The effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomised controlled trial

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Both authors contributed to the design of the trial, statistical analyses, and preparation of the manuscript. Hannah Bartlett collected the data. Both authors read and approved the final manuscript. The authors declare no competing interests.

ABSTRACT

Objective

The aim of the study is to determine the effect of lutein combined with vitamin and mineral supplementation on contrast sensitivity in people with age-related macular disease.

Design

A prospective, nine-month, double-masked randomised controlled trial.

Setting

Aston University, Birmingham, UK and a UK optometric clinical practice.

Subjects

ARM and atrophic AMD participants were randomised (using a random number generator) to either placebo (n = 10) or active (n = 15) groups. Three of the placebo group and two of the active group dropped out.

Interventions

The active group supplemented daily with 6 mg lutein combined with vitamins and minerals. The outcome measure was contrast sensitivity (CS) measured using the Pelli-Robson chart, for which the study had 80 % power at the 5 % significance level to detect a change of 0.3 log units.

Results

The CS score increased by 0.07 ± 0.07 and decreased by 0.02 ± 0.18 log units for the placebo and active groups respectively. The difference between these values is not statistically significant ($z = -0.903$, $p = 0.376$).

Conclusion

The results suggest that 6 mg of lutein supplementation in combination with other antioxidants is not beneficial for this group. Further work is required to establish optimum dosage levels.

Sponsorship

The project was sponsored by the UK College of Optometrists. Intervention and placebo tablets were provided by Quest Vitamins Ltd UK.

Descriptors

Age-related macular disease, lutein, randomised controlled trial, nutrition, antioxidants.

INTRODUCTION

There is interest in the use of nutrition as a prevention and treatment strategy for age-related macular disease (ARMD) as it is the leading cause of visual disability in the developed World (Klein et al., 1997), and because treatment options are currently lacking (Zarbin, 2004). According to an international classification and grading system (Bird et al., 1995), this condition can be divided into early (age-related maculopathy, ARM) and late (age-related macular degeneration, AMD) stages.

Interest has been raised into the protective role of the oxygenated xanthophylls group of carotenoids in the eye, particularly the retina. Lutein, zeaxanthin and its isomer meso-zeaxanthin, are the only carotenoids present in the lens [1] and retina [2] and are also known as macular pigment (MP). It has been suggested that they play a similar role in humans as in plants, as antioxidants and screeners of high-energy blue light [3].

The absorbance spectrum of MP peaks at 460 nm and it is purported to act as a broadband filter, reducing the sensitivity of the macular region to short wavelength light which is most damaging in the 440 to 460 nm range (Pease et al., 1987, Reading and Weale, 1974). Zeaxanthin is reported to be a superior photoprotector during prolonged light exposure; the shorter time-scale of protective efficacy of lutein has been attributed to oxidative damage of the carotenoid itself (Sujak et al., 1999).

The MP also acts as a scavenger of reactive oxygen species (ROS). The relatively high concentration of MP in the inner retinal layers (Snodderly et al., 1984) is very likely to indicate a photoprotective role, while the presence of MP in the rod outer segments (Sommerburg et al., 1999), is suggestive of a ROS-quenching function. Lutein and zeaxanthin have been found in higher concentration in the rod outer segments of the perifoveal retina than the peripheral retina, again lending support to their proposed protective role in ARM and AMD (Rapp et al., 2000).

This RCT was designed to investigate the effect of 6 mg lutein combined with 750 µg retinol equivalents, 250 mg vitamin C, 34 mg vitamin E, 10 mg zinc, and 0.5 mg copper on contrast sensitivity measured

using the Pelli-Robson chart (Clement Clarke International, Edinburgh Way, Harlow, Essex, CM20 2TT, UK) in ARM affected eyes. Contrast sensitivity (CS) is a particularly relevant outcome measure for those with ocular disease as it provides a measure of real-world visual function (Hyvarinen, 1995). CS may help to provide a more complete assessment of visual function in macular disease, and it has been suggested that the test may be a superior predictor of daily living activities and mobility than visual acuity (VA) alone (Jin et al., 1992, Mones and Rubin, 2005). CS is reported to be a better measure of the ability to judge distances (Rubin et al., 1994) and discriminate between objects (Scott et al., 2002) and has also been reported to detect vision loss due to AMD prior to VA testing (Hyvarinen et al., 1983). Although there is a moderate correlation between VA and CS (Rubin, Roche, Prasada-Rao and Fried, 1994, Rubin et al., 1997), these two measures are not interchangeable (Haegerstrom-Portnoy et al., 2000). The effect, however, of a six letter loss of CS has been reported to have a similar impact on self-reported visual disability as a 15 letter loss of VA (Rubin et al., 2001). The inclusion of CS in visual assessment of macular disease patient may be useful in monitoring disease progression, evaluating the benefit of treatment, and designing appropriate rehabilitation strategies (Fletcher and Schuchard, 2006).

During the design of the trial, 6 mg daily intake of lutein had been reported to be associated with a reduced risk of AMD (57 % lower risk for the highest quintile of lutein intake, 6 mg per day, relative to the lowest quintile, 0.5 mg per day) (Seddon et al., 1994). The reasons for using a multi-ingredient formulation include the fact that ARM has a multifactorial aetiology, and so may be affected by more than one nutrient, and also that nutrients are thought to work synergistically together. A relevant example of this synergism is the facilitation of vitamin A transport from the liver by zinc (Newsome et al., 1994). A review of the nutrients considered suitable for inclusion in an ocular nutritional supplement has been published (Bartlett and Eperjesi, 2004).

Methods

Protocol

The study was approved by the Aston University Human Sciences Ethical Committee (code 02/M). The tenets of the Declaration of Helsinki were followed (World Medical Association, 1997). The trial was

registered for an International Standard Randomised Controlled Trial Number (ISRCTN 78467674), and the method has been published (Bartlett and Eperjesi, 2003). Reporting of this RCT adheres to the guidelines set out in the revised CONSORT statement (Moher et al., 2001).

Recruitment

Recruitment methods employed included sending study information to local optometrists, ophthalmologists, and a specialist centre for rehabilitation of people with sight loss. Enrolment was carried out by HB, who, along with FE, was masked to group assignment.

The main research centre was Aston University, Birmingham. A secondary research centre was a UK optometric clinical practice.

Inclusion/exclusion criteria

For inclusion participants had to 1) provide written informed consent, 2) be available to attend one of the research centres, 3) present with no ocular pathology in at least one eye, or no ocular pathology other than ARM, identified using the International Classification and Grading System for Age-Related Maculopathy and Age-Related Macular Degeneration (Bird, Bressler, Bressler, Chisholm, Coscas, Davis, Dejong, Klaver, Klein, Klein, Mitchell, Sarks, Sarks, Sourbane, Taylor and Vingerling, 1995). This definition of ARM includes soft or hard drusen, and areas of increased or decreased pigment associated with these drusen. Fundus examination was used to determine the presence of ARM. Exclusion criteria included type I and II diabetes, prescribed anti-platelet or anti-coagulant medication because of possible interaction with vitamin E (The ATBC Cancer Prevention Study Group, 1994), and concurrent use of nutritional supplements that potentially raised vitamin and mineral intake above the recommended safe limits (Bartlett and Eperjesi, 2005). Those with AMD in one or both eyes were excluded.

Randomisation

Only one investigator (HB) was involved in the randomisation process, which employed the random number generator in Microsoft Excel for Windows XP. Odd and even numbers were used to identify group.

Outcome measure

Contrast sensitivity (CS) was measured using a Pelli-Robson chart (Clement Clarke International, Edinburgh Way, Harlow, Essex, CM20 2TT, UK), and scored per letter.

Masking

The study formulation and placebo tablets were produced by Quest Vitamins Ltd, and were identical in external and internal appearance, and taste. The manufacturer allocated distinguishing symbols, μ and λ to the outer packaging, which was otherwise identical. The code for the symbols was withheld by the manufacturer until all data had been collected and analysed. Throughout this report, the letters P and A will be used to refer to the placebo and active formulation respectively.

The study formulation contained the following:

Lutein esters	6mg
Retinol	750μg
Vitamin C	250mg
Vitamin E	34mg
Zinc	10mg
Copper	0.5mg

The placebo tablets contained cellulose.

Participants in both groups were instructed to take one tablet, at the same time every day, with food. They were encouraged not to alter their diets, or to change their current supplementation regime.

Follow up

Data collection took place at baseline and nine months and was carried out by HB. Data were collected between March 2003 and December 2004.

Statistical analysis

The change between baseline and nine month values was calculated. SPSS software (version 11) for Microsoft Windows XP was used for analysis. The non-parametric Mann-Whitney U test was used to determine whether the means of these values differed at the 5% significance level between the two groups.

Sample size calculation

A group size of nine was calculated to be sufficient to provide 80 % power at the 5 % significance level for CS based on an effect size of 0.3 log units and mean and standard deviation values taken from a sample of 50 ARM and atrophic AMD patients of the University optometry clinic (1.39 ± 0.22 log CS).

Participant flow

Out of the 36 people that completed enrolment questionnaires, six did not meet the inclusion criteria or decided not to enrol. The remaining 30 individuals were randomised into the treatment or placebo group; a breakdown is shown in table 1.

Insert table 1 about here.

Statistical analysis was carried out on a per protocol basis. Compliance was assessed by counting remaining tablets at the follow-up visits, and averaged 94.4 %. There was no significant difference in compliance between groups.

Baseline characteristics

Although it is not correct to test for differences between two randomly allocated groups using conventional statistical tests as any differences will have arisen by chance alone, we acknowledge that the small sample size means that there could be differences between the groups. For this reason we have reported this information. The cohort ranged in age from 55 to 82 years (mean ± SD: 69.2 ± 7.8) and 53 % were female. There was no significant difference in age or gender between groups. There was no significant difference in baseline visual acuity (VA) between active (0.20 ± 0.28) and placebo (0.08 ± 0.15) groups (t = - 1.229, p = 0.229). All participants were White British. There was no significant difference in iris colour between groups. The baseline CS scores were 1.43 ± 0.20 and 1.36 ± 0.20 log units for the placebo and active groups respectively. Both groups fell below the normal CS score reported for this age group, which is 1.65 log units and is repeatable to within ± 0.15 log units (Elliott et al., 1990).

There was no significant difference between groups for age, smoking history (pack years) and years spent living abroad. Dietary intake of lutein, vitamins C and E, retinol equivalents, and zinc was assessed using food diaries and food frequency questionnaires. Analysis of food diaries was carried out using FoodBase 2000 (The Institute of Brain Chemistry and Human Nutrition, 166-220 Holloway Road, London N7 8DB, UK) for Microsoft Windows XP. There was no difference between groups except that the P group consumed significantly more vitamin C (161.1 ± 71.0) than the A group (88.0 ± 53.7: t = 3.04, p = 0.005). There was no difference in nutritional supplementation habits between P and A groups.

Adverse effects

There were no reported adverse effects from any of the study participants.

RESULTS

Masking success

End of trial assessment using questionnaires indicated masking success. Out of those participants taking the placebo tablet, 10 % correctly guessed which tablet they were taking, and 10 % incorrectly guessed.

Out of those taking nutritional supplement, 13 % guessed correctly which tablet they were taking, and 7 % incorrectly guessed. The remaining participants did not know which group they were randomised to.

Assessment of change in baseline characteristics

All participants were asked to fill out end-of-trial food diaries and food frequency questionnaires in order to assess any change in dietary habits over the trial period. Eighty percent of the end-of-trial food frequency questionnaires and food diaries were returned by the P group and 90 % by the A group.

There was no change in dietary lutein, vitamin C, vitamin, E, or retinol for any of the groups. However, there was a significant change in mean zinc intake from 9.17 ± 2.44 mg to 11.41 ± 3.64 mg ($t = -2.912$, $df = 19$, $p = 0.04$) in the A group. There was no change in ocular health in either group.

Main outcome

The mean CS score increased by 0.07 ± 0.07 log units in the P group and decreased by 0.02 ± 0.18 log units in the A group. A Mann-Whitney test was used to compare groups because the P group data set was not normally distributed (Kolmogorov-Smirnov = 0.320, $p = 0.004$). There was no significant difference between the P and A group in the change in CS over nine months ($z = -0.903$, $p = 0.366$). There was an improvement in CS over nine months in the P group ($p = 0.03$, eta squared = 0.21), although this is not clinically significant (Elliott, Sanderson and Conkey, 1990).

DISCUSSION

The results suggest that supplementing for nine months with a formulation containing 6 mg lutein, 750 µg retinol equivalents, 250 mg vitamin C, 34 mg vitamin E, 10 mg zinc, and 0.5 mg copper does not have an effect on CS in ARM-affected eyes. This is the only RCT to investigate the effect of nutritional supplementation on visual function in people with ARM.

Other RCTs have looked at the effect of nutritional supplementation on ARM and AMD. The Age-related Eye Disease Study (AREDS) found that a formulation containing 500 mg vitamin C, 273 mg vitamin E, 15

mg beta-carotene, and 80 mg zinc was moderately effective in preventing progression to advanced AMD. This effect was only seen in those subjects with extreme intermediate drusen, large drusen or non-central geographic atrophy without advanced AMD (The AREDS Research Group, 2001). The Lutein and Antioxidant Supplement Trial (LAST) was a 12-month RCT designed to evaluate the effect of 10 mg lutein alone or 10 mg lutein combined with additional carotenoids and antioxidants/minerals on MP optical density and objective visual outcome measures in 90 subjects with AMD. Glare recovery and contrast sensitivity significantly improved with both interventions, although it is worth noting that the study population was 95.6 % male (Richer et al., 2004).

Although no positive effect of supplementation was shown in this case, the study did have 80 % power at the 5 % significance level to detect a change of 0.3 log units. This effect size was selected because the measurement of CS using the Pelli-Robson chart has been shown to be reliable to ± 0.15 log units, which means that a change of 0.3 log units can be classed as clinically significant. A change of this size would also have brought both placebo and active groups into the normal range (1.50 to 1.80 log units) (Elliott, Sanderson and Conkey, 1990) for their age.

Research into the role of xanthophylls for retinal health is ongoing. There is evidence for selective deposition of lutein in the retina (Bernstein et al., 2001, Rapp, Maple and Choi, 2000), increase of retinal and serum levels of lutein with supplementation (Berendschot et al., 2000, Hammond et al., 1997, Landrum et al., 1997), and an increased risk of ARMD with low serum (EDCCS Group, 1993) and retinal (Beatty et al., 2001, Bone et al., 2001) lutein levels. Lutein/zeaxanthin supplementation has been linked with improved visual function in patients with congenital retinal degenerations (Dagnelie et al., 2000) and with AMD (Richer, 1999).

The lack of positive effect shown by this RCT may be explained by the selected lutein dosage level. When the study was designed, the recommended daily intake of lutein was 6 mg, based on an epidemiological study that determined a 57 % reduced risk for AMD in those consuming 6 mg lutein/zeaxanthin per day

compared to those consuming 0.5 mg per day) (Seddon, Ajani, Sperduto, Hiller, Blair, Burton, Farber, Gragoudas, Haller and Miller et, 1994).

More recent work has demonstrated a general increase in macular pigment optical density (MPOD - retinal levels of lutein/zeaxanthin) response with dose (Chew et al., 2003, Landrum et al., 2004). In one study, those supplementing with 10 mg or 20 mg of lutein, but not 5 mg lutein, for 120 days had an increased response compared with those taking a placebo (Landrum, Bone, Dixon, Etienne-Levielle, Formosa and Saint-Louis, 2004). Another study showed that, in patients with varying stages of ARM and AMD, doses of 2.5 mg, 5 mg, and 10 mg lutein all induced an increase in serum levels by one month, and a peak by three months. Three-month levels ranged from 104 % to 339 % change from baseline. Macular pigment optical density levels, however, remained largely unchanged over the six-month supplementation period. In other studies the retinal response has been reported to occur after 15 weeks with increased dietary levels of corn and spinach (Hammond, Johnson, Russell, Krinsky, Yeum, Edwards and Snodderly, 1997), 140 days with 30g/d supplemental lutein or zeaxanthin, and six months with 2.4g/d supplemental lutein or zeaxanthin (Landrum, Bone, Joa, Kilburn, Moore and Sprague, 1997). The response rates appear to be variable, and this may explain why we did not find an effect on CS. Our dosage of 6 mg per day lutein may not have increased MPOD.

A putative lutein-binding protein has been found in the retinae of human eyes (Yemelyanov et al., 2001), which binds with high affinity and specificity to lutein and other xanthophylls. It has been suggested that people who are less responsive to xanthophyll supplementation may be so because of genetic differences that result in reduced or less efficient binding proteins (Landrum and Bone, 2004). This factor may have had an effect on the outcome of this trial. The protein may also act as an enzyme for the conversion of lutein to meso-zeaxanthin, which predominates over lutein and zeaxanthin at the fovea. There is no current evidence to support the suggestion that people with ARM or AMD have a reduced ability to absorb lutein or zeaxanthin at the macula.

The formulation also contained lutein esters extracted from marigold flowers, rather than pure lutein. It could be argued that this affected the results of this study. In flower petals, the pigments are stored as diesters, whereas they are found unesterified in most fruits and vegetables (Goodwin, 1980). In fact, industrial research showed that 93 % of the lutein and zeaxanthin found in fruits, vegetables, and eggs is found as lutein, rather than lutein esters (DeFreitas, 2004). Lutein esters contain two fatty acid groups that must be cleaved off before the body can use the lutein (Noy, 2000). The efficacy of this hydrolysis of lutein esters into lutein occurs with an efficacy that is well below 5 % (Breithaupt et al., 2002, Granado et al., 2002). Furthermore, a negative correlation between age and serum lutein levels in individuals consuming lutein esters has been reported that was not found in people supplementing with lutein (Chung et al., 2004). This may suggest that the ability to hydrolise lutein esters declines with age.

These factors have been used to support the argument that lutein esters are less bioavailable than pure lutein. Studies carried out to investigate differences in bioavailability between pure and esterified lutein do not support this hypothesis. One study reported no significant difference in serum lutein response between 6 mg lutein from spinach, 6 mg pure lutein, and 10.23 mg lutein esters (Chung, Rasmussen and Johnson, 2004). In another study, serum response was greater from lutein esters than pure lutein (Bowen et al., 2002). Although these studies suggest that the use of lutein esters in our formulation should not have hindered bioavailability, it is important to note that they recorded serum response rather than retinal response. Although the retinal response is related to serum response, and dietary modification affects both, the retinal response is reported to be slower than the serum response.

Although serum antioxidant checks were considered during the design of the trial, this evidence suggested that it would only provide a short-term indication of blood antioxidant levels and so would not provide any additionally useful information about compliance. The inclusion of blood testing in the protocol may also have hindered recruitment.

All participants were White British, and so the results cannot be applied to other ethnic groups.

It is difficult to explain the counterintuitive improvement in mean CS score over the trial period within the P group, although this change was not clinically significant. The trial results could have been confounded by the fact that the P group consumed almost twice as much vitamin C than the A group. The increase in dietary intake of zinc in the A group is also worth mentioning, although it could be argued that, if anything, this change would increase rather than decrease the likelihood of finding a treatment effect based on the results of trials such as AREDS (The AREDS Research Group, 2001).

The mixed antioxidant and mineral formulation does not permit investigation of the effect of specific nutrients on visual function. The rationale for using a mixed formulation is that nutrients are thought to work synergistically together. A relevant example of this synergism is the facilitation of vitamin A transport from the liver by zinc (Newsome, Miceli, Liles, Tate and Oliver, 1994).

Although this study investigated the effect of nutritional supplementation in eyes affected by ARM, there is also interest in lutein supplementation for healthy eyes. It has been hypothesised that the blue-light filter effect of lutein/zeaxanthin may reduce longitudinal chromatic aberration (Wald, 1945) The acuity hypothesis states that retinal lutein may improve visual acuity for images that are illuminated by white light by absorbing poorly focussed short wavelengths before this light is processed by the retina (Hammond et al., 2001). The findings of a study by the authors that investigated the effect of lutein and antioxidant supplementation on visual function in healthy eyes has yet to be published. Despite a lack of empirical evidence, lutein/zeaxanthin supplements are being taken by the public in an attempt to improve retinal health and vision in the absence of disease (Mares-Perlman, 1999). We are not aware of any currently published trials that have investigated this hypothesis.

The finding of no evidence of effect of nine months of nutritional supplementation on CS adds to the literature, and may suggest that daily intake of 6 mg lutein or less does not have a beneficial effect on ARM. Further clinical trials are required to investigate optimum lutein dosage levels, and we await the results of a large multi-centred RCT.

References

Bartlett H and Eperjesi F (2003): A randomised controlled trial investigating the effect of nutritional supplementation on visual function in normal, and age-related macular disease affected eyes: design and methodology [ISRCTN78467674]. BMC Nutr. J. **2**, 12.

Bartlett H and Eperjesi F (2004): An ideal ocular nutritional supplement? Ophthal. Physiol. Opt. **24**, 339-349.

Bartlett H and Eperjesi F (2005): Adverse reactions and contraindications for ocular nutritional supplements. Ophthal. Physiol. Opt.

Beatty S, Murray IJ, Henson DB, Carden D, Koh H and Boulton ME (2001): Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest. Ophthalmol. Vis. Sci. **42**, 439-446.

Berendschot TT, Goldbohm RA, Klopping WA, van de Kraats J, van Norel J and van Norren D (2000): Influence of lutein supplementation on macular pigment, assessed with two objective techniques. Invest. Ophthalmol. Vis. Sci. **41**, 3322-3326.

Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY and Katz NB (2001): Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. Exp. Eye. Res. **72**, 215-223.

Bird AEC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD et al. (1995): An International Classification and Grading System for Age- Related Maculopathy and Age-Related Macular Degeneration. Surv. Ophthalmol. **39**, 367-374.

Bone RA, Landrum JT, Mayne S, Gomez C, Tibor S and Twaroska E (2001): Macular pigment in donor eyes with and without AMD: A case-control study. Invest. Ophthalmol. Vis. Sci. **42**, 235-240.

Bowen PE, Espinosa SM, Hussain EA and Stacewicz-Sapuntzakis M (2002): Esterification does not impair lutein bioavailability in humans. J. Nutr. **132**, 3668-3673.

Breithaupt D, Bamedi A and Wirt U (2002): Carotenol fatty acid esters: easy substrates for digestive enzymes? Comparative biochemistry and physiology. Part B, Biochemistry and molecular biology **132**, 721-728.

Chew E, Ferris FL, de Monasterio F, Thompson D, Kim J, Csaky C et al. (2003): Dose ranging study of lutein supplementatio in persons over age 60. Invest. Ophthalmol. Vis. Sci. **44**, E-Abstract 968.

Chung H-Y, Rasmussen H and Johnson E (2004): Lutein bioavaliability is higher from lutein-enriched eggs that from supplements and spinach in men. J. Nutr. **134**, 1887-1893.

Dagnelie G, Zorge I and McDonald T (2000): Lutein improves visual function in some patients with retinal degeneration: a pilot study via the internet. Optometry (St. Louis, Mo.) **71**, 147-164.

EDCCS Group (1993): Antioxidant status and neovascular age-related macular degeneration. The Eye Disease Case Control Study Group. Arch. Ophthalmol. **111**, 104-109.

Elliott DB, Sanderson K and Conkey A (1990): The Reliability of the Pelli-Robson contrast sensitivity chart. Ophthal. Physiol. Opt. **10**, 21-24.

Fletcher D and Schuchard R (2006): Visual functionin patients with choroidal neovascularization resulting from age-related macular degeneration: the importance of looking beyond visual acuity Optom. Vis. Sci. **83**, 178-189.

Granado F, Olmedilla B and Blanco I (2002): Serum depletion and bioavaliability of lutein in type I diabetic patients. European Journal of Nutrition **41**, 47-53.

Haegerstrom-Portnoy G, Schneck M, Lott L and Brabyn J (2000): The relation between visual acuity and other spatial vision measures. Optom. Vis. Sci. **77**, 653-662.

Hammond BR, Jr, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB et al. (1997): Dietary modification of human macular pigment density. Invest. Ophthalmol. Vis. Sci. **38**, 1795-1801.

Hammond BR, Wooten BR and Curran-Celentano J (2001): Carotenoids in the retina and lens: Possible acute and chronic effects on human visual performance. Arch. Biochem. Biophys. **385**, 41-46.

Hyvarinen L (1995): Contrast sensitivity testing in clinical practice. Br. J. Ophthalmol. **79**, 867-868.

Hyvarinen L, Laurinen P and Rovamo J (1983): Contrast sensitivity in evaluation of visual impairment due to macular degeneration and optic nerve lesions. Acta Ophthalmologica (Copenhagen) **61**, 161-170.

Jin C, Wu D and Wu L (1992): The contrast sensitivity function in low vision. Yen Ko Hsueh Pao **8**,

Klein R, Klein B, Jensen S and Meuer S (1997): The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. Ophthalmology **104**, 7-21.

Landrum J and Bone R (2004): Dietary lutein and zeaxanthin: reducing the risk for macular degeneration. Agro Food Ind. Hi-Tech **15**, 22-25.

Landrum JT, Bone R, Dixon Z, Etienne-Levielle V, Formosa M and Saint-Louis M (2004): Influence of lutein dosage on macular pigment response. Invest. Ophthalmol. Vis. Sci. **45**, E-Abstract 1290.

Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL and Sprague KE (1997): A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp. Eye. Res. **65**, 57-62.

Mares-Perlman J (1999): Too soon for lutein supplements. Am. J. Clin. Nutr. **70**, 431-432.

Moher D, Schulz K and Altman D (2001): The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. BMC Med. Res. Methodol. **1**, 2.

Mones J and Rubin G (2005): Contrast sensitvity as an outcome measure in patients with subfoveal choroidal neovascularisation due to age-related macular degeneration. Eye **19**, 1142-1150.

Newsome D, Miceli M, Liles M, Tate D and Oliver P (1994): Antioxidants in the retinal pigment epithelium. Prog. Retin. Eye Res. **13**, 101-123.

Pease P, Adams A and Nuccio E (1987): Optical density of human macular pigment. Vis. Res. **27**, 705-710.

Rapp LM, Maple SS and Choi JH (2000): Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. Invest. Ophthalmol. Vis. Sci. **41**, 1200-1209.

Reading V and Weale R (1974): Macular pigment and chromatic aberration. J. Optom. Soc. Am. **64**, 231-238.

Richer S (1999): ARMD--pilot (case series) environmental intervention data. J. Am. Optom. Assoc. **70**, 24-36.

Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D 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**, 216-230.

Rubin G, Bandeen-Roche K, Huang G, Munoz B, CSchein O, Fried L et al. (2001): The association of multiple visual impairments with self-reported visual disability: SEE project. Invest. Ophthalmol. Vis. Sci. **42**, 64-72.

Rubin G, Roche K, Prasada-Rao P and Fried L (1994): Visual imparment and disability in older adults. Optom. Vis. Sci. **71**, 750-760.

Rubin G, West S, Munoz B, Bandeen-Roche K, KZeger S, Schein O et al. (1997): A comprehensive assessment of visual impairment in a population of older Americans. Invest. Ophthalmol. Vis. Sci. **38**, 557-568.

Scott I, Feuer W and Jacko J (2002): Impact of visual function on computer task accuracy and reaction time in a cohort of patients with age-related macular degeneration. Am J. Ophthalmol. **133**, 350-357.

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC et al. (1994): Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA **272**, 1413-1420.

Snodderly DM, Brown B, Delori F and Auran J (1984): The macular pigment I. Absorbance spectra, localisation, and discrimination from other yellow pigments in primate retinas. Invest. Ophthalmol. Vis. Sci. **25**, 660-673.

Sommerburg O, Siems W, Hurst J, Lewis J, Kliger D and van Kuijk F (1999): Lutein and zeaxanthin are associated with photoreceptors in the human retina. Curr. Eye. Res. **19**, 491-495.

Sujak A, Gabrielska J, Grudzinsnki W, Borc R, Mazurek P and Gruszecki W (1999): Lutein and Zeaxanthin as Protectors of Lipid Membranes against Oxidative Damage: The Structural Aspects. Arch. Biochem. Biophys. **15**, 301-307.

The AREDS 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. Archives of Ophthalmology **119**, 1417-1436.

The ATBC Cancer Prevention Study Group (1994): The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. New. Engl. Med. J. **330**, 1029-1035.

Wald G (1945): Human vision and the spectrum. Science **101**, 653-658.

World Medical Association (1997): Declaration of Helsinki. J. Am. Med. Assoc. **277**, 925-926.

Yemelyanov A, Katz N and Bernstein P (2001): Ligan-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retina. Exp. Eye. Res. **72**, 381-392.

Zarbin M (2004): Current concepts in the pathogenesis of age-related macular degeneration. Arch. Ophthalmol. **122**, 598-614.