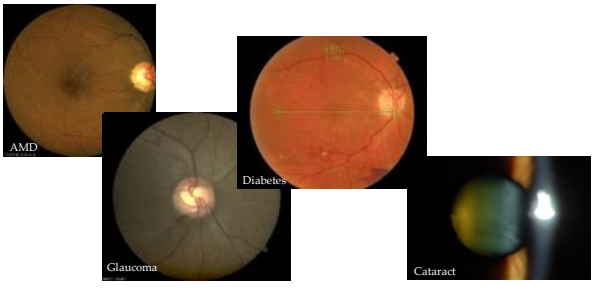
Has a relevant financial relationship with:

Innova Systems, Haag Streit, Sanofi, Optovue, ZeaVision and Guardion health Sciences/VectorVision as a speaker and/or research consultant

The content and format of this course is presented without commercial bias and does not claim superiority and commercial product or service.

2

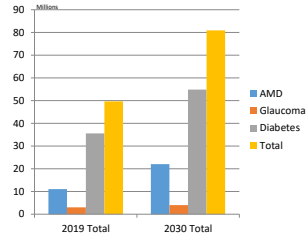
The common…



3

Medical management demands accurate diagnostic and monitoring equipment

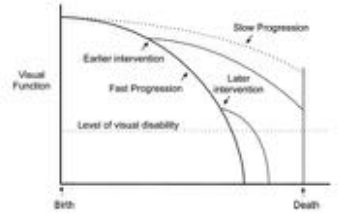
- An estimated 50 million have disease that will affect their vision
- Half of those affected don't know they have the disease
- Prevalence rates expected to increase by roughly 50% by 2030



U.S. Disease Prevalence Rates

4

Early detection and prompt treating saves irreversible vision loss



J Caprioli; Am J of Ophthalmol, February 2008

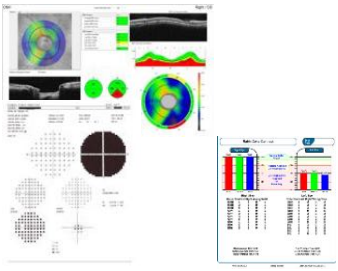
5

Ocular Structure and Visual Function

- Structure precedes functional damage
- Function precedes structural damage
- Both damage visible simultaneously

6

What is the diagnosis?

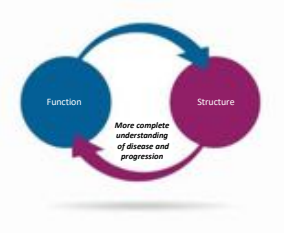


- What's the history?
- What's the clinical picture?
- What do other ancillary tests tell you?
  - Fundus, Visual fields, OCT, A1c???
  - All these tests may be needed to make the diagnosis

7

Value of Function *plus* Structure

- **Early Detection:** Function precedes structure in many conditions, highlighting problems before structural damage occurs
- **Progression:** Functional tests plays a critical role in detecting sub-clinical progression
- **Improvement:** Structural tests demonstrate stability; only functional tests can demonstrate improvement



8

Color Vision as a Biomarker of Disease

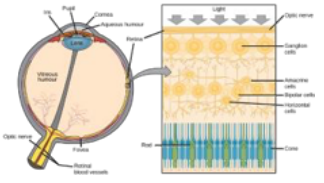
- Wet AMD
- Retinal Dystrophy
- Cataract
- Optic Neuritis
- Multiple Sclerosis
- Diabetes with or without retinopathy
- Dry AMD

Loss of color vision is a major complaint in rapidly changing disorders

Color vison is also a biomarker of slow progressing diseases even though patients are unaware of color vision change

9

Color Processing Through the Visual Pathway



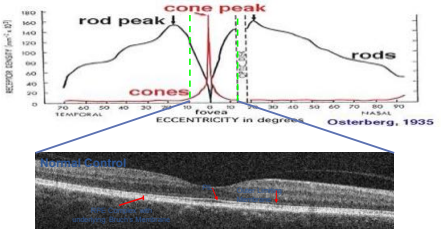
- Color perception (RGB) arises at the photoreceptor level
- Opponent color processing arises in inner nuclear layer via horizontal and bipolar cells and continues at retinal ganglion cells
- Damage to any part of the retina or visual pathway should affect color vision

10

We have enjoyed our OCTs

11

Cone Contrast Testing: Functional Compliment to the OCT



12

Color vision testing has seen some serious upgrade

13

Rabin Cone Contrast Test

- Based in science
  - Co-developed between Innova Systems and US Air Force
- Combines Cone Isolation technology and Contrast Sensitivity
- Color vision technology sensitive enough to detect subtle changes from disease
- Threshold test, similar to visual field

But just faster…

- Patent No. US 9,883,794

Simulation

14

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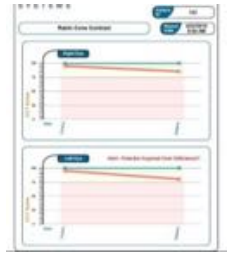
Rabin Cone Contrast Output



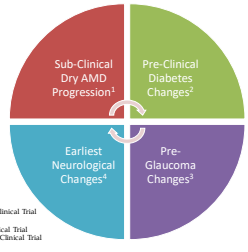
16

Color Deficient Profiles

Hereditary	Acquired
• Specific to red or green cone type	• Not as selective to specific cone type
• Single cone type	• Multiple cone types
• Moderate to severe	• Mild to severe
• Symmetry	• Often asymmetry
• No blue	• Frequently blue

17

Cone Contrast Testing: Clinical Value Beyond OCT



1. Duke Longitudinal Clinical Trial
2. Diabetes Case Study
3. Shiga Glaucoma Clinical Trial
4. UCSF Optic Neuritis Clinical Trial

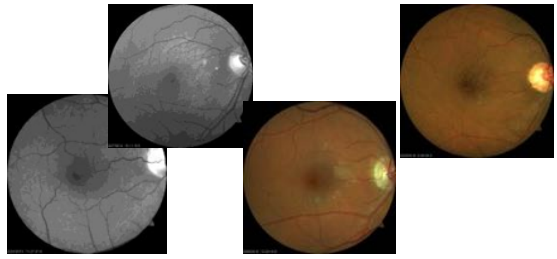
Rabin Cone Contrast identified:

- Progression in AMD when OCT showed no change
- Changes in diabetics prior to manifest retinopathy
- Earliest changes visible in ocular manifestations of multiple sclerosis
- Confirmation of pre-glaucomatous damage progression

18

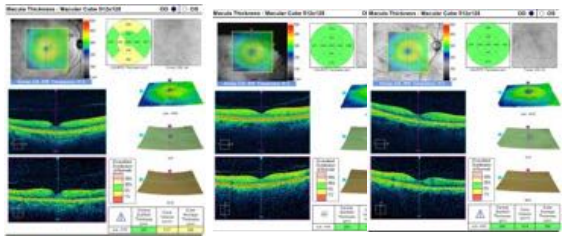
19

Structural changes



20

Do we always see thickness abnormalities?



21

Structure & Function in AMD

- Drusen may occur first but visual acuity may be normal.
- But is 20/20 vision equals no

Glare
Disability

Structure and function measurements should correlate and are not opposites

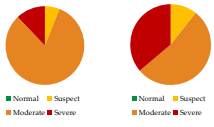
Not Structure or function BUT "StructuoFunction"

22

Cone Contrast Test Effectiveness in Staging Dry AMD

No AMD | Early Stage AMD | Intermediate AMD | Advanced AMD

"All patients in Early Stage AMD had abnormal Rabin Cone Contrast scores"



Rabin Cone CCT

Duke University 12 Month Longitudinal Study

ARVO 2022

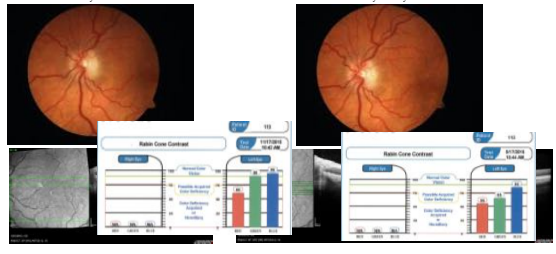
23

Intermediate Dry AMD Monitoring–What's Your Diagnosis?

What about now?

Left Eye- Nov 2015

Left Eye- May 2016



24

AMD:
Reduces Cone Contrast

Detection of Progression

- Monitor more frequently
- Early intervention initiatives
  - ✓ Nutritional changes
  - ✓ Nutritional supplement changes
    - Early and greater amount
  - ✓ Medical management when needed

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Management of Dry-AMD… current practice

Category 1	Category 2	Category 3	Category 4
No AMD	*Early Stage AMD*	*Intermediate AMD*	*Advanced AMD*
A few small or no drusen	Several small drusen or a few medium-sized drusen in one or both eyes	Many medium-sized drusen or one or more large drusen in one or both eyes	In one eye only, either a break-down of light-sensitive cells and supporting tissue in the central retinal area (advanced dry form), or abnormal and fragile blood vessels under the retina (wet form)

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antioxidants

Systematic Review

A Systematic Review of Carotenoids in the Management of Age-Related Macular Degeneration

Drake W. Lem [1], Pinakin Gunvant Davey [1,*], Dennis L. Gierhart [2] and Richard B. Rosen [3]

- Carotenoid vitamin therapy can provide visual benefits at all stages of AMD.
- Albeit these benefits may vary among different stages of disease

Antioxidants 2021, *10*, 1255. https://doi.org/10.3390/antiox10081255

27

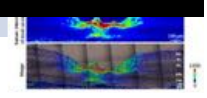
Imaging lutein and zeaxanthin in the human retina with confocal resonance Raman microscopy

Binxing Li[a], Evan W. George[a], Gregory T. Rognon[a], Aruna Gorusupudi[a], Arunkumar Ranganathan[a], Fu-Yen Chang[a], Linjia Shi[a], Jeanne M. Frederick[a], and Paul S. Bernstein[a,1]

[a]Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT 84132

Zeaxanthin	Lutein
• Mainly accumulates in the IPL, OPL, and ONL at the center of the human foveal pit • Concentrates highly in Fovea	• Distributed more diffusely across the retina at a much lower concentration relative to zeaxanthin
quenching	

Take home "the current AREDS2" formula's 10 mg of lutein and 2 mg of zeaxanthin may not be enough and greater amounts may be needed.



www.pnas.org/cgi/doi/10.1073/pnas.1922793117

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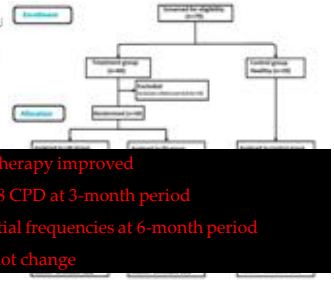
nutrients

MDPI

Visual Function and Macular Carotenoid Changes in Eyes with Retinal Drusen—An Open Label Randomized Controlled Trial to Compare a Micronized Lipid-Based Carotenoid Liquid Supplementation and AREDS-2 Formula

Pinakin Gunvant Davey [1,*], Thomas Henderson [2], Drake W. Lem [1], Rebecca Weis [2], Stephanie Amonoo-Monney [1] and David W. Evans [1]



- High dose carotenoid vitamin therapy improved
- Contrast sensitivity in 12 and 18 CPD at 3-month period
- Contrast sensitivity in ALL spatial frequencies at 6-month period
- Dark adaptation recovery did not change

- MPOD
- Dark adaptation

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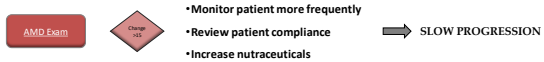
What Are You Going To Do Differently?



30

How do you monitor treatment?

- Baseline fundus photos then OCT … then … do all over in 6 months?
- So you have measured structural damage…what about the function?

- Color contrast changes
- Contrast sensitivity changes
- Dark adaptation changes
- Visual field changes
- Electrodiagnostics

Visual function changes observed in AMD

31

Diabetes and Color Vision

Old dog new tricks

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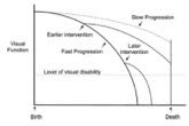
Diabetes and the Eye

Diabetic Retinopathy

- 4.2 million adults have DR in USA
- 655,000 have vision-threatening DR
- 1/3 patients with diabetes will develop retinopathy

Retina takes a good 10-15 years of beating

- During this time "looks normal" but probably not really
- Elevated blood glucose is the culprit
- Metabolic control is a must



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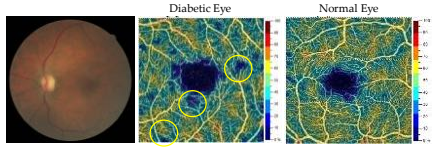
Diabetes and OCT angiography

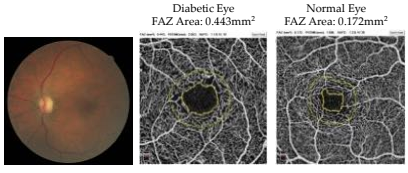
- Vascular changes



34

Identify Early Vascular Changes in Diabetic Eyes

Patients with DM more likely to have a larger FAZ than healthy eyes.

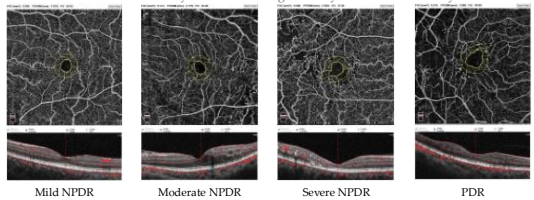


Mean Foveal avascular zone is larger in more advanced diabetic retinopathy

35

FAZ Measurements in diabetic eye disease

FAZ size and FAZ vessel density are correlated significantly with disease severity in DR.[1]



1. Nesper PL, Roberts PK, Onishi AC, et al. Quantifying Microvascular Abnormalities With Increasing Severity of Diabetic Retinopathy Using Optical Coherence Tomography Angiography. Investigative Ophthalmology & Visual Science. 2017;58(6):BIO307-BIO315. doi:10.1167/iovs.17-21787

36

Case: Diabetes Exam- What's Your Diagnosis?

Courtesy of Pinakin Davey OD, PhD

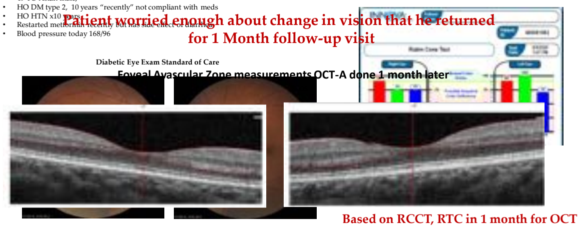
- 49 YO Asian male,
- HO DM type 2, 10 years "recently" not compliant with meds
- HO HTN x10 years
- Restarted metformin recently
- Blood pressure today 168/96

Patient worried enough about change in vision that he returned for 1 Month follow-up visit

What about now?

Diabetic Eye Exam Standard of Care

Foveal Avascular Zone measurements OCT-A done 1 month later



Based on RCCT, RTC in 1 month for OCT

37

Case: Diabetes Exam- What's Your Diagnosis?

Courtesy of Pinakin Davey OD, PhD

Adding Rabin Cone Contrast:

- Revealed changes from early diabetic eye disease which otherwise would have been missed
- Motivated patient to follow-up with 1 month visit
- Allowed for detailed discussion about diet, lower blood pressure
- Patient began diabetes nutritional supplements

0.400 0.574

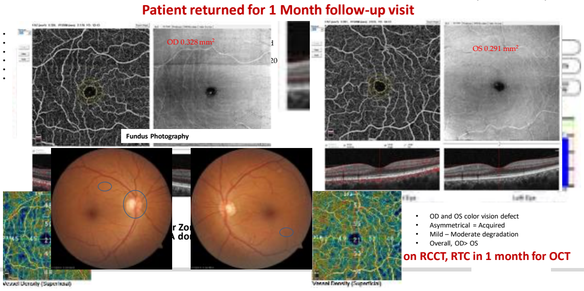
1. Nesper PL, Roberts PK, Onishi AC, et al. Quantifying Microvascular Abnormalities With Increasing Severity of Diabetic Retinopathy Using Optical Coherence Tomography Angiography. Investigative Ophthalmology & Visual Science. 2017;58(6):BIO307-BIO315. doi:10.1167/iovs.17-21787.

38

Case: Another Diabetes Exam- What's Your Diagnosis?

Courtesy of Pinakin Davey OD, PhD

Patient returned for 1 Month follow-up visit



- OD and OS color vision defect
- Asymmetrical = Acquired
- Mild – Moderate degradation
- Overall, OD> OS

on RCCT, RTC in 1 month for OCT

39

Case: Another Diabetes Exam- What's Your Diagnosis?

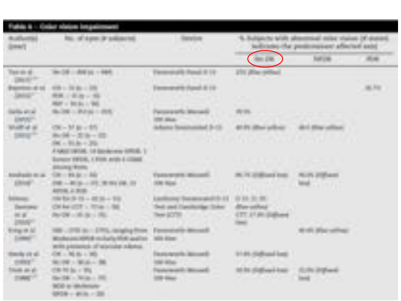
Courtesy of Pinakin Davey OD, PhD

Adding Rabin Cone Contrast:

- Revealed changes from early diabetic eye disease, prompting further exploration & leading to change in diagnosis
- Allowed for detailed discussion about diet, A1c
- Motivated patient to begin diabetes nutritional supplements
- Prompted change in monitoring frequency

40

Clinical Trials Show Color Vision as Biomarker in Diabetes



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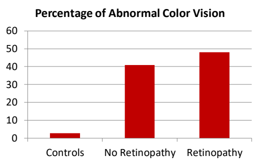
Color Vision and Neuroretinal Function in Diabetes

Wolff et al. investigates how T2DM and DR affect color vision and mfERG
84 subjects; participants included diabetics with and without retinopathy plus controls



CLINICAL STUDY RESULTS

CV
- Affected in patient with T2DM even without DR
- Central DR increases likelihood of CV deficit

mfERG
- Less frequently abnormal than CV in the absence of DR

2014 *Documenta Ophthalmologica*

42

Functional Retinal Outcomes: Prediabetes & T2DM

Karson et al. investigates how T2DM affect color vision and mfERG
43 subjects; 3 groups: Prediabetics, Type II diabetics, Controls

N. Karson et al. — Retinal function in prediabetes

Functional Retinal Outcomes in the Study Population

	Control (<= 5.6%)*	Prediabetes (5.7% - 6.4 %)*	Diabetes (>= 6.5%)*
No. Subjects	15	17	11
Avg HbA1c	5.3%	5.8%	7.0%
Color Vision Fail %	26.7%	70.6%	72.7%

CLINICAL STUDY RESULTS

CV	Prediabetic group had measurable functional changes before diabetes Color vision is the strongest biomarker
mfERG	No change in prediabetic group
CS	No change in prediabetic group

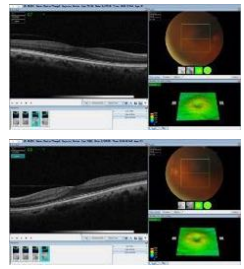
2020 *Ophthalmic & Physiological Optics*

43

Case: Diabetes Exam- What's Your Diagnosis?

- 72 y/o Indian male
- Type 2 Diabetes
- 20/25 OU
- NS1+ Cataracts OU

What about now?



Case courtesy of Becky Verma, OD

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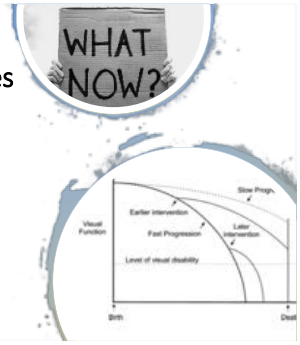
Diabetes & DR: Reduces Cone Contrast



Detection of Progression

- Monitor more frequently
- Health initiatives
  - ✓ Weight management
  - ✓ Exercise
  - ✓ Nutritional changes
  - ✓ Medical management when needed
- Nutritional supplements

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nutrients

Review

A Systematic Review of Carotenoids in the Management of Diabetic Retinopathy

Drake W. Lem [1,†], Dennis L. Gierhart [2] and Pinakin Gunvant Davey [1,*,†]

of DR, specifically in patients with type 2 or poorly managed type 1 diabetes. Meanwhile, early interventional trials with dietary carotenoid supplementation show promise in improving their levels in serum and macular pigments concomitant with benefits in visual performance. These findings provide a strong molecular basis and a line of evidence that suggests carotenoid vitamin therapy may offer enhanced neuroprotective effects with therapeutic potential to function as an adjunct nutraceutical strategy for management of diabetic retinopathy.

Nutrients **2021**, *13*, 2441. https://doi.org/10.3390/nu13072441

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Nutrients **2021**, *13*, 2441. https://doi.org/10.3390/nu13072441

47

The Diabetes Visual Function Supplement Study (DiVFuSS)

The Diabetes Visual Function Supplement Study (DiVFuSS) was designed to test the effects of a novel, multi-component nutritional supplement on visual function. Participants included patients with both type 1 and type 2 diabetes.

- 2016 *British Journal of Ophthalmology*
  - six-months
  - placebo controlled

CLINICAL STUDY RESULTS WITH DVS

Randomized, placebo-controlled study demonstrated:

- 21% improvement in color vision**
- 19% improvement in contrast sensitivity (easier to read ink on an newspaper)**
- 12% improvement in central and peripheral vision**

**Improvements were made without significantly affecting blood glucose levels

48

What Are You Going To Do Differently?

(Pre) Diabetic Exam → Score <85

- Initiate A1C testing in suspect patients & alert PCP
- Discuss lifestyle modifications earlier
- Motivate patient to begin carotenoid vitamin supplements & Omega 3's

⇒ PREVENT ONSET

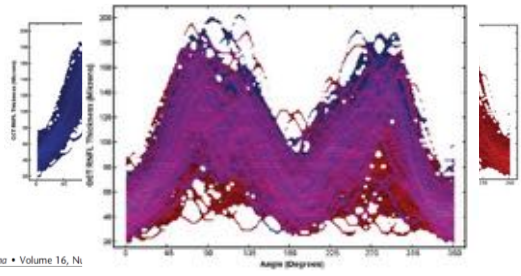
49

Glaucoma and color vision defects

Photo by Charles from Pexels

50

RNFL in a population with and without glaucoma

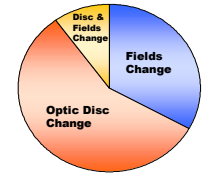


J Glaucoma • Volume 16, N

51

Does structural loss always precede functional loss in glaucoma?

- OHTS reports
- 55% of subjects reached endpoint (POAG) based on changes in the optic disc only.
- 35% of glaucoma was found by visual field changes.
- Only 10% of subjects had concurrent optic disc and visual field changes.



Kass et al., Arch Ophthalmol. 2002;120:701-703

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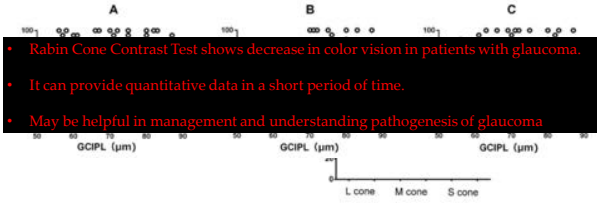
Glaucoma

Evaluation of Acquired Color Vision Deficiency in Glaucoma Using the Rabin Cone Contrast Test

Yuichi Niwa, Sanae Muraki, Fumiyuki Saito, Takayuki Minamikawa, and Masahito Ohji



- Rabin Cone Contrast Test shows decrease in color vision in patients with glaucoma.
- It can provide quantitative data in a short period of time.
- May be helpful in management and understanding pathogenesis of glaucoma

IOVS | October 2014 | Vol. 55 | No. 10 | 6687

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What Are You Going To Do Differently?

Glaucoma Exam → Change >40

- Begin treatment in borderline cases
- Adjust treatment plan

⇒ REDUCE VISION LOSS

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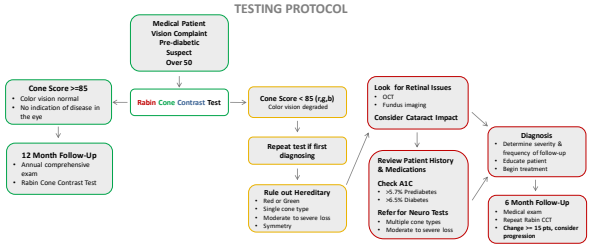
How Are You Going To Test?



55

Summary

- Adding Rabin CCT completes the comprehensive exam
- Rabin CCT allows for early detection and progression of disease
- Rabin CCT demonstrates improvement with treatment

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Carotenoids and its benefits: More than meets the eyes

Pinakin G. Davey OD, PhD, FAAO, FOWNS, FARVO
Professor & Director of Clinical Research
Western University of Health Sciences

1

Disclosures

Has a relevant financial relationship with
Sanofi, ZeaVision, Guardion Health and Innova systems as a speaker or research / consultant

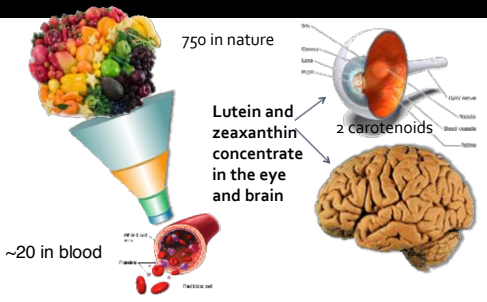
The content and format of this course is presented without commercial bias and does not claim superiority and commercial product or service.

2

Carotenoids- Xanthophylls the Basics

3

Carotenoids



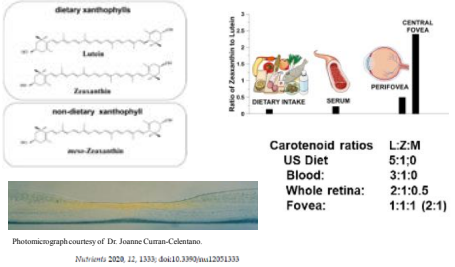
4

Macular Xanthophylls

- **Around 50 carotenoids consumed**
- **Around 20 or so see in serum**
- **Two that are obtained in diet make it to all over the body (Lutein and zeaxanthin)**
- **RPE65 converts lutein to meso-zeaxanthin in retina**

5

Carotenoids in retina-Xanthophylls

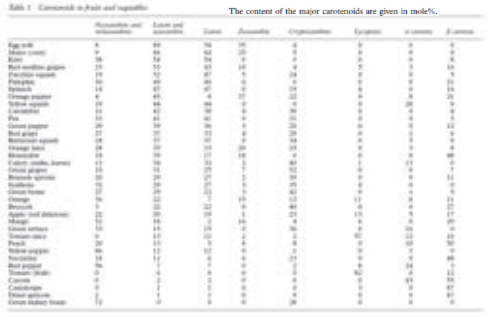


Photomicrograph courtesy of Dr. Joanne Curran-Celentano.

Nutrients **2020**, *12*, 1333; doi:10.3390/nu12051333

6

The content of the major carotenoids are given in mole%.



Br J Ophthalmol 1998;82:907–910

SCAN ME

7

Carotenoids food sources

Foods	Serving Size	Lutein + Zeaxanthin Content (mg)
Spinach, frozen (cooked)	1 cup	29.8
Kale, frozen (cooked)	1 cup	25.6
Swiss chard (cooked)	1 cup	11.0
Collard greens, frozen (cooked)	1 cup	8.9
Summer squash (cooked)	1 cup	4.0
Peas, frozen (cooked)	1 cup	3.8
Brussel sprouts, frozen (cooked)	1 cup	2.4
Broccoli, frozen (cooked)	1 cup	2.0
Edamame, frozen	1 cup	1.6
Sweet yellow corn (boiled)	1 cup	1.5
Asparagus (boiled)	0.5 cup	0.7
Avocado, raw	1 medium-size	0.4
Egg yolk, raw	1 large	0.2

Meso-zeaxanthin is not found in common food : in shrimp shells, turtle fat, and fish skin

Lem, D.W.; Gierhart, D.L.; Davey, P.G. A Systematic Review of Carotenoids in the Management of Diabetic Retinopathy. *Nutrients* **2021**, *13*, 2441. https://doi.org/10.3390/nu13072441

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nutrients MDPI

Review

Why is Zeaxanthin the Most Concentrated Xanthophyll in the Central Fovea?

Justyna Widomska *, John Paul SanGiovanni and Witold K. Subczynski

- Very potent antioxidant-particularly in region of high oxygen tension and metabolism compared to lutein
- Zeaxanthin structure more stable in the lipid bilayer membranes
- Zeaxanthin is less predisposed to destruction than lutein when counteracting oxygen singlets

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Imaging lutein and zeaxanthin in the human retina with confocal resonance Raman microscopy

Binxing Li, Evan W. George, Gregory T. Rognon, Aruna Gorusupudi, Arunkumar Ranganathan, Fu-Yen Chang, Linjia Shi, Jeanne M. Frederick, and Paul S. Bernstein

Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT 84132

Zeaxanthin	Lutein
• Mainly accumulates in the IPL, OPL, and ONL at the center of the human foveal pit • Concentrates highly in Fovea centralis • Fovea high risk of Light induced oxidate damage, singlet oxygen	• Distributed more diffusely across the retina at a much lower concentration relative to zeaxanthin • Evenly and low concentration across macula

Take home "the current AREDS2" formula's 10 mg of lutein and 2 mg of zeaxanthin may not be enough and greater amounts may be needed.

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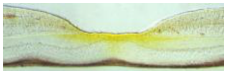
Carotenoids influence visual function

- **Optical mechanisms**
  - **Glare Disability,**
  - **Color Contrast**
  - **Visual Range**
  - **Contrast Sensitivity**
- * Biological mechanisms
  - **Glare Recovery**



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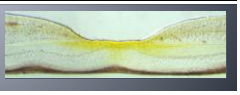
Macula pigment optical density- A biomarker



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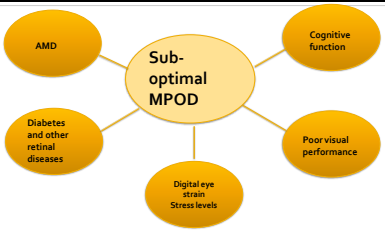
MPOD is a Biomarker



PG Davey et al, Differences in macular pigment optical density across ethnicities: A comparative study *Ther Advances in Ophthalmol 2020*

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Clinical devices

QUANTIFEYE- ZEAVISION

MAPCATSF- GUARDION HEALTH

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Measurement of Macular Pigment Optical Density

- Heterochromatic flicker Photometry- principle
- Macular pigment absorbs blue light (not green light)
- More macular pigment = longer time you see flicker
- Results are quantified via software

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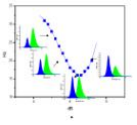
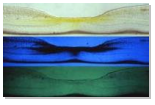
QuantifEye® MPS II Instrument
(simple efficient , 2 -3 minute test)

Centre Flickering Target





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Things I wanted to know about measuring MPOD

- Is it easy? Yes
- Do I need to perform in both eyes? NO
- Correlation between eyes?
- Is it repeatable? Excellent
- How long does it take? 2 minutes for testing
- Dominant eye? Any eye
- Can it measure changes? Yes

Yes

Davey PG et al., Clin Ophthalmol. 2016 Aug 29;10:1671-8. doi: 10.2147

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AMD classification, hypothesis and more

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Chronic disease- AMD

- **AMD in USA** 3-3.5 million 2020
- 196 million worldwide 2020; 288 million 2040
- AMD # 1 cause of legal blindness in the developed world.
- 7.1% of individuals over the age 75 years have late stage AMD

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AMD

Dry

Chronic
visual acuity remains unchanged for long, some degree vn loss, may progress to severe blindness

Wet

10-15% of AMD
Vision dramatically reduced

Advanced AMD
Geographic Atrophy
Or choroidal neovascular growth

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Classification and pathogenesis

- Although neat to classify as dry and wet
- There is overlap of pathogenesis
- The end stage of dry AMD continues into wet AMD
- So important to understand that wet AMD pathogenesis continues in the background of dry AMD
- Neovascular AMD-Anti VEGF
- Dry AMD- Vitamins

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AMD a journey

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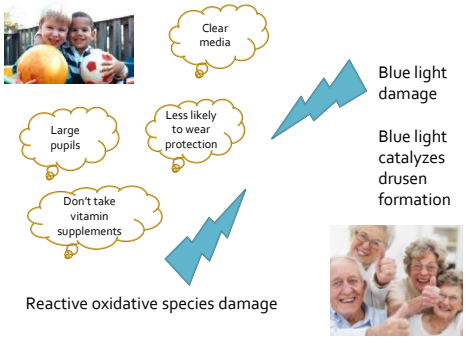

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Dry- AMD

PATHOGENESIS

- Exact pathogenesis unknown
- Oxidative damage due to higher oxygen levels and reactive oxidation species (ROS)
- Blue light
- Lifetime light exposure

NATURAL PROTECTION

- Antioxidants present in eye
  - Vitamin C
  - Zeaxanthin
  - Meso-zeaxanthin
  - Leutin

  } Natural filter of blue light
- Pupils become smaller with age
- Yellowing of lens cuts of blue

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Lutein + Zeaxanthin and Omega-3 Fatty Acids for Age-Related Macular Degeneration

The Age-Related Eye Disease Study 2 (AREDS2) Randomized Clinical Trial

- No true placebo- patients got AREDS formula
- Addition of lutein (10 mg)+zeaxanthin (2mg)+ EPA (650 mg) + DHA (350 mg) did not further reduce the risk of progression to Advanced AMD
- More lung cancer was noticed in β-carotene group compared to no β-carotene

JAMA. 2013;309(19):2005-2015
Published online May 5, 2013. doi:10.1001/jama.2013.4997

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Population of AREDS-2

- Extremely educated
- Well nourished population

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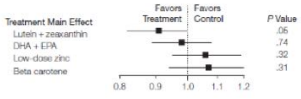
Sub-group analysis



Specially lutein and zeaxanthin was most beneficial when the individuals were taking it had lowest level to begin with

Lower MPOD is now accepted as a modifiable risk factor to AMD

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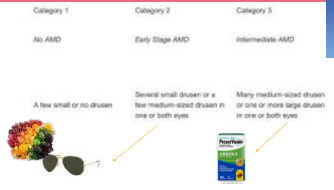
Management of Dry-AMD… current practice

Is this good enough?



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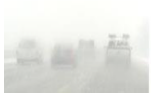
Functional vision is affected in Early AMD

Glare Disability

Glare Recovery

Color vision



Contrast (CSF)

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How do you monitor treatment?

- Baseline fundus photos then OCT … then … do all over in 6 months?
- So you have measured structural damage…what about the function?

- Contrast sensitivity changes
- Color contrast changes
- Dark adaptation changes
- Visual field changes
- Electrodiagnostics

Visual function changes observed in AMD

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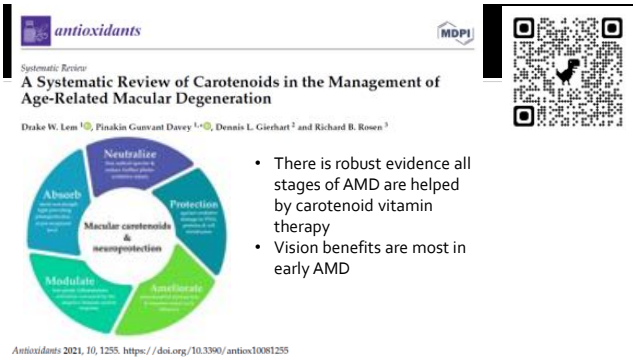

- There is robust evidence all stages of AMD are helped by carotenoid vitamin therapy
- Vision benefits are most in early AMD

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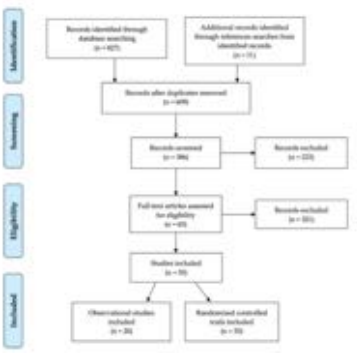

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Summary of various RCTs in AMD

- Increase in serum levels
- Increase in MPOD
- Enhanced central retinal functions mfERG
- Slight benefits to BCVA
- Contrast improvements
- Glare improvements
- Mesopic vision improvements
- Risk reduction to progression

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What can we do different than AREDS?

TESTING

- Measure MPOD
- Measure functional tests
  - Contrast sensitivity
  - Color Contrast
  - Glare function

MANAGEMENT

- More amounts of carotenoids
- NSF certification
- Omega- 3
  - Enhances carotenoid absorption
- Measure baseline MPOD
  - Check it every 3 months
  - Monitor compliance
  - Monitor uptake
- Check Functional vision tests
  - Monitor improvement

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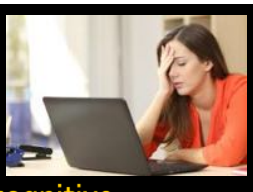

Screen time stress and cognitive performance

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Carotenoids and health ?

- Carotenoids in macula improves vision and decreases ocular fatigue- easy sell
- But not so straightforward….
- Cortisol, stress ???

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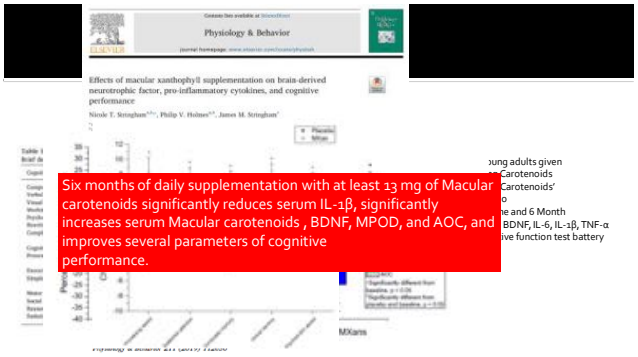

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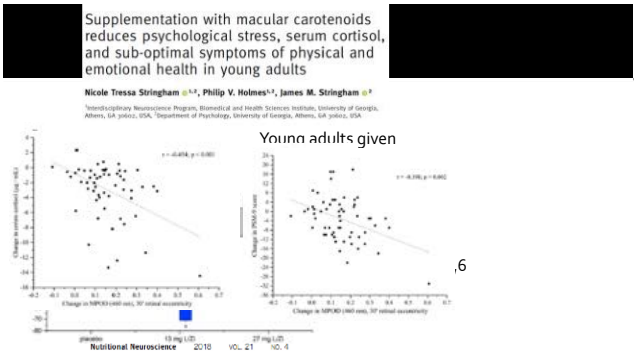

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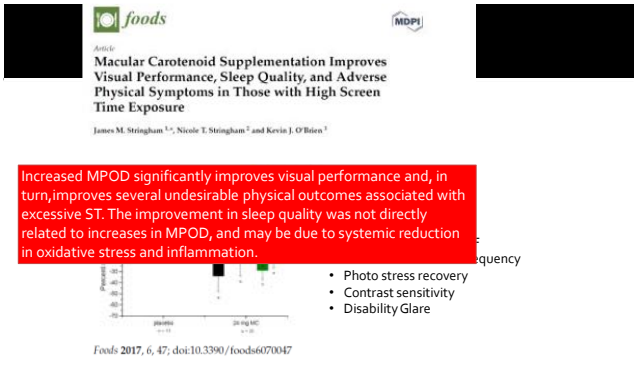

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Summary

- Carotenoid supplementation has a real role in decreasing stress and betterment.
- Dose matters
- Duration matters -6-12 months effects visible
- Don't turn your computers on unless you have taken your Lutein and Zeaxanthin
- Don't be Lazy; take your LZ (Lutein and Zeaxanthin)

D Lem and PG Davey Tackle Digital eye strain *Opt Management article*

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Association of MPOD and cognition Children and Adults

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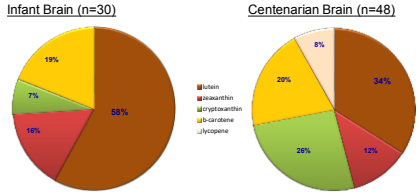
Brain carotenoid profile in infants and centenarians



Vishwanathan et al. 2014 Johnson EJ et al. 2013

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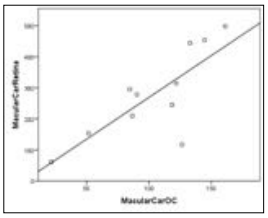
Correlation – Human retina and occipital cortex concentrations of lutein and zeaxanthin. have an amazingly strong correlation



n=11	r	*P* value
Bivariate	0.784	0.004
Age & sex adjusted	0.834	0.010
Age, sex & cognitive status adjusted	0.887	0.008

Macular pigment carotenoids = Lutein (*Meso-zeaxanthin*) + *Zeaxanthin* in the retina

Vishwanathan R, Schalch W, Johnson E J.Nutr Neurosci. 2016;19(3):95-101

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Renzi et al., The relation between serum xanthophyllls, fatty acids, macular pigment and cognitive function in the Health ABC Study. *FASEB J* 2008;22:877.5.

- n = 118 healthy older subjects in the Memphis, Tennessee area
- ages 76–85 y; equal numbers of men and women, were assessed for serum lutein and zeaxanthin, MPOD, and various measures of cognitive function.

MP was related to performance on a variety of indexes designed to assess processing **speed, accuracy, and completion ability** (P < 0.05).

45

Hammond BR et al , Effects of lutein/zeaxanthin supplementation on the cognitive function of community dwelling older adults: A Randomized, Double-Masked, Placebo-Controlled Trial, *Front. Aging Neurosci.* 9:254, 2017.

- AREDS II carotenoid dosing (12 mg LZ) was evaluated in community dwelling older adults 73.7 +/- 8.2 yrs. of age.
- Participants receiving the active LZ dietary supplement had statistically significant increases in MPOD (P<0.03)

Improvements in complex attention (p<0.02) and cognitive flexibility domains (p < 0.04) relative to study participants taking the placebo.

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Dietary carotenoids related to risk of incident Alzheimer dementia (AD) and brain AD neuropathology: a community-based cohort of older adults

Changzheng Yuan,[1,2,3] Hai Chen,[1] Yamin Wang,[4] Julie A Schneider,[5] Walter C Willett,[2,3,6] and Martha Clare Morris[4]

[1]Department of Big Data and Health Science, Zhejiang University School of Public Health, Hangzhou, Zhejiang, China; [2]Department of Nutrition, Harvard TH Chan School of Public Health, Boston, MA, USA; [3]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; [4]Rush Institute for Healthy Aging, Rush University Medical Center, Chicago, IL, USA; [5]Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA; and [6]Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, MA, USA

927 participants (No AD) were followed up for 7 years- Rush Memory and Ageing project AD neuropathy was assessed in 508 deceased participants

Results were controlled for Age, sex, education, cognitively stimulating activities, physical activities, Apolipoprotein

Conclusions: Our findings support a beneficial role of total carotenoid consumption, in particular lutein/zeaxanthin, on AD incidence that may be related to the inhibition of brain β-amyloid deposition and fibril formation.

Am J Clin Nutr 2021;113:200–208.

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Macular pigment optical density is positively associated with academic performance among preadolescent children

Results: The regression analyses revealed that MPOD improved the model, beyond the covariates, for overall academic achievement ($\Delta R^2 = 0.10$, $P < 0.01$), mathematics ($\Delta R^2 = 0.07$, $P = 0.02$), and written language composite standard scores ($\Delta R^2 = 0.15$, $P < 0.01$).

Discussion: This is the first study to demonstrate that retinal L and Z, measured as MPOD, is positively related to academic achievement in children, even after accounting for the robust effects of IQ and other demographic factors. These findings extend the positive associations observed between MPOD and cognitive abilities to a pediatric population.

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Renzi-Hammond LM et al, Effects of a Lutein and Zeaxanthin Intervention on Cognitive Function: A Randomized, Double-Masked, Placebo-Controlled Trial of Younger Healthy Adults, *Nutrients* 2017

Daily supplementation with LZ in healthy 18-30 year old, resulted in significant improvements in spatial memory ($p<0.04$), reasoning ability ($p<0.05$) and complex attention ($p<0.04$), **"above and beyond improvements due to practice effects".**

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Maternal Intake of Lutein and Zeaxanthin during Pregnancy Is Positively Associated with Offspring Verbal Intelligence and Behavior Regulation in Mid-Childhood in the Project Viva Cohort

Conclusions: Higher maternal L/Z intake during pregnancy was associated with better offspring verbal intelligence and behavior regulation ability in mid-childhood, suggesting a potential benefit during prenatal development. We did not find a benefit of higher maternal L/Z intake on other child cognitive or behavioral outcomes. Project Viva is registered at clinicaltrials.gov as NCT02820402. *J Nutr* 2021;00:1–13.

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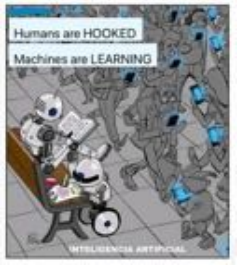
"Children" and smart devices



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Healthy eyes and Sports vision

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Visual Performance Research

Billy Hammond PhD, Emily Bovier PhD, Lisa Renzi PhD, University of Georgia; Athens, GA
A Double-Blind, Placebo-Controlled Study on The Effects of Lutein and Zeaxanthin on Neural Processing Speed and Efficiency. (Published: PLOS One, September, 2014)
N= 64 young adults aged 18-32 years.
3 arm study: 29 subjects took 20 mg of zeaxanthin (carotenoids), 25 subjects took the Eye Promise Vizual EDGE PRO supplement (26mg of zeaxanthin, 34mg of carotenoids), and 10 subjects took placebo, duration 4 months.
Purpose of the study: to determine whether improving MPOD via zeaxanthin (20 mg) or mixed carotenoid (Eye Promise Vizual EDGE PRO) supplementation improved neural efficiency and visual motor performance in young, healthy, adults.

Summary: Subjects in the zeaxanthin and EyePromise vizual EDGE arms experienced;
- **A 20% Increase in Macular Pigment Optical Density (MPOD)**
- **A 12% Improvement in Critical Flicker Fusion Threshold**
- **A 10% Improvement in Visual Motor Reaction Time**

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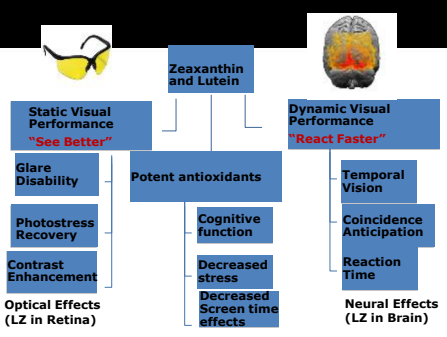

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Evaluating & Increasing Macula Pigment

- Better Visual Quality
- Better "Day and Night" Driving Vision
- Better Blue Light Protection
- Better Cognition
- Better Sports Vision
- Better Sleep and less stress
- "Better Eye Exam and Better Care"

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Global summary

- It is tough to get perfect nutrition everyday.
- Nutritional supplements can be a reliable way of augmenting your diet.
- Carotenoids are important for vision
- Maybe even more for health than we thought!
- Measuring MPOD allows for a trackable measure in various health and disease states- compliance and bioavailability measure.
- An ounce of prevention…

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Thank You!

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