

Preliminary Results in Macular Pigment Optical Density Associated with and without Zeaxanthin and Lutein Supplementation

Abstract

Purpose: To determine the change in macular pigment optical density (MPOD) after subjects with low macular pigment decide to either take lutein and zeaxanthin supplementation or forgo supplementation for one year.

Methods: One hundred and ninety eight healthy subjects, 122 women, and 76 men in a clinical setting with low MPOD scores comprised two groups, who either take Lutein (L) and Zeaxanthin (Z) supplements or do not take supplements. All were followed for 12 months.

Results: After 12 months, MPOD had increased by 0.11 AU in the supplement group and decreased to 0.06 AU in subjects who opted not to take supplements. The changes between the two groups were statistically significant ($p < 0.0001$).

Conclusion: Adding zeaxanthin and lutein, along with other minerals and antioxidants to the diet in subjects with low MPOD resulted in an increase in MPOD scores. Interestingly, in subjects that did not add supplementation to their diet the MPOD scores were reduced. Although diet can play a major role in the increase or decrease of MPOD, our data point to the possibility of enhancement of low MPOD through supplementation.

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Abbreviations: MPOD: Macular Pigment Optical Density; L: Lutein; Z: Zeaxanthin; MZ: Meso-Zeaxanthin; LAST: Lutein Antioxidant Supplementation Trial; AU: Absorption Unit; HFP: Heterochromatic Flicker Photometry; ZVF: Zeaxanthin and Visual Function; POLA: Pathologies Oculaires Lie'esa'Yage

Introduction

Age Related Macular Degeneration (ARMD) is a lifelong oxidative disease process culminating in patient's loss of vision, loss of mobility, and loss of normal life functionality [1]. The cause of this reduced function is damage to the photoreceptors in the macula [2]. Of great concern are patients who are at risk for macular degeneration yet unidentified because of the lack of accepted integrated screening techniques designed to identify patients who are predisposed to ARMD. If patients at risk for ARMD could be identified before vision loss occurs, preventive measures could be instituted. By the year 2020 it is predicted that there will be 15 million people afflicted with this life-changing disease [3]. Estimates show that roughly one in three over the age of sixty-five will be diagnosed with ARMD [4]. With increased life expectancy, this disease will continue to have a significant public health impact on the quality of life worldwide. Many studies have shown that diet and supplementation can increase Macula Pigment Optical Density (MPOD) [5-7] but no studies to date have shown that MPOD can be reduced without the ingestion of lutein or zeaxanthin either by diet or supplementation in a population without signs or symptoms of ARMD.

Factors affecting a patient's risk for ARMD include exposure to the sun, family history, gender, age, medical history, smoking, poor diet, obesity, and low levels of macular pigment. Individuals that experience excess exposure to sunlight coupled with low serum levels of antioxidants are among the highest at risk. Nolan et al. [8] summarized that any protective effect of MP depends on its ability to guard against chronic and cumulative retinal oxidative damage provoked either by phototoxic blue light or as a result of high oxygen metabolism, will need to be monitored in the young to middle-age.

While the cornea and lens absorb the shorter wavelengths of blue light, longer wavelengths of blue light do pass through to the retina causing cumulative damage over time [9]. One function of the macula pigment is to filter blue light so as not to create an inflammatory response within the retina [10]. Macular pigment is thought to protect against retinal damage by filtering out phototoxic short-wavelength visible light and by defending rod outer segment membranes from oxidative stress [11]. When individuals do not have sufficient macular pigment they are at higher risk of developing ARMD [12-14] because structures posterior to the macular pigment will have a greater exposure to the blue light compared to those of a person with normal to high levels of macular pigment. As a result, we might expect a greater incidence of ARMD among people having a low macular pigment density. In addition to the macular pigment functioning to filter high-energy blue light, it also functions as an antioxidant reducing toxic free radicals. This is important, as the retina is highly susceptible to oxidative stress given its high oxygen consumption and high metabolic turnover.

Macular pigment is a yellow compound, is preferentially distributed in the fovea in the Henle fiber layer which consists of foveal cones' axons, and in the para fovea; macular pigment is also located in the inner plexiform layers of the retina and RPE (?). It is composed mainly of two carotenoids, lutein, and zeaxanthin which are thought to act as antioxidants and filters of blue light. Zeaxanthin and lutein are not synthesized by the human body and are found in fruits and green leafy vegetables. Their concentration correlates to macular pigment optical density (MPOD) [15]. Meso-zeaxanthin (MZ) is also present in the macula due to the conversion of lutein to MZ. Unlike zeaxanthin and lutein, MZ while not a significant component of a normal diet, can nonetheless be absorbed into the serum [16]. Where as in another study Meso-zeaxanthin is synthesized through a chemical alteration of lutein and not present in serum [17].

MPOD is modified by peak blood serum levels of lutein and zeaxanthin, therefore dietary intake affects MPOD levels. Researchers have increased macular pigment density through dietary intake of foods such as spinach and corn, [18,19] and through lutein/zeaxanthin supplementation [20]. A higher dietary intake of foods rich in lutein and zeaxanthin has been associated with a lower risk of developing advanced exudative ARMD [21].

The value of antioxidant intake on ARMD progression was demonstrated by a reduction in progression of 17% in patients taking only antioxidants, a 21% reduction in progression in patients only taking zinc (Blue MT), and a 25% reduction in progression in patients taking antioxidants and zinc [22]. Richer et al. [23] point out in the Lutein Antioxidant Supplementation Trial (LAST) study that those individuals in greatest need of supplementation, those having the lowest levels of measured macular pigment optical density (MPOD), represent the population with the greatest increase in MPOD.

The purpose of this paper is to investigate whether the intake of carotenoids, lutein and zeaxanthin, can change absorption unit (AU) and thereby change MPOD tested by heterochromatic flicker photometry. This pilot study documented the effects on the MPOD of those subjects without ARMD but suffering from low MPOD levels who decided to accept the therapy of lutein and zeaxanthin and to subjects who decide to forgo the therapy. This pilot evaluation was to compare the change in MPOD in subjects with MPOD scores below 0.30 as a function of supplementation or no supplementation.

Methods

Subjects were eligible for this pilot investigation if they were over the age of 18 and had low MPOD readings. For the purpose of this study, we have defined low MPOD to be 0.30 based upon the average values in normal subjects in the literature [24-28]. The other eligibility criteria were self-identified good health and the ability to return for a second comprehensive eye examination performed 12 months after the initial examination. Subjects were contacted by mail to participate, informed of the nature of the study, and asked to sign an informed consent form approved by the IRB. The study followed the tenets of the Declaration of

Helsinki. Each subject recruited into the study was identified as having an annual comprehensive eye examination with a fundus evaluation to ensure that no observable pathologies were present.

Subjects were given the option of adding EyePromise® Restore, a dietary supplement containing Zeaxanthin, Lutein, Omega 3 fatty acids, Tocopherols and antioxidants to their daily intake. Subjects included in this study composed two groups, those who accepted the option of supplementation and those who decided not to add supplementation to their diet. The supplement was a single-dose, containing 4 mg Lutein, 8 mg Zeaxanthin, 120 mg Vitamin C (ascorbic acid), 60 IU Natural Vitamin E (d-alpha tocopherol), 125 mg Omega 3, 10 mg alpha lipoic acid, and 6 mg of mixed tocopherols. Supplemented subjects consumed one soft gel daily for one year and were instructed to take the supplement with a meal that contained at least a small amount of fat providing good bioavailability.

MPOD was measured by the QuantifEye® instrument, distributed by Zeavision. The QuantifEye® uses heterochromatic flicker photometry to determine the level of filtering properties of the macular pigment which provides diagnostic information about MPOD. The MPOD measurement is defined as absorbance which is the same as optical density unit ODU or absorbance unit AU. This is a logarithmic unit used to measure optical density, the absorbance of light transmitted through a partially absorbing substance. If T is the percentage of light transmitted, then the absorbance is defined to be $-\log_{10} T$ absorbance units. No other data were collected.

Kinkelder determined the QuantifEye device, MPS 9000 series: Tinsley Precision Instruments Ltd., Croydon, Essex, had good correlation with a fundus reflectance method. They found high agreement between test and retest measurements of QuantifEye (0.02 ± 0.18) and the fundus reflectance method. Kinkelder [29] suggested the Macuscope (MacuVision Europe Ltd., Lapworth, Solihull, UK, was not a repeatable and reliable test because of low agreement with test and retest measurements. They also found the macuscope had poor agreement with fundus reflectance measurements. Others have found similar results and is the reason why we determine the QuantifEye was the best test for our investigation [30-37]. At each visit patients were tested twice using the technique of heterochromatic flicker photometry (HFP).

Means were calculated for MPOD at the baseline and twelve-month visit. Comparisons were made between the two groups at baseline using a t-test to determine similarity at baseline. Changes over time between the two groups were also completed using a t-test, a chi-square test was used to compare categorical variables, and an analysis of variance that controlled for the baseline amount of MPOD was done.

Results

One hundred and ninety eight healthy subjects (n=198) in total, 122 women, and 76 men equally were divided into two groups: one group (n=100) opted to take the supplement and the other group (n=98), opted not to take the supplementation both group patients were followed up with ocular exams at 12 month

interval. Table 1 presents the MPOD data for the subjects for the two visits. Baseline MPOD for the subjects who supplemented was 0.27 AU ± 0.13, while those who did not supplement had a baseline MPOD of 0.25 AU ± 0.13. The two groups did not differ significantly at baseline (p-value = 0.21). After 12 months, the MPOD had increased to 0.38 AU ± 0.15 in the supplement group and had decreased to 0.19 AU ± 0.07 in those who chose not to supplement. The changes between the two groups were statistically significant (p < 0.0001) (Figure 1), even after controlling for baseline MPOD in an analysis of variance.

Table 2 categorizes subjects by their change in MPOD (no change, increase, or decrease) over the year of supplementation. Seventy-eight percent (78%) of subjects had an increase in their MPOD scores after one year of supplementation. Three-quarters (3/4) of the subjects who did not take supplements had a decrease or remained the same in their MPOD scores after one year (chi square p-value < 0.0001).

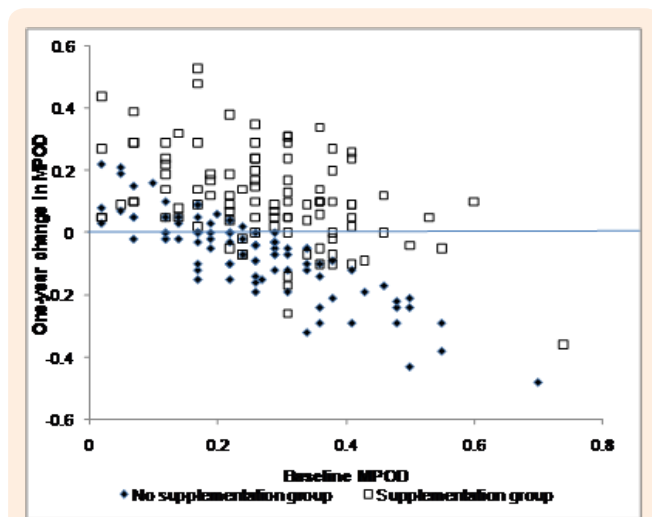


Figure 1: Baseline MPOD versus one-year change in MPOD by supplementation group.

Table 1: Mean MPOD by visit by the presence of supplementation.

	Patients who took Supplements	Patients who did not take Supplements
Number of Patients	100	98
Mean MPOD at baseline (± sd) AU	0.27 (0.13)	0.25 (0.13)
Mean MPOD at 12 months	0.39 (0.15)	0.19 (0.07)
Mean change MPOD	0.11 (0.15)	-0.06 (0.13)

Table 2: Percentage of patients who experienced a change in MPOD over one year.

One-Year Change	Patients who took Supplements n (%)	Patients who did not take Supplements n (%)
Negative Change	18 (18.0)	62 (63.3)
No Change	4 (4.0)	12 (12.2)
Positive Change	78 (78.0)	24 (24.5)

Discussion

This pilot study was designed to investigate the change in MPOD measurement for a group of subjects without ARMD but who did have low MPOD levels taking lutein/zeaxanthin supplement and for a group who did not take the supplement. This study questioned the modifiability of MPOD scores by changing subject's ingestion of antioxidant supplements. At the end of the study, those subjects who agreed to take supplements had an average MPOD of 0.39 AU. This is similar to a report from Van der Veen who studied the US population and found that MPOD scores averaged 0.33 AU, although no investigation has studied the optimal range of acceptable MPOD [38]. Additionally those subjects who did not add supplementation to their diet not only failed to show increase in macular pigment on average but in the majority of cases showed a decrease from baseline levels. MPOD is a variable measurement dependent on renewal, ingestion, absorption, metabolism, and utilization. This study only looked at the ingestion aspect of lutein and zeaxanthin.

A reduced macular pigment layer allows blue light through to the photoreceptors and reduces its protective nature. Maintaining high levels of both zeaxanthin and lutein either by supplementation or the ingestion of foods containing these carotenoids to increase the macular pigment layer may reduce this one risk factor of ARMD. While no study has evaluated subjects with low MPOD prior to the onset of ARMD, the Blue Mountain Study, Beaver Dam Study and Aged-Related Eye Disease Study have shown that a higher dietary intake of lutein and zeaxanthin is associated with a reduced risk of developing advanced ARMD [39-41]. The LAST study demonstrated that lutein in combination with other antioxidants significantly increased MPOD and glare recovery, near visual acuity, and contrast sensitivity [42]. The outcomes of the LAST study supported the notion that subtle signs of photoreceptor and retinal pigment epithelium disturbances characteristic of ARMD, such as glare recovery difficulties, degraded contrast sensitivity, scotomas, and metamorphopsia often occur long before the appearance of obvious fundus signs, when up to 80% of photoreceptors and retinal pigment epithelium complexes are already gone. The conclusion of the study raised the possibility that antioxidant intervention may be useful in patients without macular degeneration to protect the retina.

"The Zeaxanthin and Visual Function (ZVF) Study in AMD" concluded that zeaxanthin increased estimated Macular Pigment in AMD patients similar to lutein at 1 year. Researchers determined that adding zeaxanthin supplementation to an ARMD patients' vitamin regimen is logical based on the Zeaxanthin's

foveal distribution [43]. Finally the Pathologies Oculaires Lie`esa`l'Age (POLA) survey assessed the association of plasma lutein and zeaxanthin with the risk of ARMD and cataracts. The results showed that high levels of serum zeaxanthin reduced macular degeneration by 93% and nuclear cataracts by 75 % [44].

When evaluating whether the change in MPOD over the year is clinically significant consideration must be given to the magnitude of change with respect to expected measurement variability. Based upon publications by Van der Veen [37] and Bartlett [44], we believe that a change exceeding 0.08 AU represents an actual improvement (or decline in the negative direction) in MPOD that is beyond measurement error. Based on this criterion, we saw meaningful improvement in 58% of those patients taking supplements (i.e., a change in MPOD greater than 0.08). By this same criterion, we saw 39% of those who did not take supplements experience a meaningful decline in MPOD (i.e., a decrease in MPOD of more than 0.08).

An obvious limitation to our study was the fact that the subjects were not randomized into the two study group's supplement or no supplement groups. Therefore, there is the potential for bias when a subject self-selects to participate in a treatment. Because of this, and because of a lack of additional information collected, there can be alternative explanations for the change in MPOD scores. Diet was not a variable investigated. Subjects who scored low initially were educated about the advantages of vitamin supplementation and diet change. These individuals could have ingested enough antioxidants in their normal diet without supplementation to skew our results. The bioavailability of the antioxidants was also not monitored. A subject's body mass index was another variable not taken in consideration and could have skewed the results. Antioxidants have a preference to deposition in body fat over retinal deposition. Although subjects were asked if supplementation was taken daily, subjects were not monitored on the number of antioxidants ingested on a monthly basis. Also age was not a controlling variable in our population and could have biased our results as well as only testing MPOD on one eye in each subject.

Our study demonstrated in a traditional clinical setting that increasing the intake of zeaxanthin, lutein and other vitamins positively increased the measurement of MPOD using heterochromatic flicker photometry in our low MPOD population. Subsequent investigations will study the prevalence of low MPOD and how it relates to visual function such as glare recovery and photo stress. Also additional studies need to quantify the optimal range of MPOD scores to document the level of MPOD safety for protection against AMD.

Conclusion

In this traditional clinical office patient population, prescribing a dietary supplementation with zeaxanthin and lutein and other minerals and antioxidants for a year resulted in an increase in MPOD. In subjects that did not add supplementation to their diet on average reduced their level of MPOD. Although diet can play a major role in the increase or decrease of MPOD, our data point to the possibility of enhancement through supplementation.

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References

1. Hassell JB, Lamoureux EL, Keeffe JE (2006) Impact of age related macular degeneration on quality of life. *Br J Ophthalmol* 90(5): 593-596.
2. Carpentier S, Knaus M, Suh M (2009) Associations between lutein, zeaxanthin, and age related macular degeneration: an overview. *Crit Rev Food Sci Nutr* 49(4): 313-326.
3. Gottlieb JL (2002) Age-related macular degeneration. *JAMA* 288(18): 2233-2236.
4. Mares-Perlman JA, Brady WE, Klein R, Klein BE, Bowen P, et al. (1995) Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol* 113(12): 1518-1523.
5. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, et al. (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci* 42(2): 439-446.
6. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, et al. (2001) Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci* 42(1): 235-240.
7. Trieschmann M, Spital G, Lommatzsch A, van Kuijk E, Fitzke F, et al. (2003) Macular pigment: quantitative analysis on autofluorescence images. *Graefes Arch Clin Exp Ophthalmol* 241(12): 1006-1012.
8. Nolan JM, Stack J, Donovan O, Loane E, Beatty S (2007) Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res* 84(1): 61-74.
9. Schalch W, Dayhaw-Barker P, Barker FM (1992) The Carotenoids of the human retina. In: *Nutritional and Environmental Influences on the Eye*. Taylor A (Ed.), Boca Raton, FL: CRC Press, USA, pp. 215-250.
10. Ham WT, Mueller WA (1989) The photo pathology and nature of the blue-light and near-UV retinal lesion produced by lasers and other optical sources. In: *Laser Applications in Medicine and Biology*. Wolbarsht ML (Ed.), Plenum Press, New York, USA, pp. 191-246.
11. Schalch W, Dayhaw-Barker P, Barker FM (1999) The carotenoids of the human retina. In: Taylor A (Ed.), *Nutritional and Environmental Influences of the Eye*. Boca Raton, FL: CRC Press, USA, pp. 215-250.
12. Tan J, Wang J, Flood V, Rochtchina E, Smith W, et al. (2008) Dietary Antioxidants and the Long-term Incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology* 115(2): 334-341.
13. Richer S, Davenport J, Lang JC (2007) LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age related macular degeneration to dietary supplementation with xanthophylls. *Optometry* 78(5): 213-219.
14. Nolan J M, Stack J, O Donovan O, Loane E, Beatty S (2007) Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res* 84(1): 61-74.
15. Bone RA, Landrum JT, Guerra LH, Ruiz CA (2003) Lutein and Zeaxanthin Dietary Supplements Raise Macular Pigment Density and Serum Concentrations of these Carotenoids in Humans. *J Nutr* 133(4): 992-998.

16. Bone RA, Landrum JT, Cao Y, Howard AN, Alvarez-Calderon F (2007) Macular pigment response to a supplement containing meso-zeaxanthin, lutein and zeaxanthin. *Nutr Metab* 4:12.
17. Neuringer M, Sandstrom MM, Johnson EJ, Snodderly DM (2004) Nutritional manipulation of primate retinas: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest Ophthalmol Vis Sci* 45(9): 3234-3243.
18. Hammond BR Jr, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci* 38(9): 1795-1801.
19. Johnson EJ, Hammond BR, Yeum KY, Qin J, Wang XD, et al. (2000) Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr* 71(6): 1555-1562.
20. Berendschot TT, Goldbohm RA, Klöpping WA, van de Kraats J, van Norel J, et al. (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci* 41(11): 3322-3326.
21. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, et al. (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA* 272(18): 1413-1420.
22. Age-Related Eye Disease Study Research Group (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene and zinc for age related macular degeneration and vision loss: AREDS report no.8. *Arch Ophthalmol* 119(10): 1417-1436.
23. Richer S, Davenport J, and Lang J (2007) LAST II: Differential temporal responses of macular pigment optical density in patients with atrophic age related macular degeneration to dietary supplementation with xanthophylls. *Optometry* 78(5): 213-219.
24. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, et al. (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci* 42(2): 439-446.
25. Ciulla TA, Hammond BR (2004) Macular pigment density and aging, assessed in the normal elderly and those with cataracts and age related macular degeneration. *Am J Ophthalmol* 138(4): 582-587.
26. Ciulla TA, Curran-Celantano J, Cooper DA, Hammond BR, Danis RP, et al. (2001) Macular pigment optical density in a midwestern sample. *Ophthalmology* 108(4): 730-737.
27. Nolan J, O'Donovan O, Kavanagh H, Slack J, Harrison M, et al. (2004) Macular pigment and percentage of body fat. *Invest Ophthalmol Vis Sci* 45(11): 3940-3950.
28. Mares JA, LaRowe TL, Snodderly DM, Moeller SM, Gruber MJ, et al. (2006) Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. *Am J Clin Nutr* 84(5): 1107-1122.
29. de Kinkelder R, Van der Veen RL, Verbaak FD, Faber DJ, van Leeuwen TG, et al. (2011) Macular pigment optical density measurements: evaluation of a device using heterochromatic flicker photometry. *Eye (Lond)* 25(1): 105-112.
30. Hammond BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ (1997) Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci* 38(9):1795-1801.
31. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, et al. (2004) Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* 75(4): 216-230.
32. Bone RA Landrum JT (2010) Dose-dependent response of serum lutein and macular pigment optical density to supplementation with lutein esters. *Arch Biochem Biophys* 504(1): 50-55.
33. Howells O, Eperjesi F, Bartlett H (2013) Improving the repeatability of heterochromatic flicker photometry for measurement of macular pigment optical density. *Graefes Arch Clin Exp Ophthalmol* 251(3): 871-880.
34. Schalch W, Cohn W, Barker FM, Köpcke W, Mellerio J, et al. (2007) Xanthophyll accumulation in the human retina during supplementation with lutein or zeaxanthin: the LUXEA (LUtein Xanthophyll Eye Accumulation) study. *Arch Biochem Biophys* 458(2): 128-135.
35. Murray IJ, Carden D, Makridaki M (2010) The repeatability of the MPS 9000 macular pigment screener. *Br J Ophthalmol* 95(3): 431-432.
36. Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, et al. (2013) 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* 54(3): 1781-1788.
37. Van der Veen R, Berendschot TT, Hendrikse F, Carden D, Makridaki M, et al. (2009) A new desktop instrument for measuring macular pigment optical density based on a novel technique for setting flicker thresholds. *Ophthalmic Physiol Opt* 29(2): 127-137.
38. Flood V, Smith W, Wang JJ, Manzi F, Webb K, et al. (2002) Dietary antioxidant intake and incidence of early age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmology* 109(12): 2272-2278.
39. Vanden Langenberg GM, Mares-Perlman JA, Klein R, Klein BEK, Brady WE, et al. (1998) Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol* 148: 204-214.
40. Cho EY, Seddon JM, Rosner B, Willett WC, Hankinson SE (2004) Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch Ophthalmol* 122(6): 883-892.
41. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, et al. (2004) Double-masked, placebo controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* 75(4): 216-230.
42. Bone RA, Landrum JT, Fernandez L, Tarsis SL (1988) Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci* 29(6): 843-849.
43. Delcourt C, Carriere I, Delage M, Barberfer-Gateau P, Schalch W, et al. (2006) Plasma Lutein and Zeaxanthin and Other Carotenoids as Modifiable Risk Factors for Age-Related Maculopathy and Cataract: The POLA Study. *Invest Ophthalmol Vis Sci* 47(6): 2329-2335.
44. Bartlett H, Howells O, Eperjesi F (2010) The role of macular pigment assessment in clinical practice: a review. *Clin Exp Optom* 93(5): 300-308.