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

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**ABSTRACT**

5   **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**

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


**Setting**

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


15   **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.


20   **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.


25   **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).


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**Conclusion**

The results suggest that 6 mg of lutein supplementation in combination with other antioxidants is not

35 beneficial for this group. Further work is required to establish optimum dosage levels.

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**Descriptors**

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

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**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

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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).

120    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.

125    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

130    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

135    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.

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Randomisation

145  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

150  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

155  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.


160  The study formulation contained the following:

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

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The placebo tablets contained cellulose.


Participants in both groups were instructed to take one tablet, at the same time every day, with food. They

170  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.

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**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

180    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

185    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

190    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

195    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.

230 **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.

235 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 $9.17 \pm 2.44$ mg to $11.41 \pm 3.64$ 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**

240 The mean CS score increased by $0.07 \pm 0.07$ log units in the P group and decreased by $0.02 \pm 0.18$ 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

245 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

250 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

255    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

260    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

265    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.

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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

275    (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

280    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).

285  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

290  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,

295  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.

300  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

305  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

310    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

315    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.

320    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

325    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.

330    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.

335    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

340     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.

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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.

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