The effect of ocular nutritional supplements on macular pigment optical density

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This thesis describes the investigation of the effects of ocular supplements with different levels of nutrients on the macular pigment optical density (MPOD) in participants with healthy eyes.

Abstract

A review of the literature highlighted that ocular supplements are produced in various combinations of nutrients and concentrations. The ideal concentrations of nutrients such as lutein (L) have not been established. It was unclear whether different stages of eye disease require different concentrations of key nutrients, leading to the design of this study.

The primary aim was to determine the effects of ocular supplements with different concentrations of nutrients on the MPOD of healthy participants. The secondary aim was to determine L and zeaxanthin (Z) intake at the start and end of the study through completion of food diaries.

The primary study was split into two experiments. Experiment 1 was an exploratory study to determine sample size and experiment 2 the main study.

Statistical power was calculated and a sample size of 38 was specified. Block stratification for age, gender and smoking habit was applied and from 101 volunteers 42 completed the study, 31 with both sets of food diaries.

Four confounders were accounted for in the design of the study; gender, smoking habit, age and diet. Further factors that could affect comparability of results between studies were identified during the study and were not monitored; ethnicity, gastro-intestinal health, alcohol intake, body mass index and genetics. Comparisons were made between the sample population and the Sheffield general population according to recent demographic results in the public domain.

Food diaries were analysed and shown to have no statistical difference when comparing baseline to final results. The average L and Z intake for the 31 participants who returned both sets of food diaries was initially 1.96mg and 1.51mg for the final food diaries.

The effect of the two ocular supplements with different levels of xanthophyll (6mg lutein/zeaxanthin and 10mg lutein only) on MPOD was not significantly different over a four-month period.

Lutein Zeaxanthin MPOD Food diaries Healthy eyes

For Gaynor, Nicole and Thomas

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Contents

Abbreviations used

AA	Arachidonic Acid
ALA	Alpha-Linoleic Acid
AMD	Age-related macular degeneration
AREDS	Age related eye disease study
BCVA	Best corrected visual acuity
BMI	Body mass index
CCD	Charge coupled device
DHA	Docosahexaenoic acid
DS	Dioptre Sphere
EPA	Eicosapentaenoic acid
ETDRS	Early Treatment Diabetic Retinopathy Study
EURRECA	European micronutrient recommendations aligned
FFQ	Food frequency intake questionnaire
H_O	The null hypothesis
HFP	Heterochromatic flicker photometry
Hz	Hertz
iC	ICap ONS
L	Lutein
LA	Linoleic Acid
LE	Left eye
MP	Macular pigments
mg	Milligram
MPOD	Macular pigment optical density
MZ	Meso-zeaxanthin
ONS	Ocular nutritional supplements
PUFA	Poly-unsaturated fatty acids
RE	Right eye
RPE	Retinal pigment epithelium
RRS	Resonance Raman spectrometry
SLO	Scanning laser ophthalmoscope
SNPs	Single gene polymorphisms
V	Vitalux plus ONS
VA	Visual acuity
YAG	Neodymium-doped yttrium aluminium garnet laser
Z	Zeaxanthin

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Chapter 1 Introduction

In Chapter 1 an overview is provided of the macular pigment (MP), their history, sources and role within the eye.

A growing sense of awareness of ocular nutritional supplements (ONS) from within the professional press and at continuing education meetings led to an interest in age-related macular degeneration (AMD), it's relationship with macular pigment optical density (MPOD) and the measurement of MPOD and how that could lead to ONS advice in everyday clinical practice. This led to the proposal and design of the study within this thesis.

The macula was first described by Buzzi in 1783 (Whitehead et al 2006) and the xanthophylls, which are concentrated in the macular region, were described by Wald in 1945. Bone et al identified lutein (L) and zeaxanthin (Z) as the specific xanthophylls in the retina in 1985 (Bone et al 1985). Xanthophylls are the sub-category of carotenoids that have oxygen within their structure, unlike the hydrocarbon only carotenes.

There are more than 600 pigments in the carotenoid class, 30 to 50 within the human diet, of which 20 are measureable in human serum, where L exceeds Z. Only these two appear in the retina, where Z stereoisomers ((3S,3'R) Z, (3R,3'S) meso-zeaxanthin (MZ) and (3S,3'S) astaxanthin) exceed L (Bone et al1993).

L and Z have the same number of double bonds, however, the position of the double bond in L forms a more chemically reactive allylic hydroxyl end group (Anon, 2014) versus the extra conjugated double bond in Z and MZ (Figure 1.1).

Figure 1.1 The molecular structure of L, Z and MZ (Google patents, 2011)

L and Z are concentrated within leafy green vegetables, many fruits and coloured vegetables such as squash, sweet peppers, sweet corn and peas (see Table 1.1 (Sommerburg et al1998)). Consumption of these foodstuffs does vary, with African Americans consuming twice as much L (approx. 3mg/d) as Hispanic and White Americans (1-2mg/d) (Mares-Perlman, et al., 2001).

Table 1.1 Carotenoid content in fruit and vegetables in mole% (Sommerburg et al 1998)

Once ingested the serum levels are affected by body fat, oxidative stress, gender, dietary fat intake and more (Erdman et al 1993; Hammond, et al., 1997; Khachik et al 1997; Broekmans, et al., 2002; Zaripheh & Erdman, 2002). The hydrophobic molecules must bind to water-soluble lipoproteins to allow them to transfer to specific tissues. As the

retina has the highest concentration of xanthophylls of any tissue, and those xanthophylls are predominantly L and Z, there is some support for the concept of specific xanthophyll binding proteins with glutathione S-transferase pi gene (GSTP1) being identified as a strong contender for this role (Bhosale et al 2004).

Zeaxanthin is the dominant pigment at the macula, in a 2:1 to 2.4:1 ratio with L (Billsten et al 2003, Bartlett et al, 2010b); its density decreases rapidly by up to 100 fold towards the periphery of the retina; as it decreases in density, L concentrations increase (Bone, et al., 1997). This ratio of Z:L varies linearly with the ratio of rod receptor cells to cone receptor cells in the retina. Most of the Z in the central macula lutea is in the MZ form, with small quantities of astaxanthin (Landrum & Bone, 2001). The spatial extension of the MP does vary between individuals (Trieschmann et al., 2008) and it was reported that there was no correlation with spatial distribution and age measured on cadaver eyes. Other studies have found an increase in this spatial distribution of 0.06 degrees per year, measured optically (Chang et al., 2002).

Although MZ is found in the central retina it is not found within plasma or the liver. This central concentration of MZ without a ready supply within the circulatory system suggests that MZ is converted, by isomerisation, from L within the central retinal region (Bone et al 1997; Khachik et al., 2002). MZ is not found within normal human diets, further supporting the conversion of L within the retina hypothesis. The process of the isomerisation within the retina is not clearly understood. Bone et al (Bone et al 1997) suggest that this may be via an enzyme, specifically an isomerase, which they could not at the time show as occurring within the retina. The process described is labelled as ‘thermally forbidden but photochemically allowed’ which points to the problems with such a process in the retina itself.

The MP functions are purported to be blue-light filtration, which includes glare reduction (Bone & Landrum, 1984), minimization of chromatic aberration (Wooten & Hammond, 2002), enhanced fine detail distinction and contrast enhancement (Hammond et al, 1998), and cellular health maintenance by the neutralisation of reactive oxygen species (Winkler et al, 1999).

These roles are supported by the relative positioning of MP within the retina. It is suggested that MZ has a greater ‘quenching’ ability with regard to reactive oxygen species when compared to L, suiting its central predominance described above and also supporting the central retinal isomerisation hypothesis (Foote et al, 1970).

The blue-light filtration function is well supported by the work undertaken by Snodderly et al (Snodderly et al., 1984) which showed that MP if found in primarily within the Inner nuclear, inner plexiform, ganglion cell and nerve fibre layers (see Figure 1.2). Light entering the eye must pass through these layers prior to being incident upon the photoreceptors; hence the filtering of blue-light by MP is feasible (Krinsky., 2002).

Figure 1.2 Stylised representation of retinal layers and cells

L and Z are also found in the outer segments of rod photoreceptors (in perifoveal region) where there is a high concentration of polyunsaturated fatty acids (PUFAs) (Sommerburg et al., 1999). PUFAs are particularly prone to oxidative attack, and this position within the retina is supportive of the role of neutralisation of reactive oxygen species. These outer segments of the receptors are shown next to the pigment epithelium in Figure 1.2 above. It is likely that the L and Z arrive in the retina from the choroid across the pigment epithelium and up through the retina.

L and Z have been shown to be the specific xanthophylls in the retina (Bone et al, 1985). Studies have shown that supplementing with L increased plasma concentrations of L in Rhesus monkeys (Khachik, et al., 2006) and humans over 60 year of age (Rosenthal, et al., 2006) with or without AMD (Khachik et al, 2006). It has been proposed that by supplementing with ONS containing L and Z the retina would be protected against

developing AMD (Snodderly, 1995). However, this assumption has yet to be shown to be true.

Studies have shown equivocal results following supplementation. Only in studies involving late stage AMD does the effectiveness of ONS become statistically significant (Snellen et al, 2002; Cho et al, 2004). Following on from the supplements chosen for the first age related eye disease study (AREDS 1) a supplement (500mg Vit C; 400 IU Vit E; 15 mg β-carotene; 80mg of zinc oxide and 2mg cupric oxide) for late stage AMD has been commercially available, with and without β-carotene (American Academy of Ophthalmology, 2001); this was used as the base plus L, Z and omega 3 for AREDS 2.

MPOD is the amount of MP acting as a filter to a specific light wavelength spectrum (400-540nm, peaking at 460nm (Bone et al, 1992)). Supplementation has been shown to raise the level of MPOD with a 30-40% reduction in blue light reaching the photoreceptor, Bruch's membrane and retinal pigment epithelium (RPE) (Landrum et al, 1997; Bone et al, 2007). A raised serum concentration of these xanthophylls has been shown to increase to a 'saturation' level and then to plateau. This increased serum concentration was suggested to act as an indicator of likely changes to MPOD levels that would follow (Bone et al, 2003). A protective effect upon these structures leading to a slowing down, halting or regeneration in early stages of AMD has yet to be shown.

This protective mechanism is currently being investigated and is the basis upon which MPOD devices are being introduced into clinical practice. As with any innovation there will be 'early adopters' who don't necessarily get the best technology (Rogers, 1962). The clinical market for MPOD devices was in the early adopter phase when the study was carried out.

There are a number of factors that may be involved in the aetiogenesis of AMD such as oxidation, Bruch's membrane deterioration, vascular insufficiency, genetics and inflammation. This is well summarised in a previous paper (Bartlett & Eperjesi 2003).

The oxidation hypothesis suggests that the breakdown of the antioxidant system is involved in the aetiology of AMD. An antioxidant delays or prevents oxidation; which is the loss of electrons or an increase in oxidation state by a molecule, atom, or ion. The normal retinal metabolic processes and exposure to high-energy visible light (violet/blue 400 to 500nm) generates potentially damaging activated forms of oxygen known as free radicals. Free radicals are formed by oxidation. Having 'lost' an electron they have one or

more unpaired electron. Free radicals can initiate lipid peroxidation, which is thought to lead to oxidative damage to DNA, protein and carbohydrate within cells (Curcio & Millican, 1999). Retinal tissue is particularly susceptible to damage via lipid peroxidation due to four main reasons. There is an abundance of poly-unsaturated fatty acids (PUFA) in the retina especially in the macular region, within photoreceptor outer membranes that are readily oxidised (Van der Hagen et al,1993). The retina is exposed to high levels of light. Light is a strong oxidising agent, especially violet/blue light. With light and oxygen present free radical production is facilitated (Algvere et al, 2006). The retina has a higher blood flow than many tissues and is highly active metabolically. Phagocyctosis occurs within the RPE and this process produces free radicals.

The human body has several defence mechanisms against free radicals. For example antioxidant enzymes, catalase and peroxidase (Sies, 1991), facilitated by micronutrients such as selenium, zinc, manganese and copper (Bressler & Bressler, 1995). A second uses antioxidant nutrients such as vitamin E (alpha-tocopherol) (McCay, 1985), beta-carotene (Burton & Ingold, 1984) and vitamin C (ascorbate) (Sies, 1991) and central to this paper L and Z (Snodderly et al,1984). Other antioxidant compounds also contribute to this defence, such as metallathionein, melanin and glutathione (Beatty et al, 2000). Sies also discussed the compartmentalisation of free radicals from cellular components that are susceptible to oxidative damage (Sies, 1991). It has been recognised for sometime that a lack of antioxidant vitamins and minerals from dietary sources, can decrease the body's antioxidant systems efficiency, which may allow free radicals to damage cells (Machlin & Bendich, 1987).

Bruch's membrane deterioration has also been associated with AMD. With age the conductivity to fluid declines across the membrane (Chuang & Bird, 1988). This is implicated in RPE detachment from the choroid. This 'thickening' of Bruch's membrane can block nutrition and permit choroidal blood vessel proliferation, which can destroy structures as they grow (Silvestri, 1997).

Vascular insufficiency may arise when changes to the choroidal circulation impact upon normal RPE-Bruch's membrane activity, diffusion of substances and gases, removal of waste and supply of metabolites to the retina, and has been linked to AMD development (Pauleikhoff et al,1990). The build up of waste products from the visual process has been linked to RPE deterioration (Friedman, et al., 1995).

A positive family history of AMD has been shown to increase the risk of developing AMD (Smith & Mitchell, 1998). A genetic basis for AMD is supported by the occurrence of AMD within families. Given the multifactorial nature of AMD it is likely that those with the increased risk factor described above will develop AMD when exposed to relevant environmental factors (Silvestri, 1997). The relationship between a family history of AMD and MPOD being lower or higher has not been shown, although age and smoking did have a negative effect upon MPOD level (Kirby, et al., 2010). This is contrary to an earlier paper that found a relationship between low levels of serum Z and Z in MP for participants with a family history of AMD and heavy smokers (Nolan et al, 2007). The mechanism of this was discussed and it was suggested that poor capture of Z from serum or poor stabilisation within the macula were at fault. This could be due to Z depletion in the retina via its antioxidant function or poor uptake via the zeaxanthin binding protein (Pi isoform of GSTP1), which has high affinity for Z but poor affinity for L. This mechanism and its relationship with family history of AMD needs to be studied further.

Inflammation may also play a role in the development and progression of AMD (Anderson et al, 2002). Drusen (lipo-glyco-proteinaceous deposits) formation has been shown to result from a localised inflammatory response. Components of complement and other proteins involved in immune mediated processes and inflammation are present in drusen (Klein et al, 2008). Studies have also provided evidence that inflammation plays a role in the formation of choroidal neovascularisation with inflammatory cells being found on the outer surface of Bruch's membrane in eyes with neo-vascular AMD (Penfold et al, 2001). These inflammatory cells are thought to damage Bruch's membrane through release of proteolytic enzymes, oxidants and toxic oxygen compounds (Klein, et al., 2008). Inflammation may not be the initial aetiology of AMD but at an early stage it may exacerbate AMD, thus anti-inflammatory treatments can help slow progression (Wang et al, 2011).

Feigel (Feigel, B., 2009) suggested a mechanism that combined the above individual factors into a multifactoral process helping explain AMD formation. Feigel suggested that AMD is the ocular manifestation of a systemic immune and inflammatory condition, with drusen formation being the early visible sign of change. The original insult precipitating the condition is unknown with Chlamydia pneumoniae being suggested by one study group (Kalayoglu et al., 2003) but not replicated by others (Robman et al., 2007).

Ischaemia was suggested by Feigel as the primary mechanism. Ischaemia is the reduced bloodflow within the retinal environment due to an imbalance between perfusion and demand, leading to hypoxia and poor metabolite removal. As the retina is the highest oxygen consuming tissue within the body (Yu & Cringle 2005), along with the macular areas high metabolic rate, thus high oxygen demand, the retinal avascular nature it has and the confluence of watershed zones of perfusion (often first deprived in low perfusion) leaves the macular area particularly susecptible to ischaemic changes.

The alternative complement system is activated in states of ischaemia and paradoxically in reperfusion (Robbins and Cotran, 2004). Cell injury is sugested to be mediated by free radical production and the terminal membrane attact complex MAC (c5b-9) (Beatty et al., 2000). Chronic vascular insufficiency can lead to the inflammatory mediators such as cytokines, arachidonic acid and nitric oxide being associated with the retina (Osborne et al., 2004). Genetics can then help determine the onwards course of the development of the AMD with the presence or otherwise of single gene polymorphisms (SNPs). If SNPs are present it is suggested that inflammatory deposits such as membraenous debris and basal laminar deposits (which sit between the RPE cells and the RPE basement membrane) form. Without the relevant SNPs the basal laminar deposits appear more patchy and hard drusen form.

Feigel then suggests two models for early AMD development. The receptoral model focusses upon the receptors, where a slowing down of pigment regeneration occurs due to poor nutrient (e.g. vitamin A) transfer due to a thickened Bruchs membrane (Curcio et al., 2000). Other study groups have found contrary results to this and have suggested that the changes that affect the receptors are due to abnormal orientation or mishaping of the receptor, along with hypoxia leading to reduced MP density and/or photosensitivity (Elsner et al., 2002).

The second suggested model is the post-receptoral model. Lipid deposition within the choroid is suggested to increase choroidal resistance and lead to RPE decompensation. Changes to the RPE lead to choriocapillaris atrophy, basal linear deposits (primarily found between the RPE basal membrane and Bruch's membrane) and an increasingly hydrophobic Bruch's membrane all leading to poor perfusion (Feigel et al., 2007).

Very recently a study group has described how the deposits within the retina described above may form (Thompson et al., 2015). It was shown that zinc, lipids and protein were included within these deposits found beneath the RPE layer, however Thompson et al.

are suggesting that a form of calcium phosphate (hydroxyapatite) is crucial to starting these deposits forming. Spheres of hyrdoxyapatite act as binding sites for proteins and lipid in a gradual snowball effect forming larger globules of drusen over time. Further work on this and its potential for diagnostic or treatment work needs to be undertaken.

In terms of the clinical development of the condition, the first sign of metabolic changes are debris and remnants of incomplete degradation from phagocytosis of rod and cone cell membranes. These are deposited between the basement membrane of the RPE and Bruch's membrane. The photoreceptor outer segments that are not digested by the lysosomes of the RPE remain in the RPE cells as highly oxidized lipid material (lipofuscin) (Curcio & Millican, 1999). When large enough this can be seen on the retina as drusen. This can lead to drusenoid pigment epithelial detachment (PED), with inflammatory cells leading to choroidal neovascularization (Algvere & Seregard, 2002).

From the above it is clear that AMD is not one observable sign, but a spectrum of signs with progressive severity. To help answer the question about which ONS levels would be required to be offered by clinicians at specific stages of AMD development, this spectrum needs to be broken down into identifiable stages. A system to help grade the level of AMD in clinical practice and to create relevant scoring systems for the Beaver Dam Study (Klein et al, 1991), the Framingham Eye Study (Leibowitz, et al., 1980) and, although modified, the AREDS (Age-related eye disease study research group, 2005) was suggested by Klein et al (Klein et al, 1991).

This system used stereo-photographs of the disc and macula, assisting with reproducibility and monitoring change over time. Lighting conditions were specified to enhance subtle drusen recognition and a magnifying device to enhance magnification to 15x. A grid was superimposed to create nine subfields around three concentric circles at 500μm, 1,500μm and 3,000μm.

Grades were given by the observer for three prime signs, drusen, typical AMD lesions and other abnormalities. Drusen was sub-graded for size, type, area and confluence. For size, type and area on a scale of one to eight with seven and eight being cannot grade annotations for size and type. Other conditions typical of AMD included RPE degeneration, increased pigment, RPE detachment or serous detachment of sensory retina, retinal hard exudate, subretinal and/or subRPE haemorrhage, subretinal and/or subRPE fibrous tissue, geographic atrophy, retinal oedema and retinal haemorrhages. Other abnormalities included wrinkling retinopathy, branch and central retinal venous or

arterial occlusions, chorioretinal scars or degenerations and asteroid hyalosis. Agreement between graders was good with exact agreement at a minimum in drusen type at 62.5% and a maximum in geographic atrophy at 100%; agreement within one grade from 91.3% for drusen area to 100% for increased pigment. This simple yet effective grading system was commented upon and modified for non-stereo images by Sparrow et al (Sparrow et al, 1997). Further details on imaging of changes in AMD can be found within a comprehensive paper by Nivison-Smith et al (Nivison-Smith, 2014).

Summary

Within Chapter 1 an overview of the MP, their history, sources and role within the eye, AMD and grading scales has been described. In Chapter 2 primary studies relevant to the current work will be described, and seven relevant intervention studies critically reviewed.

Chapter 2 Literature review

In Chapter 1 MP were introduced and the background to the study described. In Chapter 2 previous work in this area is described and reviewed, gaps in knowledge are highlighted and the rationale for the current work explained.

A literature search for prior studies showing comparisons between ocular nutritional ONS and using different MPOD measurement devices was conducted using ISI Web of Science and PubMed. Key words and their combinations used in the search included 'macular pigment optical density', 'lutein', 'zeaxanthin', heterochromatic flicker photometry', 'fundus reflectometry', 'single wavelength reflectometry', 'Raman spectrometry', 'autofluoresence spectrophotometry' 'minimum motion spectrophotometry', 'macular imaging', 'macular degeneration', 'ocular nutritional supplements'. MPOD was then used as a secondary 'within results' filter, to improve relevance to this study (See Table 2.1). After this two-step filtration, papers were subjectively filtered. Further objective filtration, to remove, say, animal studies etc. would have been better. A review was made of titles, and relevant abstracts read with a view to include in this thesis. Relevant papers were then either printed or stored electronically for reading and extraction of pertinent data. Further papers were obtained from the references of the reviewed articles. Websites relating to the ocular nutritional supplements, along with those of professional organisations such as 'College of Optometrists' and 'American Academy of Ophthalmology' with an interest in ONS were also searched. All the articles were reviewed and relevant information was incorporated into the manuscript.

A total of 29 papers were used in the literature review, nine for ONS and 20 for MPOD measurement devices. The last search for papers was undertaken in September 2009, prior to ethical approval submission. Literature search was continued after this date to help inform the general discussion up to January 2015. A further seven papers were reviewed to support the ONS and eight for the measurement device protocol set out in the original rationale.

	Web of Knowledge		PubMed	
	Single filter	MPOD filter also	Single filter	MPOD filter also
Macular pigment optical density	159	-	145	-
Lutein	11,194	118	-	77
Zeaxanthin	7,923	91	-	65
Heterochromatic flicker photometry	225	72	-	42
Fundus reflectometry	107	4	-	6
Raman spectrometry	946	0	-	1
Autofluoresence spectrophotometry	0	0	47	0
Minimum motion spectrophotometry	0	0	18	0
Macular imaging	48	0	-	17
Macular degeneration	30,191	66	-	56
Ocular nutritional supplements	2	2	84	0

Table 2.1 Literature search results by search engine with single and secondary filters in place as of September 2009.

Studies investigating diet and AMD

A study investigating dietary intake suggested a relationship between the amount of dietary intake of carotenoids and a reduced risk of developing advanced AMD (Seddon, et al., 1994). This study was set in five ophthalmology centres in the USA with 356 participants all having advanced AMD, aged between 55 and 80 years. 520 control participants were enrolled from the same geographic location also having ocular diseases and matched on an age and gender basis. Seddon et al found that those with a higher intake of dietary L and Z had a lower odds ratio of AMD progression. A similar outcome was reported in AREDS, which also focused on dietary intake without supplementation (Age-related Eye Disease Study Research Group, 2007). 4519 participants were enrolled in this study across 11 clinical centres in the USA. They were aged between 55 and 80 and all fell into one of four AMD severity grades, except the controls who had drusen, but less than 15um in size. All underwent a food frequency questionnaire prior to joining the study. These results lead to the proposition that supplementation with relevant ONS could also increase macular pigment.

Ocular nutritional supplements and AMD

Intervention studies carried out in 2007 and 2008 found no significant benefit to visual performance (contrast sensitivity) from a 6mg concentration of L over a nine and 18

month period (Bartlett & Eperjesi 2007; Bartlett & Eperjesi, 2008). The 2007 nine month study had 10 participants in the control and 15 in the active group all with AMD, with seven and 13 respectively completing the study. All were recruited from local eye-care centres. The 2008 18 month study enrolled 25 participants to the placebo group and 21 to the active group, with 15 and 14 respectively completing the 18 months, had healthy eyes free from AMD and other pathology

Seven ONS intervention studies were found during the literature search that measured MPOD as in this study. A detailed critical review of each of these studies is available below. The intervention studies are Huang et al (Huang, et al., 2013), the LUTEGA study (Dawczynski et al, 2013), Loughman et al (Loughman et al, 2012), the zeaxanthin visual function (ZVF) study (Richer, et al., 2011), the collaborative optical macular pigment assessment (COMPASS) study (Nolan, et al., 2011), the lutein nutrition effects measured by autofluoresence (LUNA) study (Trieschmann, et al., 2007) and the lutein antioxidant supplement trial LAST II (Richer et al, 2007).

Of these studies the majority were carried out providing ONS interventions to participants with AMD (Huang et al, LUTEGA, ZVF, LUNA and LAST II) with only two requiring as part of their inclusion criteria participants with healthy eyes (Loughman et al and COMPASS), both from the same research group. This supports the choice within this study to undertake review of participants without ocular disease.

A decrease in MPOD with age has been suggested by one study (Huang et al) but was specifically shown not to be the case by one other (LASTII). Interestingly Huang et al also found that serum levels of carotenoids increased with age, which may point to greater issues with transfer of nutrients from the serum into the cells with age. There is some evidence that participants with the lowest MPOD levels tend to show the greatest increase in MPOD levels after supplementation (Huang et al, LUNA, LASTII). This was only contradicted in one study (COMPASS). As COMPASS showed very slow retinal uptake of carotenoids and used the ester forms of the carotenoids this may account for why the different outcome was noted.

Of interest is the finding of the LUNA study where it was shown that the three upper quartiles all showed a plateauing of MPOD rise after a period of supplementation. This may point to the potential of MPOD becoming ‘saturated’, unable to take up more carotenoid and so may point to an upper limit of MPOD required to protect the macula. In

support of this concept COMPASS points to the ‘superfluous’ nature of a MPOD of greater than 0.3 for visual performance.

Visual function was measured in several different ways across these seven studies from best corrected visual acuity (BCVA), to a broad spectrum of ‘visual performance criteria’ and back to visual acuity (VA) (see Table 2.2). LUTEGA (BCVA) found an improvement following supplementation, as did COMPASS (mesopic contrast sensitivity and light/dark adaptation). Loughman et al (broad spectrum of parameters) found no significant improvement with 20mg L whereas with 10mg L and 10mg MZ there was significant change. ZVF found that primarily with Z, but also with L and L & Z, foveal acuity increased, whereas in the parafovea Z had little effect, but L and L & Z had a significant effect upon visual function.

Across the seven studies all found some significant improvement in MPOD with supplementation (see table 2.2). Huang et al with 20mg L but not with 10mg, Loughman et al with 10mg L & 10mg MZ but not 20mg L, LUTEGA with both 20 and 10mg L, and in this study both concentrations included omega 3. ZVF showed raised MPOD for L 9mg and Z 8mg separately, but no significant change for a combined L and Z ONS, which they ascribe to competition for receptor sites at a 1:1 ratio of these carotenoids, which supports the 5:1 ratio found in this study and in nature. COMPASS and LUNA described the MPOD improvement with 12mg L & 1mg Z and LAST II with L on its own or with other antioxidants. There were some groups that did not respond to ONS at all, with 20% of participants showing no response in COMPASS (24 participants) and the lowest quartile (27 participants) in LUNA not responding.

As one of the reviewed papers (Dawczynski et al, 2013) included Omega-3 in its formulation of supplement as well as omega-3 being included in part of the AREDS 2 protocol it would be good to briefly review its role. Omega-3 content (especially Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)) of the diet of participants was unknown which prevents knowledge of how available it was and how easily it was taken up within the body. MPOD has been shown to vary positively with higher levels of omega-3 long chain PUFAs (Delyfer, et al., 2012) and AMD risks are decreased by high intake of these omega-3s (Merle, et al., 2011). Dawczynski et al described increased MPOD and BCVA for two concentrations of L/Z/EPA/DHA compared to placebo (Dawczynski et al, 2013). However AREDS 2 found no significant benefit from omega-3 intake in terms of AMD progression. Conversion from short chain essential

omega-3 Alpha-Linoleic Acid (ALA) to long chain EPA and DHA varies due to a number of factors.

The omega-6 Linoleic Acid (LA) competes, by sheer volume within the diet, for the desaturase enzyme that converts ALA to EPA, resulting in more Arachidonic Acid (AA) than EPA being formed with it's potentially pro and anti-inflammatory nature. Conversion to EPA and then onwards to DHA from ALA is also dependent upon gender and age. Younger women convert at a higher rate (21% EPA and 9% DHA) (Burdge & Wootton, 2002) than younger men (8% EPA and 0-4% DHA) (Burdge et al, 2002). This has been attributed to the oestrogen uplift found in young women when compared to young men (Giltay et al, 2004). This also helps explain why the reduction of conversion with age also occurs. Thus EPA and DHA are considered conditionally essential fatty acids and their effects upon eye health deserves further investigation (Queques & Souied 2014).

Limitations in these studies are detailed in the critical review below but include limited comparability due to ethnic origin, inability to compare results for participants with AMD with those without AMD, use of ester form L with its different absorption characteristics compared with the non-esterified form, instrument limitations, gender bias within the participant groups, ratio of L:Z and receptor site competition, and prior ONS washout times not being sufficient.

Study	Statistically significant MPOD increase	No statistically significant MPOD increase	Non responder reporting	Visual function improvements reported	MPOD measure	Time period of supplementation	Eye health	Age range in years	Number of participants
Huang (Huang et al 2013)	20mgL	10mgL	No	-	FAFS	48 weeks	AMD	>50	108
Loughman (Loughman et al 2012)	10mgL & 10mgMZ (Combined)	20mgL	No	VA Contrast sensitivity Photostress recovery Ocular straylight	HFP	6 months	Healthy	18 – 70	36
LUTEGA (Dawczynski et al 2013)	20mgL and 10mgL (separate, both with omega-3)	-	No	BCVA	FR	12 months	Non-exudative AMD	70 +/- 10	172
ZVF (Richer et al 2013)	9mgL and 8mgZ (separate)	9mgL & 8mgZ (combined)	No	Foveal acuity (with 8mgZ, 9mgL and 9mgL & 8mgZ) Parafoveal acuity (with 9mgL and 9mgL & 8mgZ)	HFP	12 months	Early/moderate AMD	74.9 +/- 10	60
COMPASS (Nolan et al 2011)	12mgL & 1mgZ (Combined)	-	20%	Mesopic contrast sensitivity Light/dark adaptation	HFP	12 months	Healthy	18 – 41	121
LUNA (Treischman et al 2007)	12mgL & 1mgZ (Combined)	-	25%	-	FAFS	6 months	AMD	71.5 +/- 7.1	136
LAST II (Richer et al 2007)	10mgL (with and without antioxidants)	-	No	-	HFP	12 months	Atrophic AMD	74.7 +/- 7.4	90

Table 2.2 Summary of study parameters discussed. FAFS – Fundus autofluoresence spectrometry, HFP - Heterochromatic Flicker Photometry, FR – Fundus Reflectometry

Critical review detail of seven intervention studies

Serum and macular response to ONS with AMD (Huang, et al., 2013)

Subjects: 330 subjects were involved in the study, 212 screened via telephone calls and 108 via retinal image review, all of Asian Chinese ethnic origin following advertising to enrol on the study. Inclusion criteria were that the subject was over 50 years of age, had a visual acuity of 0.25 (6/24) or better, had not taken any L or Z ONS, was in good health and had early AMD. Exclusion criteria were the presence of other ocular disease or abnormal digestive conditions.

Experimental design: The study was for a 48 week period and was a double blind random assigned placebo controlled study. It used three ONS, Low L (LL) at 10mg, High L (HL) at 20mg and L and Z at 10mg each. A food frequency questionnaire was recorded at the start and the finish of the 48 week period. Recorded detail included gender, age, smoking habit, waist size, height, weight, auto-fluorescence and retinal images (at 0, 24 and 48 weeks) and fasting bloods (at 0, 4, 12, 24 and 48 weeks) including total cholesterol, triglyceride, high density lipids and low density lipids.

Outcomes: Auto-fluorescence was measured by two ophthalmologists using the Heidelberg Retina Angiograph (HRA-II, Heidelberg Engineering, Heidelberg, Germany), after dilation with tropicamide to ensure a 6mm pupil; this was on one eye only. Serum levels were measured using the Hewlett-Packard/Agilent Technology model High Performance Liquid Chromatography system with a C_{30} column.

Results: Baseline MPOD was inversely correlated with age (R=-0.28 p=0.04) and baseline serum was positively correlated with age (R=0.21 p=0.034) with L/Z intake not being significantly different with age. Serum levels did increase with supplementation, whereas there was no significant change with the placebo group. Final HL level of MPOD was significantly higher than LL or L and Z (p=0.05) whereas LL and L and Z had no significant difference. Lowest level MPOD at baseline increased the most at final reading. Serum levels compared with MPOD showed positive correlation in all supplement groups with HL showing a positive correlation 0.49 (p=0.01), but no significant relation was found for LL or L and Z.

Limitations: The limited geographical and ethnic nature of the paper could create bias and reduce the ability to cross compare results. It is also necessary to query the ability to state that there are no side effects. It would be recommended that further studies with larger cohorts, especially including elderly subjects with liver and kidney diseases. The role of Z was also not established and needs further studies to clarify.

The LUTEGA study: L, Z and Omega-3 ONS on MPOD with AMD (Dawczynski et al, 2013)

Subjects: 273 subjects were identified as suitable for inclusion in this study, 172 started and 145 completed the 12 month study. Ethnic origin was not stated, but the population was all hospital based and had non-exudative AMD (Early stages). Age range was from 50 to 95 years and the subjects had to have had no ONS in the last six months containing any L, Z or omega-3. Exclusion criteria included marked retinal pigment epithelial changes or neovascularization, sub-retinal haemorrhages geographic atrophy, missing fixation, optic nerve disease, unstable glaucoma with intra-ocular pressure greater than 25mmHg, history of retina-vitreous surgery and advanced cataract.

Experimental design: The 12 month randomised parallel assigned double blind intervention study randomly assigned participants to one of three groups; D1 (10mg L/ 1mg Z/ 100mg Docosahexaenoic acid (DHA)/ 30mg Eicosapentaenoic acid (EPA)/ vitamin C 60mg, vitamin E 20mg/ Zinc 10mg and copper 0.25mg), D2 (20mg L/ 2mg Z/ 200mg DHA/ 60mg EPA/ vitamin C 120mg/vitamin E 40mg/ Zinc 20mg and copper 0.5mg) or P (placebo). The study group all underwent a full ophthalmologic examination including medical history and blood pressure measurement. Data was recorded for body mass index, iris colour, smoking habit, ametropia, systemic diseases and gender. Baseline and follow-up visits included best corrected visual acuity (BCVA), Amsler grid, MPOD, Retinal photography and auto-fluorescence, slit lamp biomicroscopy, optical coherence tomography and blood samples at 1, 3, 6 and 12 months. A simplified food questionnaire was also completed.

Outcomes: BCVA was measured by Early Treatment Diabetic Retinopathy Study (ETDRS) charts (4m) MPOD, retinal images and autofluoresence by Visucam NM/FA (Carl Zeiss Meditec, Jena, Germany) Optical coherence tomography by Cirrus 4.0 OCT (Carl Zeiss Meditec, Jena, Germany). The ONS used was FloraGLO Lutein (Kemin Food L.C, Des Moines, IA USA).

Results: At 12 months in both cohorts D1 and D2 the MPOD measurement parameters (volume, area, max OD and mean OD) increased significantly ($p<0.001$). Volume was found to drop in the placebo group over the same period supporting supplementation of some nature. BCVA displayed a significant improvement in both D2 ($p=0.006$) and D1 ($p=0.038$) when compared to P.

Limitations: The study focused on one AMD group and therefore doesn't provide information on other groups, such as advanced (exudative) AMD or those without AMD. FloraGLO L is extracted from Marigolds, which means that it is in an ester format, which changes the retinal and serum uptake levels, reducing it's comparability to studies with unesterified L. The ethnicity of the cohort is unknown. The effects upon younger age groups are not studied, primarily due to the low numbers suffering with AMD at earlier ages. The validity of the instrument used to measure MPOD may be questioned as it is known to not show results higher than 0.5 optical density units, even though a substantial number of these subjects have yielded greater values (up to 0.9) on the Macular Densitometer (Nolan & Beatty, 2013).

Impact of macular pigment augmentation on visual performance using different carotenoid formulations (Loughman et al, 2012)

Subjects: 36 participants were enrolled, 19 male and 17 female with an average age of 51+/- 13 years, with 32 completing the six month study. Ethnic origin was not stated. Inclusion criteria were to be within the 18 -70 year age group, have a spectacle prescription of less than six dioptres, to have no ocular or systemic pathology prior to the study, a BCVA of 20/60 or better and to have not taken L/Z/MZ within 12 months of the start of the study. The study eye was the eye with the better VA or if both were equal then the right eye was chosen.

Experimental design: This six month study was single masked, placebo controlled with random assignment to groups. Three groups were created 1) 20mg L 2mg Z 0mg MZ, 2) 10mg L 2mg Z, 10mg MZ and 3) Placebo. Data collected were MPOD, Iris colour, lifestyle and demographic data along with serum concentrations and BMI. Visual performance was also measured with a logMAR ETDRS test chart, Contrast sensitivity, Photostress recovery time and ocular straylight. Visits were recorded at baseline three months and six months.

Outcomes: MPOD was measured using the Macular Densitometer using heterochromatic flicker photometry (HFP), logMAR ETDRS test chart (Test Chart 2000 Pro, Thompson software solutions, Hatfield, UK), Contrast sensitivity (Optec6500 Vision Tester, Stereo Optical Co Inc., Chicago, IL, USA) Photostress recovery time (Humphrey visual field analyser 745i, Carl Zeiss Meditec Inc., Dublin) and ocular straylight (OCULUS Optikgeråte GmbH, Wetzlar, Germany). The ONS used within the study were 1) Ultralutein (Nature plus, Melville, NY USA) 2) Macushield, (Macuvision Europe Ltd, Solihull, UK) and 3) (G & G Food supplies Ltd, West Sussex, UK).

Results: There was no significant difference in MPOD, serum carotenoid and visual performance at baseline measurement between the three groups. At the three and six month visits there was no significant change to groups 1) and 3) in MPOD, serum levels of carotenoid and visual performance. A significant improvement was noted in MPOD, serum carotenoid and visual performance in group 2) at three and six months ($p<0.05$).

Limitations: The single masking with subtle visual differences between the interventions permitted the investigator to know which intervention was used by the participants. Although they were not involved in any of the randomisation, recruitment or for data analysis, this could have had a biasing effect upon the results of the study. The ethnic origin of participants is not known and so how applicable this cohort is to specific populations is unknown.

The Zeaxanthin and Visual Function Study (ZVF) (Richer, et al., 2011)

Subjects: 60 participants were enrolled in this study. 57 were male and 3 female with an average age of 74.9 +/- 10 years. These were recruited from the DVA Medical Eye Centre Clinic, Chicago. Fees were paid to participants in this study with a per visit $25 travel allowance and a $100 completion of study payment. Inclusion criteria included the need to have mild to moderate AMD with measureable defects on contrast sensitivity or glare disturbances, Amsler chart abnormalities, subjective functional night driving or reading disturbances that they wanted to improve. Exclusion criteria of high risk of developing advanced AMD and treatment for this was available, prior L or Z ONS supplementation beyond 250µg/day within the last six months, any active co-morbidities or dementia/schizoid problems and any retinotoxic medication.

Experimental design: This 12 month study was a randomised double-blind, placebo-controlled prospective intervention study. It used three supplement groups 1) 8mg Z

(n=25), 2) 8mg Z and 9mg L (n=25) and 3) 9mg L ('Faux placebo', n=10). Visits were made at baseline, four, eight and 12 months. Demographic parameters were measured – Age, gender, months since AMD diagnosed, smoking pack years, alcohol, physical activity, diabetes, Iris colour. Physical parameters included – BMI, hand grip and body fat percentage. A Food Frequency Intake Questionnaire was administered. Skin carotenoid level was measured along with Cataract grade and a self-administered vision questionnaire. MPOD and retinal autofluoresence were also measured. Foveal testing involved best refraction and VA, whereas parafoveal testing included the ChromaTest System ®, Low-contrast near VA, Distance contrast sensitivity, Photostress glare recovery and Visual field scotomas.

Outcomes: The food frequency intake questionnaire (FFQ) was the Harvard School of Public Health FFQ version GP88. Skin carotenoids were measured with the Biophotonic® Scanner (Pharmanex Inc., Provo Utah). Cataracts were scored against the Lens Opacification Cataract Scale (LOCSIII) and the self administered vision function questionnaire was the NEI VFQ25. MPOD was measured suing the Quantifeye® MPS9000 using HFP (ZeaVision Inc., Chesterfield, Missouri USA) Retinal autofluoresence was measured with the Kowa Digital VK2® system (KOWA Optimed, Tokyo, Japan). VA was measured with the ETDRS distance visual acuity video projection system (M&S Technologies, Smart Systems II, Park Ridge, Illinois, USA). The parafoveal tests were carried out with the ChromaTest® System (CH Electronics, Bromley, UK), the 10% Weber fraction Colenbrander Mixed Contrast Reading Card®**,** Contrast Sensitivity with the Functional Vision Analyser (Stereo Optical Co Inc., Chicago IL, USA), Photostress glare recovery with the KOWA AS14B Night Vision Tester (KOWA Optimed, Tokyo, Japan) and the scotomas with the SimulEyes Kinetic Visual field test (Rush Ophthalmics, Gold Beach Oregon, USA).

Results: 90% of participants attended for two of the three follow up visits, with a 96% 'pill uptake'. Nine were lost to follow up. MPOD at baseline showed no statistical difference between the three groups. At 12 months all three groups had increased the MPOD level with statistical significance for 1) Z (p=0.03) and 3) L (p=0.03) whereas 2) L and Z the result was near significance but just fell short at p=0.06. All these results were for the trend across the 12 months. Skin carotenoid was significantly raised in 3) L (p=0.02) and 1) Z (p=0.04) and especially so for 2) L and Z (p=0.008). There were 27% non-responders to skin carotenoid change with 75% of these being within the 1) Z group. Foveal near VA had significant changes with 2) L and Z and 3) L having p=0.05, whereas

the prime carotenoid at the fovea 1) Z having p=0.001. Parafoveal near vision had significant changes in 2) L and Z (p=0.02) and 3) L (p=0.04) whereas 1) Z had no significant change.

Limitations: Competition at receptor sites for L and Z when presented in equal amounts may have distorted the results for 2) as naturally the carotenoids are found in a 5:1 ratio, which points towards the benefits of this being included in future ONS in that ratio. Cataracts were present in most of the participants that cause problems for imaging and visual performance. The tedious nature of the testing battery led at least one participant to drop out. The 3D data was only available on 16% of subjects, primarily as a result of the cataracts causing problems with the imaging. Serum and skin carotenoid levels not good predictors of MPOD. This study was primarily male and so is not necessarily applicable to the female population. As the authors clearly state, and we would concur wholeheartedly with, a better objective testing system for MPOD is much needed.

The COMPASS study (Nolan, et al., 2011)

Subjects: 121 participants were enrolled on the study with no eye disease present. They were aged between 18 and 41. The inclusion criteria were that they had to have good general and ocular health and have a VA of 20/30 or better in the test eye (dominant eye or right eye if equal).

Experimental design: This was a randomised placebo controlled clinical trial. Visits were at baseline, three, six and 12 months. Two groups were created using A (active) L 12mg, Z 1 mg (ester) 120mg vitamin C 17.6mg vitamin E, 10mg zinc and 40µg selenium, and P (placebo) cellulose, lactose and magnesium sterate. Serum carotenoid levels were measured as a compliance check. Demographic data was recorded: general health, smoking habit, alcohol, exercise, BMI, blood pressure, ethnicity, marital status, education and occupation. Vision data was recorded: time since last sight test, spectacle or contact lens use, history of ocular treatment/surgery, occlusion therapy or visual training in childhood, family history of eye disease, current visual problems, computer associated asthenopia and history of headaches. A food frequency questionnaire was also completed. Refractive error and eye dominance were recorded along with glare disability, a visual function questionnaire, contrast sensitivity, photostress recovery, MPOD and fundus photography.

Outcomes: Serum carotenoid was measured using high performance liquid chromatography (HPLC). The food frequency questionnaire was developed by the Scottish Collaborative Group at University of Aberdeen (Scotland, UK). Refractive error measured on the Test Chart 2000 Pro (Thompson Software Solutions) using a Sloan ETDRS letterset. Ocular dominance was measured using the Miles test. Glare disability was assessed with the Functional Vision Analyser (Stereo Optical Co Inc., Chicago, IL, USA). The Visual function questionnaire was the VFNq30. Contrast sensitivity was measured with the Metropsis Visual Stimulus Generation device (Cambridge Research Systems Ltd, Cambridge, UK), Photostress with the macular automated photostress test using the Humphrey field analyser (745i, Carl Zeiss Meditec Inc., Dublin, CA, USA). MPOD measurements were carried out with the Macular Densitometer using HFP. Fundus photography was taken using the Nidek AFC-230.

Results: MPOD was significantly higher in group A at each visit and at eccentricities of 0.25^0, 0.5^0 and 1.75^0 ($p<0.05$) whereas group P was not significantly changed ($p>0.05$). Visual performance was unchanged except for at visit 4 (12 months) for 'Daily task comparative analysis' with $p=0.03$. In the high MPOD tertile when compared to the low MPOD tertile, there was a borderline significant improvement to mesopic contrast sensitivity and light/dark adaptation ($p=0.05$). This study did not find a greater increase in MPOD levels of the lowest MPOD scores at baseline. They also found a 20% non-responder rate.

Limitations: It has been previously stated that an MPOD of greater than 0.3 is superfluous to visual performance so the data may be testing a 'pool' of higher MPOD score thus showing little visual performance change. Visual performance was considered in a consulting room environment that does not reflect the normal day to day external environment, therefore the results can only be interpreted across a limited range of glare intensities. Compliance tests showed that on average 1.6 tablets were consumed per day compared with the two as per protocol, with 95% consuming at least one tablet a day. This was similar in both groups, thus the group comparison is valid, but the concentrations consumed were lower than the planned amounts. The serum non-responders were discussed and suggestions on why were non-compliance with taking tablets, gastrointestinal absorption attenuation and not taking the tablets with fat or oil. The group were self selected which can lead to a distortion in the cohort against the general population. The Z was delivered in an ester form which can provide problems with bioavailability. There was a significant difference between the two groups for smoking habit which could act as a confounder to the study results. The study results

were broken down into tertiles, which when compared to quintiles could yield a difference in results. Frequently quintiles have been used in this form of study. The inclusion of antioxidants in the L and Z formulation could have been the reason why the results differed rather than L and Z. Including these antioxidants in the placebo would have covered this potential confounder. The change in daily task comparative analysis was a subjective test and so could have had some change accounted for in change in attitude or desire to please the investigator, as with the Hawthorne effect. The retinal uptake of the carotenoids was slow when compared to similar studies, this could have been down to the concentrations used, using an ester form of Z, the matrix of the tablets, inclusion of antioxidants, the serum response or non-compliance.

The LUNA study (Trieschmann, et al., 2007)

Subjects: 136 participants were enrolled on this study. 108 into the Intervention (I) group, and 28 into the control (C) group. The I group had a mean age of 71.5 +/- 7.1 years ranged over 51-87 years with 92.6% displaying AMD. The C group had a mean age of 71.0 +/- 8.1 years, ranged over 57 – 83 years with 89.3% with AMD. The male to female ratio was 40:68. Inclusion criteria were to be aged over 50 years, with no/minimal lens opacities, no prior supplementation and good general health. Exclusion criteria were no atrophic changes with AMD, central retinal pigment epithelial problems or choroidal neovascular changes. Test eye was the eye with the best retinal auto-fluorescence image, the eye with the best VA or the right eye.

Experimental design: This six month study was a non-randomised open label controlled intervention study. MPOD, serum carotenoid and retinal auto-fluorescence were all recorded at each visit as baseline, six, 12, 18, 24 and three months post cessation of ONS in group I. Group C were tested at baseline and at one further visit which ranged from week 10 to week 50 with an average of week 29.4 +/- 9.3 weeks. The I group intervention was Ocuvite lutein (12mg L, 1mg Z (both ester form), 120mg vitamin C, 17.6mg vitamin E, 10mg zinc and 40µg selenium). The C group did not receive a placebo. Biochemical markers were measured at baseline and study exit for total cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL) and zinc.

Outcomes: MPOD was measured using the Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg, Germany) using retinal auto-fluorescence. Serum carotenoid was measured using the 116 liquid chromatograph (Beckman Coulter GmbH, Krefeld,

Germany). Biochemical markers were measured with: Total cholesterol and triglycerides with COHD-PAP and GPO-PAP (Roche diagnostics, Mannheim, Germany), HDL PEG modified enzymes (Roche Diagnostics) LDL the Friedwald formula, with all being measured using the Boehringer Mannheim/Hitachi automatic analyser type 747. Serum zinc was measured with the Perkin-Elmer 2380 atomic absorption spectrophotometer.

Results: The I group had a final average MPOD of 0.504, and increase or 0.1 +/- 0.009 with p=0.0008. The C group ended with an average MPOD of 0.525 up 0.03 +/- 0.02 with p>0.05. The lowest baseline MPOD individuals were noted as increasing in MPOD level more than those with a higher baseline MPOD. The results suggest that MPOD is saturable with the upper three quartiles plateauing (Q2 at 0.59 +/- 0.04, Q3 and Q4 at 0.64 +/- 0.03). The lowest quartile for MPOD did not respond to supplementation.

Limitations: The C group did not follow the same measurement regimen as the I group, with only one follow up visit. There was no control for dietary changes happening during the study. The study was not randomised and was open label, without placebo. The L and Z were supplied in the ester form which can cause problems with bioavailability. The auto-fluorescence measurement is dependent upon the assumption that the two wavelengths used and validated against a ‘normal’ population holds true with a population with AMD.

The LAST II study (Richer et al, 2007)

Subjects: 90 participants were enrolled on this study with an average age of 74.7 +/- 7.4 years, with 76 completing the study. Male female ratio was 86:4. The participants had to have atrophic AMD with one of the three: Reduced contrast sensitivity, photostress recovery abnormality and Amsler grid defect. Exclusion criteria were cataract or retinal surgery within six month of the start of the study, use of photosensitive drugs and not meeting the ophthalmic/visual criteria.

Experimental design: The study was a prospective 12 month randomised double-masked placebo-controlled intervention study undertaken in an urban mid-west USA veteran’s hospital. The intervention groups were L 10mg L, L+ 10mg L plus a broad spectrum of antioxidants and P placebo of maltodextrin. The L supplied was in a non-esterified form. Data gathered included: gender, age, AMD duration, smoking habit, alcohol, caffeine, BMI, nutrition, Iris colour, lens opacity grading, AREDS disease stage, MPOD, VA,

contrast sensitivity function, glare recovery and Amsler grid. Visits were at baseline, four, eight and 12 months.

Outcomes: MPOD was measured by HFP (MacularMetrics, Rehoboth, Massachusetts, USA), based on an average of four readings at each visit and nutrition using the Harvard School of Public Health Food Frequency Intake Questionnaire.

Results: L+ and L groups had increased MPOD measurements at twelve months whereas the P group had shown a slight decline in MPOD at the same point. This was statistically significant compared to the P group (L p=0.0007, L+ p=0.0166) but the difference between L and L+ was not significant (p=0.73). MPOD did not vary significantly with age nor BMI. The lowest baseline MPOD scores changed most when L or L+ were consumed, whereas the upper Q of MPOD scores at baseline showed little difference in change when comparing L and L+.

Limitations: The decline in MPOD for the P group is suggested to be not allowing sufficient washout of any prior ONS before beginning the study. A broader range of ages and BMIs should be reviewed prior to stating that there are no significant differences in either two variables. The population studied was male dominated (95.6%) and so cannot be immediately applicable to the female population which is reportedly at greater risk. The authors do call for a better objective device to measure MPOD, this includes an accurate, reproducible, non-invasive, rapid-capture of the level and distribution of MPOD.

Review of MPOD measurement devices

A literature review of in vivo MPOD measurement has been carried out (Howells et al, 2011). Numerous methods have been suggested for measurement of MPOD, which may account for some variations in reports on MPOD reports at different levels of AMD (Bernstein et al, 2010):

1. Heretrochromatic flicker photometry (Snodderly, et al., 2004)
2. Fundus reflectometry (Berendschot & van Norren, 2004) and single wavelength reflectometry (Schweitzer et al 2010)
3. Raman spectrometry (Bernstein et al, 1998)
4. Autofluoresence spectrophotometry (Delori et al, 2001)
5. Minimum motion photometry (Robson, et al., 2003)
6. Imaging (Berendschot & van Norren, 2006)

The first four have received particular attention.

Heterochromatic flicker photometry (HFP)

This technique involves an alternating test stimulus of wavelengths absorbed by the macular carotenoids (circa 460nm – blue) and one that is not absorbed (often 540nm – green). This alternation is perceived as flicker, which the subject adjusts until, either the perception of flicker is extinguished (from perceived to not perceived) or first appears (from not perceived to perceived). The minimal/extinguished or just perceived flicker intensity of the blue light is recorded and the test repeated at eccentric fixation at a point which assumes that MPOD is minimal. Central MPOD level is then calculated as log(foveal/eccentric). L and Z levels are measured specifically (Snodderly, et al., 2004)

HFP is a minimally invasive technique, without requirement for dilation. It is however a psychophysical technique and therefore requires training for the subject and good attention during the test. A 'test run' is normally included in the routine with this method. Normal corrected VA is required to fixate the central target (hence problems arise with media opacities) and the long and medium wavelength cone ratios are assumed to be similar at both measurement locations (central and eccentric). This assumption may not hold true with irregular ratios being present in normal subjects and a suggestion that age and macular disease can accentuate the difference (Hofer et al, 2005). The assumption that the eccentric fixation point will be in a position where macular carotenoids will be minimal or non-existent is also challenged with some individuals having measurable amounts of carotenoid beyond the normally assumed reference point of 7^0 (Bhosale et al, 2007)

Fundus reflectometry

A fundus camera, fitted with charge-coupled device (CCD) and scanning laser ophthalmoscope (SLO) obtains two images at the fovea and parafovea using both blue (480-488nm) and green (515-540nm) light (Kilbride et al, 1989; Wustemeyer et al, 2002). As MP absorbs blue light more than green, subtraction of the aligned green and blue images, following log transformation, allows density differences to be approximated. The method is objective but suffers from being none chemically specific in that there are absorbers within the eye other than MP and so all attenuation cannot be put down to MP alone. The equipment (often) requires dilation of the subject's pupils and is expensive, necessitating some technical expertise.

Spectral reflectance, covering a small central area using multiple wavelengths requires neither reference point nor dilation, and a pilot study has suggested it could be used to create separate measurements for both L and Z (van de Kraats et al, 2008).

Raman spectrometry

Resonance Raman Spectrometry (RRS) measures the bind excitation within molecules which is directly proportionate to MPOD (Bernstein et al,1998). A 1mm spot of argon laser light is fixated and this excites the MP for ~0.2s. The Raman scattered light is quantified after subtraction of background fluorescence. The linear association with MP is demonstrated by excised retinal tissue samples supporting the measurements (Ermakov et al, 2001). RRS is highly chemical specific. Light scatter by the lens or absorbance can attenuate the Raman signal and wide pupil dilation is normally required. This along with high light levels and expensive equipment has limited its commercial use.

Fundus autofluoresence spectrophotometry

MPOD is measured by determining the MP attenuation of lipofuscin fluorescence in the RPE. Lipofuscin absorbs in the blue wavelength region, overlapping that of the MP, and it emits in the orange-red region, beyond MP absorption (Sparrow et al,1999). As there is virtually no fluorescence from MP (Gellermann et al, 2002) it is possible to excite lipofuscin emission within and outside the range of macula pigment. This excitation occurs usually at 488nm and 514nm (argon laser) or 532nm (YAG laser). 488nm is absorbed by MP and lipofuscin, whereas 514 and 532nm are less absorbed by the MP but do excite the lipofuscin. Confounding lens fluorescence can be overcome by changing the higher wavelength to ~650nm and above (Sharifzadeh et al, 2006). Again the MPOD level is calculated by taking the difference in lipofuscin fluorescence intensities at foveal and extra foveal sites (Delori et al, 2001). Uniformity of lipofuscin distribution is assumed, so in cases of significant AMD where this may no longer be true, dual wavelength imaging at lipofuscin absorbing levels is advised.

This method can be carried out without dilation, it is objective, quick, requires minimal subject training and minimizes media opacity confounders. It has been shown to positively correlate with measurements by psychophysical techniques such as HFP (Delori et al, 2001). There are few standard fundus cameras that are capable of measuring fluorescence and so this would incur further investment and as such is moderately expensive.

Measurement in clinical practice

HFP was the most easily accessible form of MPOD measurement available for clinical practice when the study was started (Bartlett et al, 2010b), thus this paper will concentrate on it alone. Since then a camera based system using single wavelength

reflectometry has been introduced (Carl Zeiss Meditec Visucam 200 Carl Zeiss 509 Coldhams Lane Cambridge CB1 3JS UK). There is also a further system currently under development using fundus autofluoresence (Heidelberg Engineering Ltd. Breakspear Park Suite F Breakspear Way Hemel Hempstead Hertfordshire HP2 4TZ United Kingdom). Due to the nature of HFP the yellowing of the crystalline lens with age should not affect the MPOD readings (Madridaki et al, 2009).

The two commercially available devices that measure MPOD via HFP are the MacuScope (Macuvision Europe Ltd, Lapworth, Solihull, UK) and the MPS9000 (Tinsley Precision Instruments Ltd, Croydon, Essex, UK) also known as the Quantifeye device in the USA. The two devices have taken opposite approaches to the end point measurement and because of this both have advantages and disadvantages.

The MacuScope

This device is described as being portable, which may be true in its absolute sense. The subject observes flickering stimuli comprising of two different, alternating wavelengths. The MP absorbs the wavelength stimulus of 465nm, the other is not absorbed at 550nm. The luminance ratio between the two is reduced until flicker is minimized or as perceived by the subject, extinguished. Thus the MacuScope takes the subject from a condition of flickering to one of not flickering. This is assessed for one central and one peripheral location. As MP is assumed to be maximal at the central location, and minimal at the peripheral one, then the luminance ratio between these two locations is used to determine the MPOD value.

From a subject's point of view this method is good as it is carried out in a short period of time, preventing fatigue. The downside for the neophyte is that the determination of the point where flicker is extinguished is difficult,

The repeatability and reproducibility of this device has been reviewed (Bartlett et al, 2010a). The authors concluded that the mean MPOD reading was 0.47±0.14 (male 0.49±0.17 and female 0.45±0.11, which were not significantly different) and that there was a negative correlation with age, which is supported by others (Kocak et al, 2010). The discussion concluded on the suggestion that changes of less than 0.58 could be down to measurement noise hence the poor repeatability and reproducibility of the device, a level of change that would make the device of limited use. A similar picture emerges when comparing two different operators, displaying 0.49 change required to be classed as clinically significant. Figures relating to HFP measurement using bespoke

research centre equipment have been reported upon with markedly better reproducibility of 0.08 and repeatability of 0.09 (Beatty et al, 2001). A 2010 study focused on inter-eye reproducibility using this device with variations to the mean scores across visits, without supplementation, and between eyes and the results are shown in Table 2.3 (Hagen et al, 2010).

Table 2.3 Inter-eye reproducibility of the MacuScope device (Hagen et al, 2010)

The conclusion from this paper was that their results differed with higher mean and weaker inter-ocular correlations than shown in previous studies and reinforced the suggestion that reproducibility is poor.

The Macular pigment screener 9000 (MPS9000)

This device works by taking the subject from a point where no flicker is detected to one where flicker is first perceived, the opposite route from the MacuScope. The alternation of the stimuli begins from a starting level of 60Hz and is decreased by 6Hz per second (van der Veen et al, 2009). 60Hz is set as the start point as it is above the critical flicker fusion frequency and thus the subject perceives no flicker initially. A sequence of blue-green ratios are presented and these are inverse yoked to maintain overall luminance. Sensitivity to flicker is determined by a built in pre-test routine, establishing the initial luminance contrast of the two light sources to be established. This 30 second routine ensures that the subject was in the middle of their individual flicker sensitivity range prior to the main test.

From a subject's perspective this method is good as the determination of the end point (when flicker starts) appears to be more intuitive. This device is more portable, but does require an external PC connection.

As with the MacuScope device the MPS9000 has been subject to review (Bartlett et al, 2010c). A mean MPOD of 0.35±0.14 was reported (male 0.45±0.19 and female 0.33±0.12 which displayed significant difference p=0.022). Repeatability of readings

within 0.33 units cannot be classed as clinically significant, as it is very likely to be due to instrument noise, and 0.26 units for reproducibility.

At the time this study was designed there were only two clinical based devices available the MacuScope and the MPS9000, and the MPS9000 device became available approximately six months after the MacuScope had been purchased in preparation for this study. Both have problems with wide variations in repeatability and reproducibility (Bartlett et al 2010a, b & c). After this design period the third device (Visucam 200) became available. This has been challenged with problems of inaccuracies in measurement over 0.50 (Nolan & Beatty, 2013). Thus all three devices have been criticised.

In summary of the above:

- Dietary intake of carotenoids reduces risks of developing advanced AMD
- Visual performance at levels of supplementation over 6mg L tends to improve
- Most studies have been conducted on participants with AMD
- Changes to MPOD with age are not clear and cannot be assumed
- The lowest MPOD groups may respond more to supplementation with carotenoids
- Some participants do not respond to supplementation
- Plateauing of MPOD increase occurs in many cases
- 0.3 density units may be all that is required for most eyes
- In most studies supplementing with L levels around 10mg or more enhances MPOD levels significantly
- Clinical devices available for measuring MPOD at the time of the study were all criticised and better, more reliable devices are sought.

A gap in knowledge that was identified was which concentration of L and Z is best to offer patients with healthy eyes. Further, If the premise can be accepted that protecting the photoreceptors, Bruch's membrane and the RPE is beneficial and that supplements have been shown to increase the MPOD (Landrum et al,1997; Bone et al, 2007) then it could be useful to measure baselines for subjects in practice and then to follow this up whilst supplementing to see if the supplementation is having the desired effect.

Rationale

Ocular nutritional supplements are now commonly found within optometric practice. Which ocular supplement to offer and in what mix of constituent nutrients has not been the subject of clear scientific review and has been left to the clinician to decide. Although there is some guidance on when to offer supplementation on subjects with AMD there is little on those subjects who are healthy or just pre-AMD. From attending peer review sessions and clinical conferences the impression is given that general nutritional advice with safety levels and adverse effects from ONS is not part of the core understanding of the majority of the clinician population. The evidence for which concentration of L/Z to provide to subjects was not well reported on. To help provide guidance to clinicians this study was designed to assess if there was any difference in effect of two concentrations of L/Z. A block stratified randomised trial has been designed to investigate this research question and to attempt to make an original and significant contribution to the literature.

Summary

Chapter 2 covers a cross section of the current literature, and a critical review of ONS intervention papers, which focus upon subjects with and without AMD. The rationale behind the study is also stated. In Chapter 3, the first experimental chapter, the sample size required for the whole study is determined.

Chapter 3 Experiment 1 – An exploratory study to determine sample size

Chapter 2 reviewed pertinent papers on the use of ONS in AMD up to January 2015 and explained the rationale for the studies in this thesis. Experiment 1 sets out to determine the sample size required to give the main study 80% power. In this chapter the methods used and the rationale behind these choices for Experiment 1 are stated.

The Null Hypotheses (H_O) is that there is no significant difference between the two sample populations being measured. Thus within experimental design there is a need to prove significant difference built in.

Because statistics does not deal in absolutes but in significant variation there could be errors arising from methods used, hence the need to report on methods used, levels of significance chosen and allowing for repetition of the experiments. Type I errors are those where the difference between two populations is declared significant even when the H_O is in reality true i.e. a false positive. Type II is when the two populations are significantly different, but the H_O is accepted incorrectly i.e. a false negative. In either case the problem for the researcher is that they end up with an invalid result on which to base further work.

Power in statistics is the probability of finding a difference that does exist (as opposed to finding a difference that does not exist i.e. Type I error) and so is concerned predominantly that a Type II error does not exist. Thus power is equal to 1- (false negative rate). Power is often designated by π and the false negative rate as β. Hence π= 1- β. A standard power of 80%, assumed in this study, shows that the weight was 4:1 in favour of ensuring that a false negative reading was not found by mistake.

This ratio can be varied in some circumstances, such as in some medical tests where the chance of a false negative, i.e. telling the patient that ‘all is well’ when it is not, outweighs the risks of finding a false positive. It would also be possible that in a study with many covariates sample size may need to vary for each parameter being studied to provide a given power.

At the time of setting up this study the effect size of ONS supplementation upon MPOD was not clearly understood, hence calculating the effect size from mean and standard deviation of this initial cohort allowed a sample size calculation to be undertaken and so attempt to achieve 80% power.

Method

Subjects

Participants were recruited by:

1. Poster asking for volunteers in the waiting rooms of the practice (Appendix C).
2. A printed invitation handed to all patients attending for an eye examination at the practice during the recruitment period requesting volunteers (Appendix D).
3. Article published in the Sheffield Star Newspaper.

Participant eligibility

Inclusion criteria were vision of 20/25 (6/7.5) or greater in each eye, able to attend a community optometry clinic in SE Sheffield, willing to commit to four months of ONS and completing food diaries. Exclusion criteria were any sign of macular changes, type 1 or 2 diabetes, due to potential problems with any retinopathy, clinical signs in the eye of any other eye or systemic disease, anyone taking anti-platelet and anticoagulant medication due to vitamin E inclusion in ONS (see Appendix A, vitamin E, anticlotting effect), those under age 16 at the start of the intervention and those already taking supplementation containing L and Z. A comparison of inclusion and exclusion criteria used from prime comparative studies discussed within this thesis is in Table 3.1

Recruitment

Participants were recruited from January 2010 with the final measurement being recorded in late June 2010. The sample size trial ended at this time, although recruitment for experiment 2 continued.

Settings

The centre for this study was Specsavers Opticians 50D Crystal Peaks, Sheffield, South Yorkshire, S20 7PN. Administration (enrolment, stratification and data storage) was carried out by JMM, data collection by CB and data analysis by GTV. Food diaries were scored and reviewed by GTV and review of process and analysis by FE. JMM is a dispensing Optician and Manager, CB is an exam lane assistant and GTV is an Optometrist and Director of Specsavers Opticians at Crystal Peaks, Sheffield UK. FE an experienced researcher at Aston University.

Inclusion Criteria	Vasey 2014	B&E 2008	B&E 2007	AREDS 2	Huang	ZVF	LUNA	LUTEGA	Loughman	LAST II	COMPASS
Visual acuity	20/25	-	-	-	20/30	Y	-	-	20/60	-	20/30
Able to complete study	Y	Y	Y	Y	-	-	-	-	-	-	-
AMD	-	-	Y	Y	Y	Y	Y	Y (dry)	-	Y	-
Age	16+	-	-	50-85	50+	-	50+	50-95	18-70	-	18-41
Clear media	-	-	-	-	Y	-	Y	Y	-	-	-
Exclusion criteria											
Macular changes	Y	Y	-	-	-	Y(adv)	-	Y (wet)	Y	-	Y
Diabetes	Y	Y	Y	-	-	Y	-	Y	Y	-	Y
Other disease	Y	Y	Y	Y	Y	Y	-	Y	Y	-	Y
Anticoagulant/platelet meds	Y	Y	Y	-	-	-	-	-	Y	-	Y
Already on ONS	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	_
Dietary absorption problems	-	Y	-	-	-	-	-	-	Y	-	Y
IOP >= 26mmHg	-	-	-	Y	-	-	-	Y	-	-	-
Eye surgery	-	-	-	-	-	-	-	Y	-	Y	-
Prescription	-	-	-	-	-	-	-	-	<6DS	-	-

Table 3.1 Inclusion and exclusion criteria from prime comparative studies discussed within this thesis Vasey 2014 (This thesis), B&E 2008 (Bartlett & Eperjesi, 2008), B&E 2007 (Bartlett & Eperjesi, 2007), AREDS 2 (The Age-related Eye Disease Study 2 Research Group, 2013), Huang (Huang, et al., 2013), ZVF (Richer, et al., 2011), LUNA (Trieschmann, et al., 2007), LUTEGA (Dawczynski et al, 2013), Loughman (Loughman et al, 2012), LAST II (Richer et al, 2007), COMPASS (Nolan, et al., 2011). Y = Yes criteria used, Y(adv) Yes advanced AMD, Y(wet) Yes neovascular AMD, Y(dry) Yes dry AMD and <6DS Less than six dioptre sphere.

The participant journey is shown in figure 3.1 Reporting of this study adheres to the consolidated standards of reporting trials (CONSORT) statement (Schulz et al., 2010).

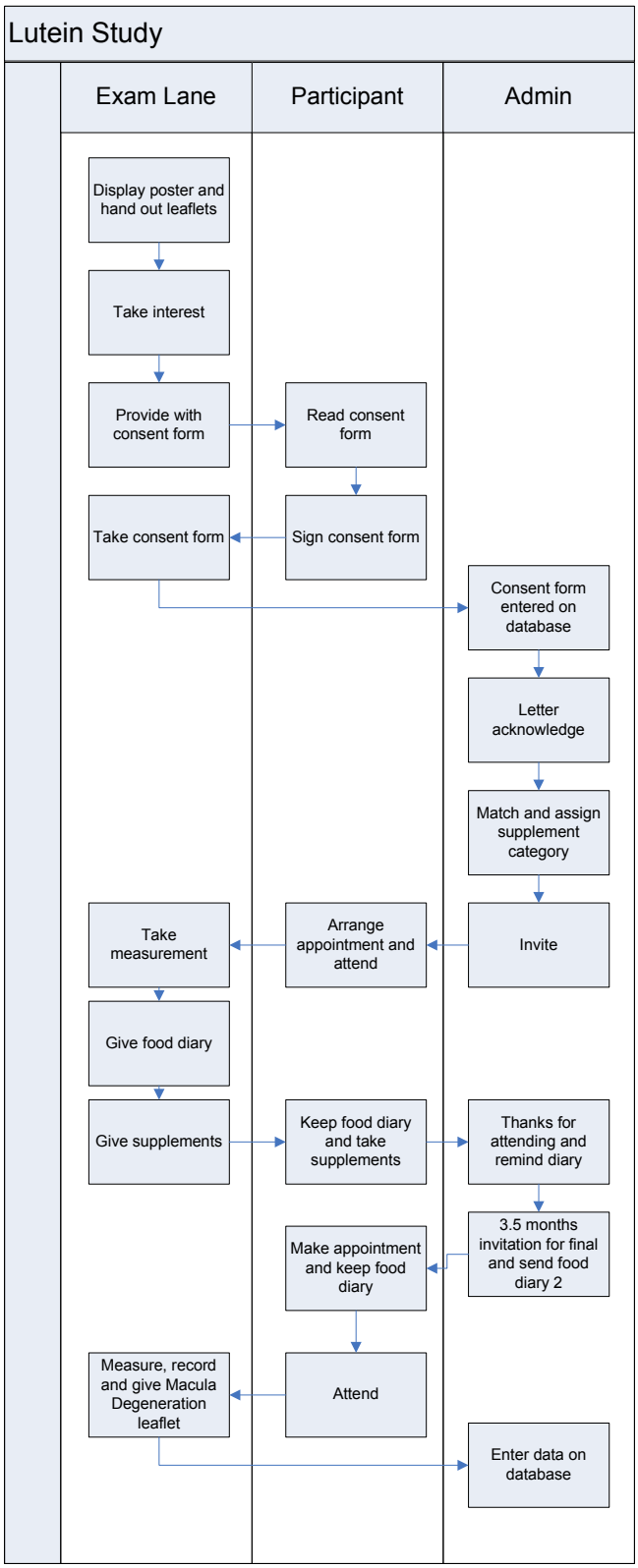


Figure 3.1 Flow diagram of participant pathway

With multifactorial experiments there can be elements, which, if not controlled for, can lead to misleading outcomes; these are known as confounders. To help reduce the influence that confounders could have on this study a choice was made to create groups, thus allowing matched pairs to be created, allowing for:

Gender – The amount required as a recommended daily intake is lower in females when compared to males across the board. Gender matching reduces any potential confounding effects of this.

Smoking habit – The smoking of tobacco has been shown to have an effect upon micronutrient uptake and availability and so matching pairs with similar smoking habits reduces any confounding effect (Delcourt et al., 1998).

Age – Some studies have shown that age has an inverse relationship with MPOD (Nolan, et al., 2010) whereas others have shown no relationship between age and MPOD (Bartlett et al, 2010c; Berrow et al, 2011). As this is not conclusively proven either way groups were chosen by age group (decades of life) to reduce any potential confounding effect.

The following potential confounder was controlled by maintaining food diaries:

Diet – Dietary sources of L and Z vary in quantity and quality in diets across the western world, and even within towns and cities. It is not unknown for participants in studies looking at nutrition to change their dietary intake due to the observation they are undergoing (Hawthorne effect) (McCarney et al, 2007), thus a food diary was requested at the start of the trial and at the end of the trial, checking to see if the diet had changed. With this effect reduced the potential confounding would be reduced substantially.

Materials

Participants in both groups were instructed to follow the manufacturers instructions as written on the side of the container supplied. These two ocular supplements were chosen as they were both commercially available at the time of the study design, both were used within clinical practice within the UK, and had been for some years without reported problems (Khachik, et al., 2006; Connolly et al, 2011), and they had different levels of micronutrients, especially L, which allowed the study to assess the different effects of these levels on the MPOD. Clinicians were supplying these two ocular supplements

without knowledge if the different levels of L had any significantly different effect upon MPOD.

Two ONS were provided:
iCaps **(iC)**(Mid-Optic Ltd 1A Meteor Business Park, Meteor Center, Mansfield Road, Derby DE21 4ST – now only available in 10mg L form not 6mg used in study), a once daily tablet containing L/Z 6mg, vitamin A 800µg (Retinol equivalent) vitamin E 50mg (αTocopherol equivalent) vitamin C 125mg, vitamin B2 1.4mg, zinc 20mg, selenium 55µg, copper 1000µg and manganese 2mg.

Vitalux plus **(V)** (Mid-Optic Ltd 1A Meteor Business Park, Meteor Centre, Mansfield Road, Derby DE21 4ST), a once daily capsule containing L 10mg, vitamin C 60mg, vitamin E 20mg, zinc 10mg, copper 0.25mg and vitamin B3 10mg. The oils used in preparation were lipids, wheat germ oil and glycerol.

The MacuScope (Macuvision Europe Ltd, 122 Station Lane, Solihull, B74 6JJ UK) was selected as this was already in use in the practice where the study was based. With this device the subject observes flickering stimuli comprising of two different, alternating wavelengths. The MP absorbs the wavelength stimulus of 465nm, the other is not absorbed at 550nm. The luminance ratio between the two is reduced until flicker is minimized or as perceived by the subject, extinguished. Thus the MacuScope takes the subject from a condition of flickering to one of not flickering. This is assessed for one central and one peripheral location. As MP is assumed to be maximal at the central location, and minimal at the peripheral one, then the luminance ratios between these two locations is used to determine the MPOD value. The study had already been designed and data collection had commenced, including the sample size calculation, when the paper reviewing the repeatability of such devices was published. Alternative devices do now exist and are to be found in clinical practice.

Procedures

Study design

The effect size was not known at the start of the study and so a number of participants had to be chosen to establish this. Pilot studies can have as few as 10-15 participants to be sufficient to establish statistical power (Herzog, 2008). This is why the first 14 participants who met eligibility criteria and completed the second (four month) MPOD measurement were selected for determination of sample size. These participants became

part of Experiment 2 as the study progressed. There were no changes to the design between Experiment 1 and Experiment 2

Outcomes

The MacuScope system is a HFP device. This device measures from perception to non-perception (extinguished flicker). The results are stated as log(foveal/eccentric). The foveal and eccentric measures are of the luminance or intensity of the light of the relevant wavelength (Bone & Landrum, 2004). In this device the resulting 'score' is not continuous but is stepped. The range of steps included in this cohort were 0, 0.069, 0.138, 0.207, 0.276, 0.345, 0.414, 0.483 and 0.552. These outcomes are presented without units due to the calculation method outlined above.

Sequence – method used

Recruitment methods were implemented. Volunteers contacted JMM (administrator) who provided informed consent paperwork and entered details on a secure system. Block stratification was carried out and once a fortnight randomised allocation to intervention was completed using matched pairs.

The participants name was written upon a bag, and the allocated intervention was enclosed in this bag and sealed. The sealed bag was held securely in the data collection area. A letter was sent inviting the participant in for the baseline reading, this was followed up with a telephone call if they did not attend within two weeks.

On arrival at the research centre the test protocol used during the data collection was as follows:

The participant is seated comfortably in front of the device and the data collector describe what they will want the participant to do, and whilst doing so aligns the height of the device with the seated position of the participants eye, whilst leant forward engaging with the device.

Instructions given: 'I'd like you to put your right eye up against the cup of the eyepiece, and I will move the device to make it comfortable for you.' Information about the measurement was then shared: 'You will see a black fixation target, which we will focus for you, along with a blue light. Whilst looking at the black fixation target you may notice that the blue light is flickering. I will change the settings on the instrument and ask if you can still see the blue light flickering, again whilst looking at the black fixation target. Try and keep your eyes still, fixed on the black fixation target and blink only after you have

told me if the blue light is flickering or not. It is likely that at some point the blue light will stop flickering. We will run a test first so that you can see this in action. Once we have tested the central measurement we will move the settings to test the peripheral or outer measurement, but you still look at the black fixation target.' Confirmation is sought that the instructions are understood and an opportunity to ask any questions is offered.

Once comfortably in position the data collector will click 'Begin' on screen to start the test. Eye selection (Right or Left) is chosen on screen and the participant placed the selected eye up to eyepiece, centring it for an unobstructed view. They were instructed to look straight ahead at the blue flickering light of the central fixation target. The crosshairs of the fixation target was focused using buttons on screen, asking the participant when the cross hairs are focused. If focus could not be achieved then the prescription was checked. If the prescription was greater than +/-6DS (Dioptre Sphere) then the participant was instructed to wear their habitual correction to undertake the test.

The participant was instructed to remain as still as possible; look straight ahead at the fixation target (central) and to ignore 'shimmering' at the edges of the flicker. They were informed that this was the practice test.

'The Start' button was clicked to begin the 'Fovea test'. The 'Less' button was gently pressed, a few seconds was waited and the participant asked if the blue flickering was slowing or the blue darkening, if so the process was repeated until it had stopped. The participant was regularly encouraged to fixate the central fixation target; oblique gaze would restart flickering. The question asked was 'Is the blue light still flickering or has it stopped?' If flickering was still present: 'Blink once and stare at the black fixation target' change the setting and repeat question. If flickering had ceased: 'That's great, that is what is involved in taking this measurement, now we will run the measurement to record the data. Any questions?'

The 'Back' button was pressed and the test to record was now run, instructing the participant that this is now the actual test. The process was repeated with encouragement. When the 'flickering stopped' point was reached during this test, the 'Save' button was clicked. The device then advanced to the parafoveal test.

The participant was instructed to view the outer crosshair fixation, to the right for the RE and to the left for LE repeating the process with encouragement. Once flickering stopped during the parafoveal test the 'Save' button was clicked.

The screen then displayed the foveal and parafoveal luminance results along with the difference between the two expressed as MPOD. It also categorised this result into low (0.1 – 0.25), medium (0.25 – 0.75) and high (0.75 – 1.00). This was now printed. The whole process, without the trial test procedure, was now repeated for the left eye, with result printed for this eye also. A copy of a print out is shown at Figure 3.2.

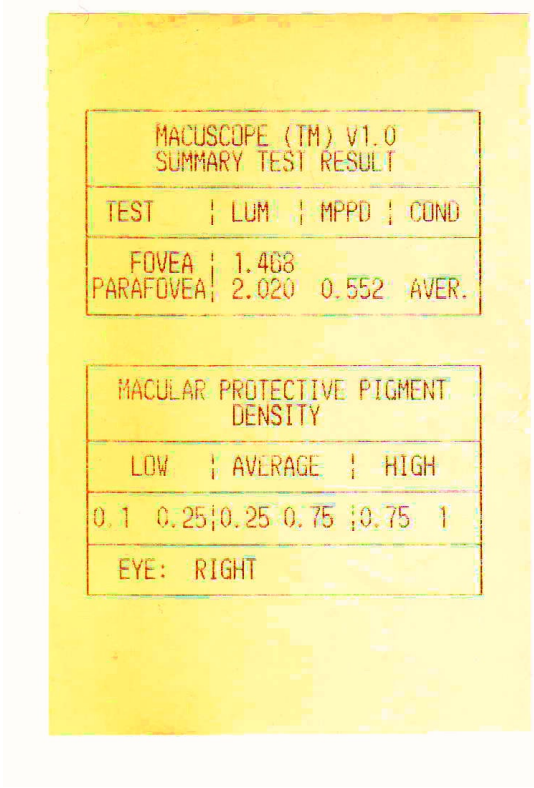


Figure 3.2 MacuScope print out of results

The print out had the patients name annotated at the head of the print out and the RE and LE results were stapled together. These were then placed in an envelope by the data collector (CB), awaiting collection by the administrator (JMM).

The sealed bag was given to the participant and they were directed to follow the instructions provided by the manufacturer (CB). A food diary was also included and was discussed with the participant. The food diaries were received back by the administrator (JMM).

At the three month point a letter was sent out to the participant reminding them that the final reading was due shortly and the second food diary was enclosed for the participant to complete during the final two weeks. Two weeks before the end date another letter

was sent asking them to attend the practice on a specified date, four months after the baseline reading and to remind them about the food diary. If on the due date they did not attend then a phone call was made to encourage them to re-attend.

On re-attending a measurement of MPOD was taken and printed off, with the name of the participant being written across the top of the slip. This followed the same protocol as discussed above. The slip was handed to the administrator (JMM). On receipt of slips the administrator would complete a sheet with both readings. These readings were entered upon a spreadsheet held by the administrator. Follow up of non-attendees continued for up to one month after the final reading original date.

After all readings were received the data and spreadsheet were given to the data analyser (GTV).

Although food diaries had been completed, they were not analysed for this experiment, which was designed to determine the sample size for the second experiment.

Concealment mechanism

The tablets were placed in a sealed bag with instructions and a food diary. The name of the participant was written on the sealed bag and these were passed over to data collection. Thus the type of supplement provided was only known to the administrator and the individual participant. Neither data collection nor data analysis were aware prior to the end of the data collection period.

Implementation

Random number generation, block stratification, enrolment and assignment of interventions were carried out by the administrator (JMM).

Masking

The data gathering and the data analysis function were both masked to the randomisation of intervention provided. Participants were not masked permitting them alone access to the relevant data sheets for the supplement taken. Only after sufficient data were accrued and final measurements had been recorded was access to the information provided for data analysis (GTV).

The study was approved by the Aston University Ethical Committee. The tenets of the Declaration of Helsinki were followed. See Appendix K

Statistical analysis

Sample size

Following the successful completion of this cohort (n=14, an *a priori* estimation) a sample size calculation was conducted using G power 3 (University of Dusseldorf http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/download-and-register) Significance 0.05 and power 80% (standard). Mean and standard deviation were used to calculate effect size.

Randomisation and allocation

Block stratification was used to reduce any effect that a potential confounder may have, thus the blocks of age, gender and smoking preferences were created. Only one investigator (JMM) was involved in the block stratification and randomization process, and they took no role in measurement nor data analysis.

Once a cohort had been gathered into the block stratified groups the matching of pairs was, when possible, carried out using random number generation utilizing the online source Random.org (Numbers, Integer generator) (http://www.random.org/integer). Each participant was given a number derived from the time of arrival into the block stratified group. The numbers available to match were entered into the random number generator and the pairs matched using the numbers adjacent to one another in the resultant list. Any remaining participants were rolled over to the next iteration of this process.

Statistics

For each participant a baseline and four month reading was taken using the MacuScope device. The difference between these two data points was calculated for both the right and left eye of each participant. A matched pair Wilcoxon sign rank test (one tailed) was used to determine whether these values differ at the 5% significance level between the two interventions (http://www.vassarstats.net/wilcoxon.html). A one tailed test was used rather than two tailed as the only likely difference would be in one direction.

The sample data were also tested for normality of distribution using the Shapiro-Wilk test (http://sdittami.altervista.org/shapirotest/ShapiroTest.html). Normal distribution was not assumed as not all met the Shapiro-Wilk normality test, hence a non-parametric test was used, however with the number of pairs in the study the Wilcoxon sign rank test does assume an approximation to a normal distribution in the calculation of the Z and p value.

Results

This section sets out the results found from experiment 1 with numbers needed to prevent a Type II error (when the two populations are significantly different, but the H_O is accepted incorrectly i.e. a false negative.), graphic illustration of the results for each eye and each intervention for the experiment, a comparison against the population of the area this was carried out in and the statistical analysis of the data (Table B1.1).

Outcomes

Data was entered into G power 3, using data for the RE only (Mean of 0.089, standard deviation of 0.215, effect size calculated 0.414). The number of participants required at 80% power to avoid a type II error (failure to reject null hypothesis) was 19 per study intervention group, 38 participants in total.

A summary of the results for each eye and with each ONS is shown graphically in Figure 3.3 and in numbers (providing mean, one standard deviation and minimum and maximum results) in Table 3.2.

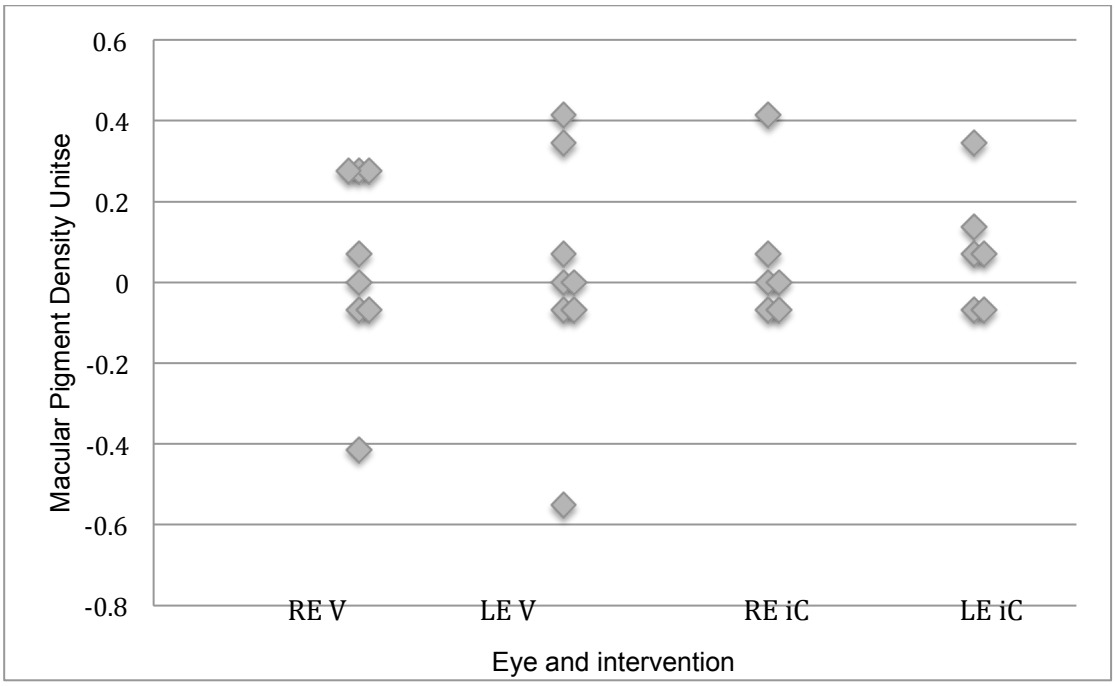


Figure 3.3 Scatter chart comparing change results of right eye (RE) and left eye (LE) for both interventions among the first cohort. (V = Vitalux plus, iC = iCaps)

	RE V	RE iC	LE V	LE iC
Mean	0.043	0.017	0.058	0.081
Standard Deviation	0.239	0.294	0.182	0.154
+1 SD	0.282	0.312	0.240	0.234
-1 SD	-0.196	-0.277	-0.125	-0.073
Max	0.276	0.414	0.414	0.345
Min	-0.414	-0.552	-0.069	-0.069
Shapiro-Wilk normality test	Normal	Normal	Rejected	Normal

Table 3.2. Data set for Figure 3.3 first cohort (V = Vitalux plus, iC = iCaps SD = Standard deviation, Max = Maximum change, Min = Minimum change)

The RE displayed more variability between participant readings than the LE for both iC and V. The range of change was greater for iC than V in the RE and similar for both ONS in the LE. The mean change for the RE was lower than that of the LE.

All of the interventions had a mean positive shift, with no one intervention being better than the other. The LE displayed, on average, more of a positive shift than the RE.

Statistical analysis of the MPOD data for differences in the change in readings for each eye gave Z values (a measure of how far from the mean, in standard deviations, the result is) of RE -1.43, LE -1.00 with its corresponding p value (a measure of probability) of RE 0.0764, LE 0.1587 (one tailed), which when compared to the criteria of statistical significance at the 95% level suggests that the results were not significantly different.

Discussion

The device used to measure MPOD, the MacuScope, reports in the manual the following contraindications for use of the device:

- BCVA worse than 20/40 (6/12)
- Yellowing of lens/dense cataracts
- Problems with fixation of target e.g. Nystagmus
- Uncorrected spectacle prescription beyond +/- 6DS

Part of the inclusion criteria for this study was a corrected VA of 20/25 or better (6/7.5). Yellowing of the lens was minimised as a contraindication by using the two sites on the retina (Foveal and Parafoveal) that would both have the same degree of yellowing affecting the results. By subtracting the two results the yellowing factor should be removed. Given the age range of participants within this study some will have had lens yellowing. Conditions causing fixation problems were screened for during enrolment and were excluded. Uncorrected prescriptions beyond +/- 6DS can be overcome by advising the use of the participant's habitual correction. Within this cohort there was no uncorrected spectacle prescription as all candidates could be corrected using the in built focussing system. All of these factors place this cohort well within the parameters of the device.

The results displayed a difference between the RE and LE, with a wider range of change results for RE when compared to the LE (RE V 0.69, RE iC 0.96, against LE V 0.48, LE iC 0.42). The largest change was in the RE with iC at 0.96 which was a third higher than for V in the same eye (0.69).

All four outcomes displayed a small mean positive shift in MPOD with the LE moving further than the RE. This does seem counterintuitive on first glance, however it could be explained by the nature of testing with HFP. Even though in this study the data measurer (CB) was kept in place, offered consistent advice and gave trial runs before taking measurements, many participants needed a period of adaptation to fully understand the measurement process and their part in it. In an attempt to be overcome this adaptation period advice and trial runs prior to 'going live' with measurements were adopted. From the results in this first experiment it would appear that this was not sufficient to overcome the problem. As will be seen in Chapter 4 this disparity between eyes disappears.

In this initial cohort the LE results were very similar which is why the Z value was 1.00 and p value 0.1587 (one tailed) which shows no statistically significant difference between the two interventions.

As there was a greater range of change in the RE the Z value was 1.43, and p value 0.0764, which lies 2.64% outside of statistical significance, so again no statistically significant difference, but a difference that may yield a statistically significant result in the whole cohort. These changes compare well with the review carried out by Bartlett et al in 2010 (Bartlett et al, 2010b) suggesting that supplementation effects a maximal 0.1 uplift to MPOD across many studies on the subject.

The power calculation produced a result of 19 participants in each group of the study, 38 participants in total. This compares to the other primarily discussed papers within this study as shown on Table 3.3.

Study	Sample size discussed	Numbers needed	Numbers enrolled
Huang, et al., 2013	N	-	108
Nolan, et al., 2011	Y	91	121
Richer, et al., 2011	N	-	60
Richer et al , 2007	N	-	90
AREDS 2, 2013	Y	4,000	4,203
Bartlett & Eperjesi, 2008	Y	26	46
Bartlett & Eperjesi, 2007	Y	9	25
Trieschmann, et al., 2007	N	-	136
Dawczynski et al, 2013	N	-	172
Loughman et al, 2012	Y	11	36
Vasey 2014	Y	38	100

Table 3.3. Statistical power discussed in studies reviewed within this paper

It cannot be stated that in the studies not showing sample power calculations, none were undertaken. The omission of this information may be down to word count requirements for journal publication. For all those reporting sample size calculation all met their target sample populations.

The trend certainly appears to be to over fill the number of participants that is set by the sample size calculation. As the calculated sample size is the minimum required to control for Type II errors, more participants should simply enhance the precision of the outcome of the effect being investigated. The sample size calculated also relies upon assumptions and educated guesses based upon methods chosen and means and standard deviation

produced therefrom. Thus the figure can be considered a bare minimum. Other statistical tests may require larger samples to be considered and so should also be borne in mind in calculating sample sizes for studies. Practically it also allows for drop-outs leaving the sample size suitable to prevent Type II errors. In this study recruitment ceased once 21 participants had undergone the process in each group, 42 in total.

The source of the initial data used to input into the sample size calculation is important, as the effect size can have a large bearing on the outcome of the calculation.

Power has three influencing factors and so knowing any two of them the third can be deduced:

1. Significance criterion
2. Effect size
3. Sample size

Within this experiment the significance criterion was set at what is a common standard of 5% (0.05 or 1 in 20). The magnitude of the effect was calculated from the first 14 participants and so the sample size was found.

It is important to undertake an *a priori* power calculation as in this study, as this will determine before the study begins, or is finalised as in this case, the sample size required.

Power calculations are also important in satisfying the reader's ability to rely upon the outcomes stated in a study. Larger sample sizes can detect smaller effect sizes, which could lead to inappropriate conclusions being drawn. Sample sizes that are too small can lead to inconclusive results. Adopting *a priori* sample size setting this study also prevents the opportunity of data fishing/data dredging which would lead to poor conclusions.

The sample size calculated in Experiment 1 was used in Experiment 2 to define how many participants would be needed to prevent a Type II error. From the above comfort is provided that the results of this study will provide information which can be relied upon and which conforms to best practice and statistical standards.

Limitations in experiment

During the data collection process the author did not collect the data himself, relying upon a trained and experienced operator instead. This could possibly introduce issues for research if the operator was not research focused but time pressured in a work

environment instead. As an example the individual could become familiar that most participants report extinguishing of flicker after, say, three ‘presses’ of the button and therefore not encourage the participant to the true point of flicker extinction.

This process could have been monitored during data collection to help prevent these errors creeping in. There is no evidence that this occurred, but it is worthy of note, especially as the author could have carried out the data collection himself. Initially the rationale of the author was to maintain maximum masking by not being involved in data collection, hence not biasing the results knowingly or unwittingly. The masking could however be maintained by separating the data capture from the supply of the supplements. The advantage of this method would have been that the researcher would have an interest in encouraging the best extinguish of flicker point for all participants.

It may have been useful to include spectacle prescription and VA against other parameters in the study and this may be considered for future study design. Within this study there was no uncorrected VA whilst using the device (inclusion criteria of 20/25 minimum VA) and all spectacle prescriptions were within the +/- 6DS requirements of the device focussing system.

Summary

This first experiment provided data to allow a sample size calculation. To provide 80% power at 0.05 significance 38 participants in total were required, 19 in each arm of the study. In the Chapter 4 - experiment 2, comparison of two different concentrations of L, is described.

Chapter 4 Experiment 2 - The effect of two ocular nutritional supplements on MPOD

In Chapter 3 the sample size was established. Experiment 2 poses the null hypothesis that the effect of the use of two different concentrations of L in ONS in clinical practice has no significant difference on MPOD.

Previous studies have set out a range of L, Z and MZ ONS combinations and have tested them for statistical significance. 20mg L does significantly change MPOD according to some authors (Huang, et al., 2013; Dawczynski et al, 2013) whereas one other shows that it doesn't (Loughman et al, 2012). However others show that 10mg L with 10mg MZ does significantly change MPOD, and Dawczynski et al agree, so long as it has Omega 3 PUFAs included in the ONS. Another study showed that 10mg L with or without antioxidants will raise MPOD (Richer et al, 2007) and this raising of MPOD is shown with 12mg L and 1mg Z (Trieschmann, et al., 2007; Nolan, et al., 2011). Finally the study that helps understand the ratio of L:Z best used in ONS found that 9mg L or 8mg Z raise MPOD, but when combined they do not (Richer, et al., 2011). So a gap appears to be present, does a 10mg L ONS raise MPOD statistically significantly compared to a 6mg L/Z ONS, especially in a group with healthy eyes? The 6mg L ONS has been reviewed by Bartlett and Eperjesi with its effect upon visual performance, but not its effect upon MPOD (Bartlett & Eperjesi, 2007; Bartlett & Eperjesi, 2008).

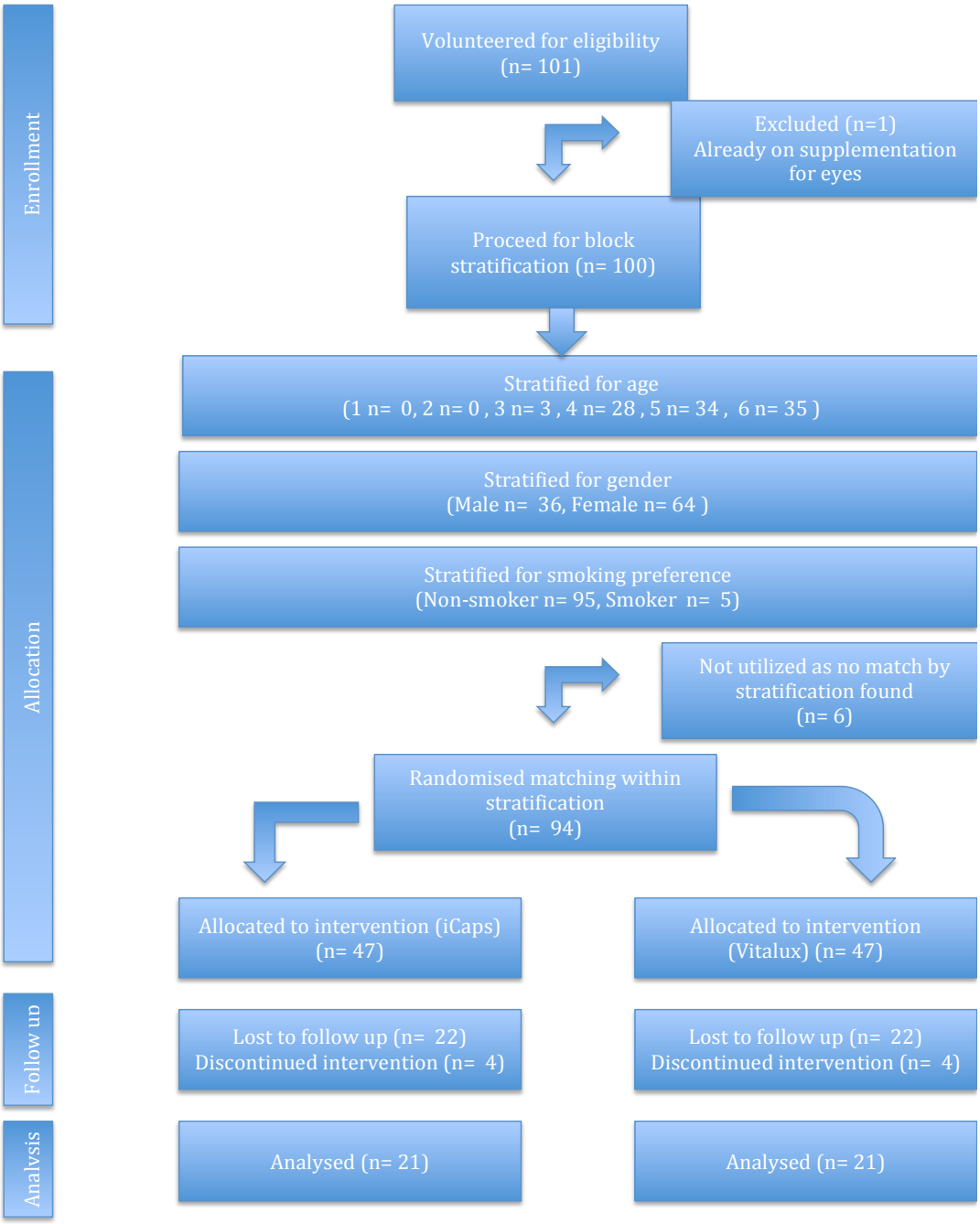


Figure 4.1 CONSORT statement 2010 flow diagram. Age groups 1= 16-19, 2 = 20-29, 3 = 30-39, 4 = 40-49, 5 = 50-59 and 6 = 60+

Method

Subjects

As in Experiment 1 described in Chapter 3 participants were recruited through posters, printed invitations and an article published in a local newspaper. Those with macular changes, under age 16 at the start of the intervention and those already taking supplementation including L and Z were excluded. Recruitment took place between January 2010 and June 2011 with the final measurement recorded in September 2011. All data collection took place at Specsavers Opticians, 50D Crystal Peaks, Sheffield, South Yorkshire S20 7PN. Administration was carried out by JMM, data collection by CB and data analysis by GTV. Food diaries were scored and reviewed by GTV and review of process and analysis by FE.

Gender, age, smoking habit and diet were all controlled to reduce confounding. Reporting of this study adheres to the consolidated standards of reporting trials (CONSORT) statement (Schulz et al., 2010) (Figure 4.1).

Materials

As with experiment 1 the same two interventions were provided iCaps (Mid-Optic Ltd 1A Meteor Business Park, Meteor Centre, Mansfield Road, Derby DE21 4ST – now only available in 10mg L form not 6mg used in study) and Vitalux plus (Mid-Optic Ltd 1A Meteor Business Park, Meteor Centre, Mansfield Road, Derby DE21 4ST). The MacuScope (Macuvision Europe Ltd, 122 Station Lane, Solihull, B74 6JJ UK) was used to measure MPOD.

Procedures

The study was a block stratified randomised trial with two equal cohorts undergoing intervention consecutively. There were no changes to the trial once underway.

As with experiment 1 (Chapter 3) the outcomes remained constant and the sequence for participant involvement conformed to that shown in Figure 3.

The tablets were placed in a sealed bag with instructions and a food diary. The name of the participant was written on the sealed bag and these were given to CB ready for data collection. Thus the type of supplement provided was only known to the administrator JMM and the individual participant. Neither CB (data collection) nor (GTV) data analysis were aware prior to the end of the data collection period. Random number generation, block stratification, enrolment and assignment of interventions were carried out by JMM.

The data gathering and the data analysis function were both masked to the randomisation of intervention provided. Participants were not masked, in that they could see the type of ONS provided once they returned to their homes, permitting them alone access to the relevant data sheets for the supplement taken. Only after sufficient data sets were accrued and final outcomes had been recorded did GTV (data analysis) have access to the information held. The study was approved by the Aston University Ethical Committee. The tenets of the Declaration of Helsinki were followed. See Appendix K

Statistical Analysis

The sample size calculated in Experiment 1 was for 42 participants, 21 in each intervention arm.

The change between baseline and four month readings was calculated for both eyes individually and a one tailed Wilcoxon sign rank test chosen to calculate if a significant difference arose. The same test was applied to the food diaries, with a two-tailed test used for this calculation. As with experiment 1 (Chapter 3) block stratification and food diaries were used for the four confounders originally identified. Only one investigator was involved in allocation to blocks and the randomisation process. Random number generation was carried out using the online source Random.org (Numbers, Integer generator) (http://ww.random.org/integer). Participants in both groups were instructed to follow the manufacturers instructions as written on the side of the container supplied.

Results

This section sets out the results found from experiment 2 with graphic illustration of the results for each eye and each intervention for the whole cohort (Figure 4.2), a comparison against the population of the area this was carried out in (Table B1.1), a comparison of the food diary results for the cohort and the statistical analysis of the data.

Outcomes

The recruited sample was 100. 6 were not matched within block stratification and so did not enter the study. 22 in each group (44 total) were lost to follow up, and 4 in each group (8 in total) discontinued the intervention.

A summary of the results for each eye and with each ONS is shown graphically in Figure 4.2. and in numbers (providing mean, one standard deviation and minimum and maximum results) in Table 4.1.

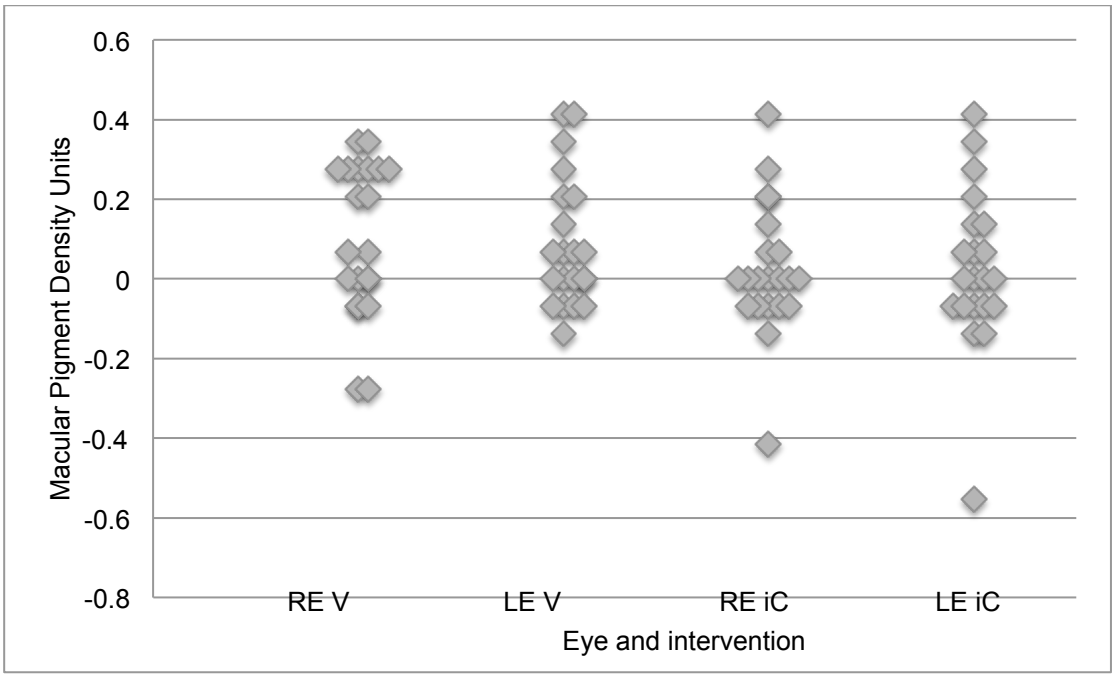


Figure 4.2. Scatter chart comparing change results of right eye (RE) and left eye (LE) for both interventions for 42 participants. (V= Vitalux plus, iC = iCaps)

	RE V	RE iC	LE V	LE iC
Mean	0.102	0.023	0.089	0.026
Standard deviation	0.192	0.168	0.163	0.202
+1 SD	0.294	0.191	0.252	0.228
-1 SD	-0.090	-0.145	-0.075	-0.175
Max	0.345	0.414	0.414	0.414
Min	-0.276	-0.414	-0.138	-0.552
Shapiro-Wilk normality test	Rejected	Rejected	Rejected	Rejected

Table 4.1. Data set for Figure 4.2 Whole study (V= Vitalux plus, iC = iCaps, SD = Standard deviation, Max = Maximum change, Min = Minimum change)

The mean results were all positive with V increasing more in both eyes than iC. There is no longer a LE differential against the RE as was apparent in Chapter 3. iC displayed lower minimum figures in both eyes compared to V. The maximum increase in MPOD figures was consistent across iC and V LE at 0.41 with V RE close behind with 0.34 (only one step in measurement apart). Non-parametric tests were adopted due to the rejection of all populations from being normal using Shapiro-Wilk normality test.

The MPOD data yielded a Z value (a measure of how far from the mean, in standard deviations, the result is) of RE 1.50, LE 0.97 with the corresponding p values of RE 0.07, LE 0.17 (one tailed), which when compared to the criteria of statistical significance at the 95% level suggests that the results were not statistically significantly different.

A Post Hoc power calculation (G power 3) was undertaken using change data from the RE of the whole cohort (42 participants) to check final power (Mean 0.062, standard deviation 0.430, effect size calculated 0.145.) The final sample size required was calculated to be 296 (148 per study arm). This gave a final power of 23.57% from the sample size actually used (42).

A comparison of means was calculated for the initial(14):final(42) cohort and the initial(14):follow-on(28). The initial:final means were not statistically significantly different ($p= 0.619$) and the initial:follow-on means were also not statistically significantly different ($p= 0.800$).

At baseline 15 out of 100 participants had an MPOD of 0.3 or greater (15%), whereas of the completing cohort nine of 42 participants had an MPOD of 0.3 or greater at baseline

measure (21.4%). 38 participants had a baseline MPOD for the RE within 0.1 of 0.3. Thus 47% of baseline MPOD readings for the RE were below 0.2.

Discussion

A sample of 42 participants, 21 in each group, were recruited to test if there would be any significant increase in MPOD from using a 10mg L ONS when compared with a 6mg L/Z ONS. This was designed so that advice could be provided to clinicians as to which dose of L should be provided to their patients in primary care practices. There was no significant difference between V (L 10mg) and iC (L/Z 6mg) on MPOD found within this study. The p value for the right eye was close to significance (0.0668). A one tailed test was selected as any change in outcomes should show an increase in MPOD from using a higher dose of L and Z. A two tailed test would suggest that a higher dose of L and Z could yield a decrease in MPOD when compared to a lower dose, which is not evidenced in the literature.

Seddon et al, in a non-intervention study highlighted a link between reduced AMD development and higher intake of dietary carotenoids (Seddon, et al., 1994), therefore the idea of supplementing with carotenoids would appear to make sense to help reduce the progression of AMD within clinical practice. Although the link to preventing progression of 'normal' to AMD is not yet proven, work on how a 'normal' eye responds to carotenoid supplementation would be useful in potentially establishing this.

In 2007 and 2008 there was no significant visual performance change found in AMD and 'normal' patients when using a 6mg L ONS, however these studies didn't report on MPOD levels (Bartlett & Eperjesi, 2007; Bartlett & Eperjesi, 2008). With no MPOD data the understanding of the retinal uptake of L into the MP was not developed, as the paper concentrated on visual changes effected by L, this is a multifactorial function of the eye. As the study group found no change to vision with the level of supplementation chosen (6mg L) this helped establish a minimum L concentration for this study and by studying its effect upon MPOD helped fill in some of these data that had not previously been observed.

Some studies have shown significant changes in MPOD from high concentration L and no significance from lower L concentration. Huang et al (Huang, et al., 2013) found that 20mg of L provided a significantly 'higher plateau' of MPOD and serum levels of L when compared with L 10mg, L/Z 10mg each or the placebo group over a 48 week period in a group with early AMD. Loughman et al (Loughman et al, 2012) reported no significant changes for supplementation with 20mg L, 2mg Z and the placebo group, but did report significant improvements to MPOD, serum carotenoid and visual performance from the 10mg L, 2mg Z and 10mg MZ group. This seems counterintuitive unless the MZ had an

enhancing or separate action on MPOD. Certainly this would benefit from further investigation. The effect of the 20mg L group from Loughman et al's work does back up the findings within this study of no significant difference to MPOD. From these two studies there is some agreement, that in the absence of MZ 10mg L does not significantly improve MPOD levels.

The LUTEGA study (Dawczynski et al, 2013) with early AMD participants showed that both L 10mg and L 20mg ONS had significantly higher MPOD reading after just one month that was maintained over the study period of 12 months, whereas the placebo group had a gentle decline on MPOD levels over the same period. However in agreement with this current study the LUTEGA study did not find a significant difference between L 10mg and L 20mg groups, and they recommended the L 10mg concentration with omega-3 long chain PUFAs. The omega-3 may account for the significance at 10mg L when compared with this study and this would be worth investigating further.

Two studies found significant MPOD increases using similar levels of L. The COMPASS study (Nolan, et al., 2011) with participants with healthy eyes found a significant improvement in MPOD for the 12mg L and 1mg Z, with the L in an ester form which may be less bioavailable (see discussion Chapter 6) when compared to a placebo. This helped to establish that taking a supplement was beneficial within a healthy population for increasing MPOD. The study did not contrast the effect upon MPOD of different levels of ONS. This contrast was attempted in the current study and in the LUTEGA study (Dawczynski et al, 2013). In the LUNA study group (Trieschmann, et al., 2007) and COMPASS (Nolan et al, 2011) the participants with AMD responded to an ONS (12mg L and 1mg Z with L in an ester form which may be less bioavailable (see discussion in Chapter 6)) and MPOD did increase significantly when compared to a placebo control group. Again this shows that ONS are beneficial for raising MPOD, but does not differentiate between concentrations of carotenoids.

As both of these studies (LUNA and COMPASS) used the esterified form, which may be less bioavailable, it may be argued that they are more comparable to 10mg L in a non-esterified form, thus it may be seen to be directly contradictory of the results of this current study. The COMPASS study had very slow retinal uptake, unlike any of the other studies, and didn't control for smoking habit. The LUNA study had no dietary control, was not randomised nor masked. The measurement method was based upon an assumption about autofluoresence wavelengths used in a normal population, when in the LUNA study the population had AMD and so the assumption may not hold.

Two further studies with comparable levels of L to this study did show significant change to MPOD. The ZVF study (Richer, et al., 2011) working with participants with AMD showed statistically significant changes to MPOD for both the L (9mg) and Z groups (8mg) and a result very close to significance for the combined L and Z group. The LAST II study (Richer et al, 2007) with participants with AMD showed that L (10mg) and L+ (10mg plus broad spectrum of antioxidants) did raise MPOD significantly when compared to a placebo group. This helped establish that L can raise MPOD in AMD subjects. However the effect of antioxidants, being present or not, did not significantly alter the MPOD level. Both of these studies were based upon populations with AMD, which may help account for the differences in the results between them and this study. The maculae of populations with AMD may require, and therefore seek to take up, more L and Z, hence yielding a significant change from baseline.

This study with 42 healthy eye participants showed no statistically significant change in MPOD between 6mg L/Z and 10mg L.

From the comparisons above it can be seen that the effect upon MPOD would also benefit from more work in the three groups, preventative (no signs of AMD), early AMD and advanced AMD. A broader age range, clarity on bio-availability in study cohorts and potentially the bold step of a longitudinal study would be advantageous to the clinician when deciding upon which level of L to offer to subjects within each distinct group and age range.

Clinicians within primary care practice have, over the last decade or so, become increasingly aware of nutritional effects upon the eye. This has stemmed from an increase in clinical articles in the professional and national press, and presentations at clinical conferences on nutrition, supplements and eye disease. The formation of the Ocular Nutrition Society (http://www.ocularnutritionsociety.org) in 2010 is evidence the ‘normalising’ of nutrition within optometric clinical practice. From personal discussions with colleagues at clinical conferences and within the workplace there is some anecdotal medico-legal pressure felt within primary care to discuss nutritional advice with some groups of patients at higher risk of developing specific conditions (cataract, AMD, smokers) on a defensive basis, i.e. fear of legal action being taken if advice has not been provided.

With the knowledge of carotenoids developing within the peer reviewed publications and the introduction of devices for clinical use in measuring MPOD the primary care

practitioner could at last begin to quantify risk factors within the clinic. Whilst many of the early devices used for this have been criticised for poor repeatability new devices are becoming available and their introduction will be driven by clinician and patient interest in this area.

The clinician has been left to 'muddle' through with patient advice. Clinicians may be driven by many factors to provide this advice such as fear of litigation, possibly preventing future AMD, acting in the patient's best interest in the absence of evidence to the contrary and financial gain. Whatever the reasons the clinician has been unable to access data on which concentration of L and Z to provide to patients. The original AREDS study did not include these xanthophylls in the formulation. AREDS 2 included them at 10mg L and 2mg Z, which showed a small improvement in late stage AMD over that recorded in AREDS 1.

The Ocular Nutrition Society position statement (http://www.ocularnutritionsociety.org) does make reference to L concentrations in excess of 10mg may be toxic (reportedly by E Chew, Lead Investigator) but this may simply refer to the papers that the lead investigator was involved in showing no toxicity below 10mg (Rosenthal, et al., 2006; Khachik, et al., 2006) or the suggested genotoxic effect (the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer) suggested by Kalariya et al (Kalariya et al, 2009). Given that this study found no significant difference between the two concentrations of L to the MPOD over a four month period then to provide the same effect, help reduce costs, save resources and stay within proven non-toxic boundaries opting for the 6mg of L may be a sensible choice. It is interesting to note that during the running of the study one of the interventions (iC) altered its formulation from 6mg to 10mg L.

Limitations

The data produced from this study have been generated by a device that has been criticised for repeatability and reproducibility (Bartlett et al, 2010a) and as such wide variations in MPOD readings could be accredited to 'noise' and the small changes in MPOD over the four month period reported here must be questioned. The time period for reporting of four months is shorter than many other studies and a period of six to 12 months should be considered for future similar studies, enhancing comparability between studies. Longer study periods may also lead to results that are statistically significant if dose dependent differential MPOD enhancement was happening. This was shown by one study group who found no significant results at three months, but statistically significant

differences at six months for the same cohort (Liew et al, 2006). Omega 3 and MZ were not used in this study, which limits the direct comparison with some other studies. Other potential limitations to comparability such as ethnicity, gastrointestinal health, alcohol intake, body mass index and genetics may have been present, and may have affected the results. Randomisation of allocation did help in reducing these effects. These limitations are discussed in more detail in Chapter 6.

Although the two study groups (initial and whole) were found to have no statistically significant differences between means (using means, standard deviations and group size) the recalculation of power using post hoc did provide an area of concern. The final power of the results was 23.57%, well below the 80% aimed for initially. This variation came about with the final mean being lower (though not statistically significantly so) at 0.0624 compared with 0.089 (initial) and a higher standard deviation of 0.4301 compare with 0.215. This changed the effect size from 0.414 to 0.145, hence the increase in numbers needed to achieve the 80% power desired.

Summary

Chapter 4 is a report of the findings of Experiment 2, where 6mg L ONS and 10mg ONS supplementation in healthy eyes were compared. No statistically significant difference was found between the outcomes which measured MPOD. In the next chapter, experiment 3, the food diaries recorded during the study are discussed along with the outcomes from these diaries.

Chapter 5 Experiment 3 - Food diaries

Chapter 4 provided details of current research into L supplementation and its effect upon AMD along with more evidence of which dose of L and Z to provide to patients (Experiment 2). This experiment determined what the L and Z intake of the sample was, checked to see if this varied between the initial and final measurements for the group and to compare the results with other groups.

When undertaking the literature review for Chapter 2 it was noted that several studies included food diaries in their study design (Bartlett & Eperjesi, 2007; Richer et al, 2007; Bartlett & Eperjesi, 2008; Nolan, et al., 2011; Richer, et al., 2011; Huang, et al., 2013). All chose to use a food frequency intake questionnaire (FFQ) administered at the start and the end of the studies. Food diaries were chosen in addition to FFQ by one study group (Bartlett & Eperjesi, 2007; Bartlett & Eperjesi, 2008). In the current study a food diary was adopted so that it could be self administered by the participant over one week at the start and finish of the study period. Two other groups chose to measure baseline data using serum L and Z levels (Loughman et al, 2012; Dawczynski et al , 2013).

Measuring food intake can highlight changes in dietary intake that could have a confounding effect upon the results and so provide useful information to check that intake has been stable. Some participants can attempt to change their diet simply because it is being monitored, or to please the researcher. This is known as the Hawthorne effect (McCarney et al, 2007). For example, a person might increase their L and Z intake by changing diet. This could lead to an increase in MPOD which without food diary information could be attributed to nutritional supplementation.

The aim of this part of the body of work was to determine L and Z intake for study participants, to compare L and Z intake at the start and end of the study to determine if there were any changes and to compare the dietary L and Z intake with participants in other studies.

Method

Subjects

As in Experiment 1 described in Chapter 3 participants were recruited through posters, printed invitations and an article published in a local newspaper. Those with macular changes, under age 16 at the start of the intervention and those already taking supplementation including L and Z were excluded. Recruitment took place between

January 2010 and June 2011 with the final measurement recorded in September 2011. All data collection took place at Specsavers Opticians, 50D Crystal Peaks, Sheffield, South Yorkshire S20 7PN. Administration was carried out by JMM, data collection by CB and data analysis by GTV. Food diaries were scored (Figure 5.1) and reviewed by GTV and review of process and analysis by FE.

Procedures

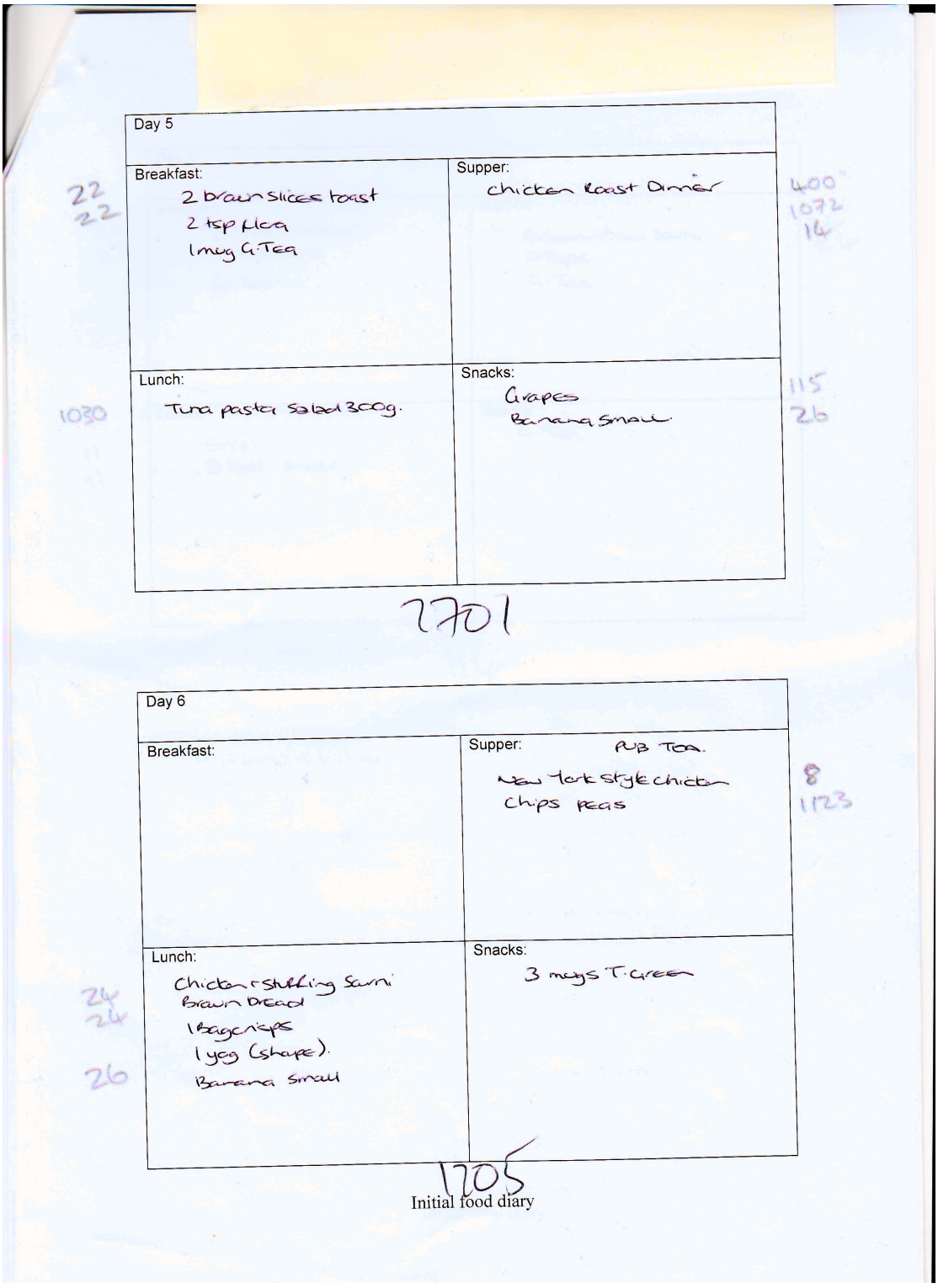
The food diary was placed in a sealed bag with the intervention for each participant, and the participants name was written on the bag. This was handed to the participant at the initial MPOD measurement session. The food diary was completed by the participant across the next seven days. This was returned by post to the administrator (JMM), and held in secure storage. The final food diary was dispatched with the letter asking for the participant to return and this diary was completed over the seven days leading up to the final MPOD measurement. It was returned when the participant attended for the reading, again being stored securely by JMM.

Only after sufficient data sets were accrued and final outcomes had been recorded did GTV (data analysis) have access to the information held. The L and Z content of the diary entries was annotated onto the diaries using the USDA National Nutrient Database for Standard Reference, Release 24 (USDA, 2013). As no weights or quantities were specified it was assumed, for consistency that the amounts used were as specified in the USDA database. The study was approved by the Aston University Ethical Committee. The tenets of the Declaration of Helsinki were followed. See Appendix K

Statistical Analysis

The Sample size calculated in Experiment 1 was for 42 participants, 21 in each intervention group.

Participants in both groups were instructed to follow the instructions found with the food diaries. Results between unpaired results (i.e. different sample sizes) for gender, intervention given, smoking status and age band were calculated using one-way ANOVA with independent variables (http://www.vassarstats.net/anova1u.html). The Spearman's correlation coefficient was calculated using an online calculator socscistatistics.com (http://www.socscistatistics.com/tests/spearman/Default3.aspx). Comparison of means for two populations was also undertaken within each confounder population comparing initial and final results (http://www.quantitativeskills.com/sisa/statistics/t-test.htm).



Initial food diary

Figure 5.1 Example page from food diary annotated with L and Z content of foods stated.

Results

The food diaries yielded the results as per Table 5.2 Figures are stated as daily in milligrams (mg).

Within this study the female participants had on average a numerically higher intake of L and Z at both the start (Male 1.26mg to female 2.39mg) and finish (1.22mg to 1.70mg) of the study. Female participants accounted for the highest individual intakes across each age group represented (40-49, 4.31mg, 50-59, 2.96 mg and 60+ 12.83mg). Female participants also had the highest individual variation in intake (0.56mg to 12.83mg) between group members and between food diary readings (drop of 0.70mg. Male participants appear to have, in comparison to this drop in female intake, a relatively stable L and Z intake dropping only 5.94% of the amount that the female participants did (0.04mg to 0.70mg). The percentage drop compared to initial readings for males and females was 3.27% and 29.03% respectively. The difference in results between male and female participants was not statistically different for the initial or final results ($p=0.17$ initial, $p=0.24$ final).

Smokers had, on average, a lower intake of L and Z at both first and final diary readings. The smoker's change from initial to final reading was greater than the non-smokers change. There was no statistical significance in the difference between the two populations for smoking habit for either the initial or final food diary results ($p=0.50$ initial, $p=0.44$ final).

Advancing age showed an increased drop off of L and Z content in diet across the study with a decrease in the minimum amounts in diet also with age. There was no statistically significant difference between the three age groups from ether the initial or final food diaries ($p=0.89$ initial, $p=0.91$ final).

There was no suggestion that the type of intervention taken had any effect upon dietary intake across the period. There was no statistically significant difference between the two groups initially or finally ($p=0.43$ initial, $p=0.52$ final). The data for iC and V has been included in table 5.1 for completeness.

The total food diary data, comparing all initial readings against all final readings, yielded a p value (a measure of probability) of $p=0.12$ (two tailed), which when compared to the

criteria of statistical significance at the 95% level suggests that the results total initial and total final food diary results were not statistically significantly different.

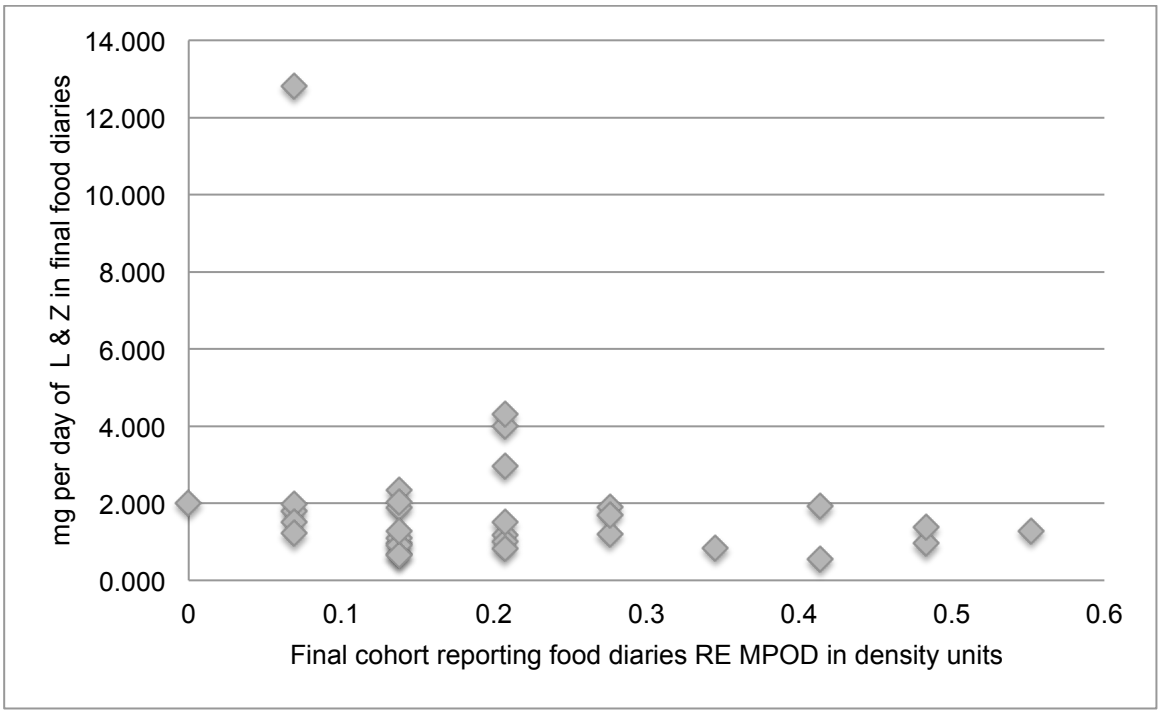


Figure 5.2 Scatter graph of final MPOD readings RE against Final food diary intake of L & Z in mg per day

Final MPOD readings for the 31 participants returning food diaries had a mean of 0.211 with a standard deviation of 0.138. For the same group the food diary L and Z intake mean was 1.96 with a standard deviation of 2.20 mg per day. A Spearman's correlation coefficient was also calculated for final RE MPOD readings and final food diary L and Z content of diet (see Figure 5.2). The result was -0.232 (p=0.210) (two tailed) which shows no statistical correlation between the two data sets.

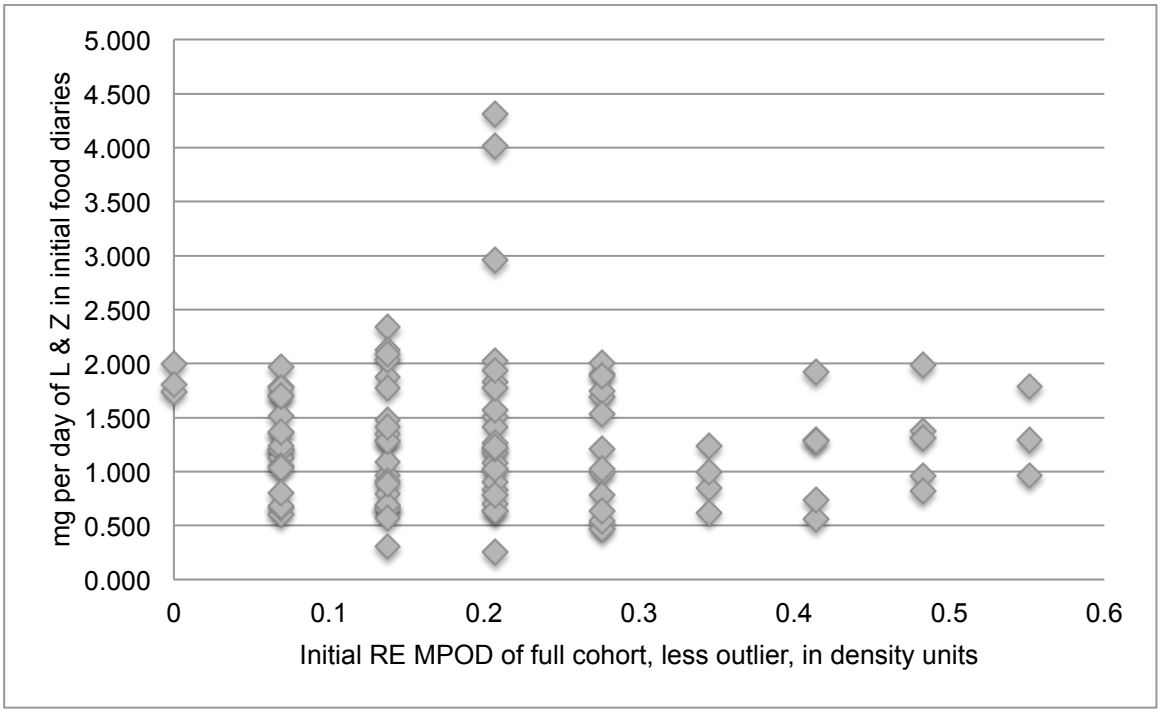


Figure 5.3 Scatter graph showing RE initial MPOD readings against initial food diary intake of L&Z in mg per day (Excluding 'outlier data of 12.829 food diary score and 0.069 initial MPOD).

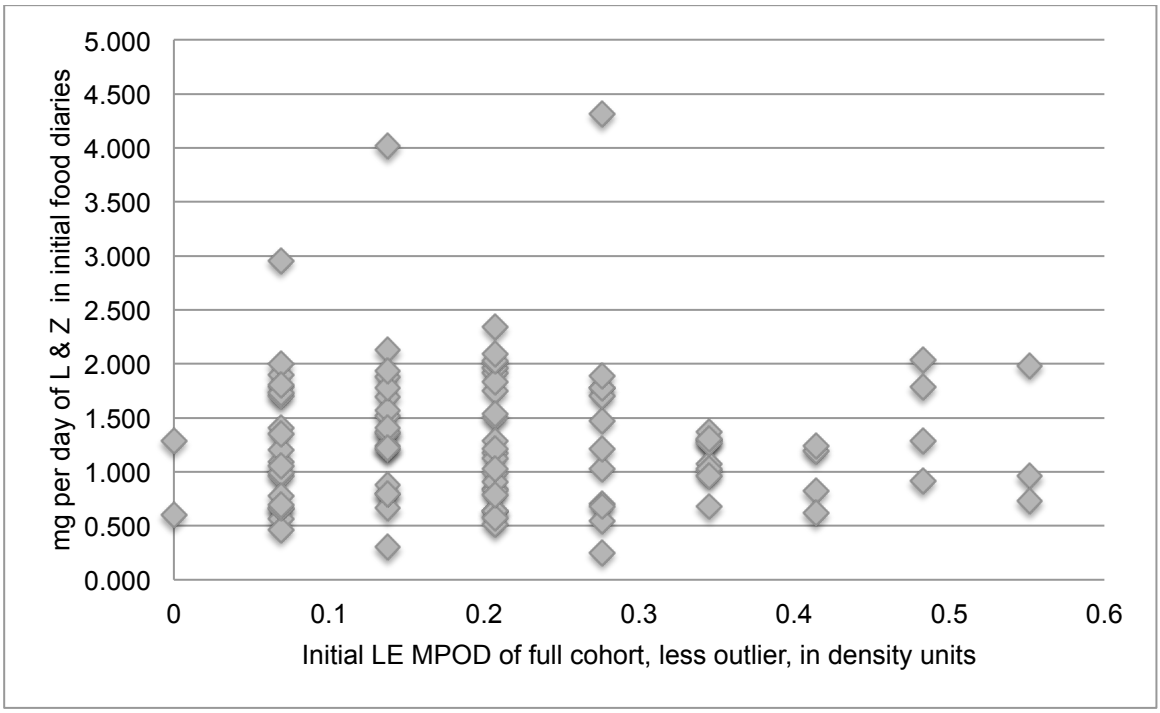


Figure 5.4 Scatter graph showing LE initial MPOD readings against initial food diary intake of L&Z in mg per day (Excluding 'outlier data of 12.829 food diary score and 0.138 initial MPOD).

A Spearman's correlation coefficient was also calculated for initial RE MPOD readings and initial food diary L and Z content of diet and then also for the LE (see Figure 5.3 & 5.4). The result was RE -0.092 (p=0.367) and LE -0.021 (p=0.842) (two tailed) which shows no statistical correlation between the two data sets.

	Numbers	Gender		Smoking habit		Age			
		Male	Female	Smoke	Non smoke	30-39	40-49	50-59	60+
Full baseline cohort (A)	100	39	61	22	78	2	32	30	38
Non-returners (B)	58	22	36	14	44	2	22	16	18
Returning cohort with final food diary (C)	31	12	19	5	34	0	7	7	17
Returning cohort without final food diary (D)	11	5	6	3	8	0	3	5	3
Returning cohort with or without food diary (E)	42	17	25	8	26	0	10	12	20

Table 5.1 Number of participants at different stages of the study also showing gender, smoking habit and age differences.

Upon calculating the difference between two means for statistically significant differences for each of the 'sub-cohorts' above against each other the majority yielded no statistically

significant difference. The results that did show statistically significant difference were L&Z intake between the genders for the 'returning cohort with final food diary (C)', 'returning cohort with or without food diary (E)' and 'full baseline cohort' (A). In each of these cases the female L&Z intake was statistically significantly higher than the male intake with respective p values of 0.047(C), 0.048(E) and 0.01(A). There was also a statistically significant difference between two cohorts within the female gender category with 'full baseline cohort (A)' compared to 'returning cohort without final food diary (D)' with a p value of 0.004. In this case the 'full baseline cohort (A)' female L&Z intake was statistically significantly higher than the 'returning cohort without food diary (D)'.

The food intake compared to smoking habit did not yield any statistically significant differences.

When reviewing the age categories for their L&Z intake only one result was statistically significant where the 'non-returners (B)' compared to the 'full baseline cohort (A)' intakes figures for the 50-59 year old group showed statistically significantly higher L&Z intake in the 'full baseline cohort (A)' with a p value of 0.017.

The 'non-returners (B)' compared to the 'full baseline cohort (A)' for females L&Z intake has a p value of 0.076. When comparing the baseline MPOD reading for the five cohorts mentioned in Table 5.1 above, the 'full baseline cohort (A)' compared with the 'non-returners (B)' had a p value of 0.055. Neither meets the standard 5% for statistically significant differences, but are worthy of mention within the discussion section.

Figures for levels of MPOD at the baseline measure show that 15% (15 of 100) were already measuring over 0.3 for MPOD and for the final cohort 21.40% (9 of 42). 38% of participants (38 of 100) were measured with their MPOD being within 0.1 of 0.3 at baseline.

	Gender of participant		Ocular nutritional supplement given		Smoking status		Age in years at the start of the intervention		
	Male	Female	iCaps	Vitalux plus	Smoker	Non-smoker	40 - 49	50 - 59	60+
Number of participants in category	12	19	16	15	5	26	7	7	17
Average initial diary intake	1.26	2.39	1.65	2.28	1.34	2.08	1.80	1.69	2.13
Average final diary intake	1.22	1.70	1.39	1.65	1.16	1.58	1.59	1.36	1.55
Difference between initial and Final diary intake	-0.04	-0.70	-0.26	-0.63	-0.18	-0.49	-0.21	-0.33	-0.58
Minimum daily intake	0.66	0.56	0.66	0.56	0.69	0.56	0.68	0.83	0.56
Maximum daily intake	2.34	12.83	4.31	12.83	2.04	12.83	4.31	2.96	12.83
ANOVA p value initial results	0.17		0.43		0.50		0.89		
ANOVA p value final results	0.24		0.52		0.44		0.91		

Table 5.2 Food diary summary results for daily dietary intake of L and Z (mg).

Discussion

Food diaries were kept by participants for the first week and final week of the study period. These were a meal by meal daily record for seven days. On receipt of both diaries the L and Z content was annotated on the diary and totalled for the week and divided by seven to provide a mg per day figure (see Figure 5.1). These results are shown (Table 5.2.) and have been subjected to tests for statistical significance for differences between initial and final food diaries. All other quoted papers use the daily L or L and Z dietary intake in mg, which makes the results directly comparable in this parameter.

The overall L and Z intake was compared with other papers. Some declared that there was no statistically significant difference between baseline and final readings without providing amounts found (Bartlett & Eperjesi 2007; Huang et al, 2013). L and Z content of dietary intake does vary considerably across the literature with ranges provided, 1.9 – 3.0mg (Richer et al, 2007), 2.5 – 4.0mg (Richer, et al., 2011), 1.16 – 1.36mg (Nolan, et al., 2011) and 10.7 – 14.1mg (Bartlett & Eperjesi 2008). This compares to this study with an average daily range of 1.16 – 2.39mg (see Table 5.2). This study compares well with the Richer and Nolan groups, but does differ from the Bartlett & Eperjesi 2008 paper. In the two Richer et al group studies (Richer et al, 2007; Richer et al, 2011) and the Bartlett & Eperjesi 2008 (Bartlett & Eperjesi 2008) study L only was reported on, whereas in the Nolan et al group (Nolan et al, 2011) and this current study L and Z were measured from the food diaries. Some of these differences may be explained by the nurtient database used alongside the food diary/FFQ chosen. In this current study assumptions had to be made about weights and cooking methods which may lead to a different L and Z score from other studies. This does point to a need to have a standarised method of L and Z content capture which could be adopted by any future study, enhancing comparability.

Studies involving supplementation can attract volunteer participants who have an interest in health matters and so can be more aware of what goes into their diet. Most mainstream dietary advice does follow the consumption of dark green leafy vegetables such as spinach and kale as part of the 5-a-day, 7-a-day, low Glycaemic Index or low fat diet. This may account for the apparent ‘outlier’ position of the Bartlett & Eperjesi paper (Bartlett & Eperjesi 2008) as seen in Figure 5.5. However the differences may also be accounted for, at least to some extent, in differences in software used in calculating the L and Z content of the diets

Figure 5.5 Range of daily average intake of L and Z (mg) for the current study and four comparative studies (B&E 2008, Bartlett & Eperjesi 2008)

Comparing the study samples in Figure 5.5 against the quintiles provided by Seddon et al (Seddon et al, 1994) where the upper quintile (the fifth) consumed 5.76mg L and Z a day, most of these studies fall below this figure. Nolan et al's average falls within the second quintile, this study between the third and fourth quintile, Richer et al 2007 the foruth quintile and Richer 2011 between the fourth and fifth. The Bartlett and Eperjesi paper from 2008 has an average consumption almost double that of the Seddon et al paper.

One danger of a simple comparison of Figure 5.5, without taking into account different methods of measuring L and Z content of diet and geographic location, is that just because four out of five lie within a simlar 'band' they must be correct. This is certainly not true, and particant profile becomes very important in understanding where differences may lie.

Taking Seddon et al's suggestion that the most benefit comes from consuming approximately 6mg L and Z per day Figure 5.4 does suggest that most study populations are not achieveing this daily intake.

Such differences in dietary intake of L and Z may have an effect upon the uptake of any supplemental L and Z. To hypothesise, the individual with a low L and Z intake may

actively uptake any supplemental L and Z whereas those with higher levels of intake may not actively do so. This could support the suggestion that having an MPOD level above 0.3 is superfluous to human needs by Nolan et al (Nolan et al., 2011). If this were to be found to be correct then the lack of response of vision to 6mg L supplementation from the Bartlett & Eperjesi paper (Bartlett & Eperjesi 2008) on healthy eyes could be partially due to having such high levels of L in the diet already.

In this study 15% of baseline MPOD measures (of 100) were above 0.3 and 21.40% of the final cohort (of 42) were above 0.3. Again if the suggestion that any MPOD above 0.3 is superfluous then almost a quarter of the participants in this study may not have been 'actively' taking up the L and Z in the ONS by the end of the study. Also there were 38 participants whose MPOD measured as being within 0.1 of 0.3 at baseline. Following on from the work of Bartlett et al (Bartlett et al., 2010b) this group could be raised to a level described by Nolan above (Nolan et al., 2011) as maximal for human needs. This leaves 47 participants in this study potentially still below the 0.3 MPOD figure even after the positive effects of supplementation.

The range of MPOD measures found within this study does also lead to a query on the categorisation set up by MacuScope for their results print out (See Figure 3.2). This would suggest that an average MPOD result would lie between 0.25 and 0.75 density units, whereas in this study over 50% of baseline MPOD results were below 0.25 and none over 0.75, even at the end of the study.

The gender results may support a female stereotype of being more conscientious about dietary intake with average intakes at initial and final readings being higher than for males. The age groups show that the female participants also yielded the highest L and Z mg levels for each age group, although without a trend. There was however a greater variety of L and Z content within the female diets with a daily range from 0.56mg to 12.83mg. Male daily dietary intake was more stable across the period with a variation of 0.04 mg compared with 0.70mg. Testing for statistically significant differences provide p values of 0.17 for the initial diaries and 0.24 for the final diaries, which shows no statistically significant differences between the groups. There is very little comment in other papers in regard to gender differences. The work by Wenzel et al (Wenzel et al, 2007) on spouses with concordant BMI, diet and serum L and Z, did show different MPOD levels begins to shed light on gender differences and this may provide a useful source of investigation in the future.

Statistically significant differences were found within the gender analysis with female participants having statistically higher baseline L and Z intakes compared to males for the cohorts returning for the second MPOD measure, either with the food diary completed (C) or as a whole cohort of returners (E), and the full baseline cohort (A) (See Table 5.1). This difference would be worth monitoring in future studies to determine if this is a trend. Even within the female participants of the cohorts there was a statistically significant differences between the full baseline (A) and returning without food diary cohorts (D). In this case the full baseline cohort (A) of females had higher baseline L and Z intake. This may suggest that those who did not complete the final food diary were prone to misreporting intake or were genuinely taking in less L and Z in their diet. This does open an interesting avenue for future work with compliance and dietary intake being compared.

Smokers had a lower average L and Z dietary intake compared to non-smokers (daily initial 1.34mg to 2.08mg, final 1.16mg to 1.58mg) which given the connection between smoking and reduced antioxidant levels and AMD (Kirby, et al., 2010; Buitendijk, et al., 2013) does cause some concern. This was not found to be statistically significant with a p value of 0.50 for the initial and 0.44 for the final comparison. Given the low numbers of smokers in this group this should be reviewed in other studies to confirm that no statistical significance exists. Whilst in this study no statistically significant difference at baseline was discovered, Nolan et al (Nolan, et al., 2011) did find a statistically significant difference in smoking habits between those asigned to different treatment arms. This supports the decision to include smoking in the block stratification of this study.

Age trends showed a reduced minimum level of L and Z and an increased drop off rate between initial and final readings. This trend may have been replicated within averages also had it not been for the unusual consumption of 89.8mg of L and Z by one of the older age group participants. During the initial food diary recording period this participant consumed raw spinach every day, sometimes more than once. Their final food diary also contained a substantial intake of spinach. Some studies have found an inverse relationship between MPOD and age (Huang et al, 2013) but others have found no relationship between the two (Richer et al, 2007; Nolan et al, 2011). Although the raw data may appear enticing, the lack of statistically significant difference (p=0.89 initial and 0.91 final) does show that this study found no difference in dietary intake of L and Z with age.

In only one age category (50-59) was an inter-cohort statistically significant difference apparent when comparing the full baseline cohort (A) against the non-returners (B). This

may suggest that future work on a low rate of L and Z intake and its effect upon compliance with study instructions would be of interest. Two results were close to statistical significance as reported within the results section, both of which are comparisons between the full baseline cohort (A) and the non-returners (B) with lower female L and Z intake and lower initial MPOD measures being involved. These would be worth investigating further to determine if they do have an effect upon the final outcome of studies. This may help bring clarity to the discussion on study population differences from the actual population, and how markers, such as compliance or initial MPOD, may help define groups that would benefit most from supplementation.

In all cases the final reading was lower on average than the initial reading. Prior studies have often measured L and Z content of diet for baseline data only with comparisons in the initial groups to ensure, or report on, any statistically significant differences. One other paper that did offer final L and Z intake data showed a drop in L content of diet across the study period (Richer et al, 2011). The average drop in this current study was 0.37mg daily compared to the Richer et al paper of 0.91mg daily. Participants may have their attention temporarily focussed upon their diet and so may change what they eat in the initial food diary compared to what a normal diet may be like for them. Given the uneven nature of the content of L and Z within foodstuffs, if the participants chose to eat more spinach, as an example, this would considerably raise their L and Z intake whilst eating spinach, but if after four months the interest had waned then the food diary would be considerably lower in L and Z. This change of diet, whether caused by increased awareness or a desire to please the research team is known as the Hawthorne effect.

The significance of this can be discounted by the p value of 0.12, which shows no statistically significant difference in food diaries between initial and final visits. From this a degree of comfort can be drawn that the study results overall were not affected by changes to dietary intakes across the study period. The drop in L and Z scores from initial to final food diaries, even though not significant, could be due as mentioned above to the initial interest in taking a supplement, promoted by a practitioner, waning over a four month period where it may have been difficult to impute use of the supplements to their individual wellbeing (van der Kruk et al, 2008). Most of the papers reviewed found a drop off in L and Z dietary intake across the study period in line with this study.

This presents any clinician offering ONS to their patients with a dilemma, in that they may well enhance any innate desire within their patients to supplement to prevent future symptoms, but this enhanced desire may wane over the months following interaction with

the practitioner. A system that would support these patients in the long term and continue to reinvigorate their desire to prevent future problems would be useful. This may well lie within the remit of social media as a more personalised and accessible medium for many people. Certainly this was used within other studies to ensure compliance and attendance via telephone calls and text messaging (Nolan, et al., 2011; Loughman et al, 2012).

The Shapiro-Wilk normality tests carried out showed that all four populations were rejected for normality hence non-parametric tests were adopted. A Spearman's correlation coefficient was calculated, comparing the initial RE MPOD readings with the initial food diary results and then also for the LE. It may be thought that those with a high L and Z intake would have a higher MPOD reading. The resulting Spearman's correlation coefficient was RE -0.092 (p=0.367) and LE -0.021 (p=0.842) which means that there is no statistically significant correlation between the initial MPOD readings and food diary L and Z intake for either eye (see Figure 5.3 and 5.4). The negative relationship in the RE and LE, even mild as it appears here, suggests that in this study the higher the initial intake of L and Z, the lower the initial MPOD was. This points to the relationship between dietary intake of L and Z and MPOD levels being a complex one with numerous factors, such as bioavailability of L and Z in the diet, serum uptake, non-responders and as yet unknown factors, being involved.

The food diary results were skewed due to the uneven distribution of L and Z within the diet. Spinach and kale eaters ingested significantly more L and Z than none eaters, and these were more likely to be eaten by women compared to men. This helps to account for some of the wide variation between the sexes and for maximum values and the range of results for females. There are gender differences in the way individuals respond to exercise (Donnelly & Smith, 2005) and the attitudes towards diet and willingness to take up 'healthier' eating habits also varies between the sexes (Herbert, et al., 1997; Beardsworth et al, 2002; Rieker & Bird, 2005; Davy et al, 2006). Women are more likely to diet to lose weight, whereas men exercise more, women were more likely to take advice on diet from family, magazines, professionals and books whereas men tended to rely upon classes, friends, instinct and television. Differences in self-assessment and beliefs that have been shown to be statistically significant include eating too much sugar, the importance of limitation of carbohydrates and fats and the recognition that weight loss is needed.

Concerns over accuracy of reporting in the food diaries were raised by the significant lack of information about alcohol and chocolate/sweets consumed in most food diaries and the

lack of information about weights of food consumed and a vagueness of description (Westerterp & Goris, 2002). Thus assumptions had to be made about how to score the food diaries, which may have affected the results. The method of cooking was also not reported, but can have a significant effect upon how much L/Z is available for uptake (Yadav & Sehgal, 2003).

L and Z content of foods is not frequently included within many nutrition database programmes, which could help standardise collection and reporting if they were present. The food diaries in this study were marked up using the USDA nutritional database for L and Z release 24 (USDA, 2013). Due to the lack of information about quantities the database quantity figures were taken as assumptive amounts consumed. This may have over standardised the food diaries with different people taking different quantities. However, without evidence to the contrary, this was taken as a sensible way forward to gain food diary data. Within the database there is some reflection of different quantities that may be used, in the case of spinach, in salads compared to cooked dishes, and this was applied in accord with the description in the food diary.

Different FFQ were used, two using the Harvard school of public health FFQ (Richer et al, 2007; Richer, et al., 2011), one the Scottish collaborative group at the University of Aberdeen FFQ (Nolan, et al., 2011) and three others not declaring which FFQ was used (Bartlett & Eperjesi 2007; Bartlett & Eperjesi 2008; Huang, et al., 2013). Each of these FFQs examines the intake slightly differently and so comparision between studies is not available directly. Some form of standardisation of database here would be useful for comparisons between studies.

The 31% response rate for completion of both sections of the food diaries was disappointing and was the point of greatest resistance to compliance for participants. The statistical analysis suggests that there was no significant difference between the initial and final food diary results ($p=0.12$). This would suggest that changes to diet did not enhance or adversely affect the results from supplementation.

Use of a FFQ may help go someway to help address some of the challenges faced within this study (Richer, et al., 2011; Nolan, et al., 2011). An advantage of the FFQ is that the research team administers it at visits by the participants. This allows for collection of data from each participant. Within this study the prime complaint that restricted compliance was the week long recording of food data. More food diaries would have been collected,

however the week long recording did allow for accuracy in data collected rather than attempting to recall what food had been eaten over the preceding week.

Within this current study food diaries were given to participants at the initial visit, or posted to them for the final visit. This meant that the participant could complete the diary at home. Wolffsohn et al pointed to some benefits of this method of data collection as being the most cost effective and with the highest level of internal consistency of any of the methods they studied (Wolffsohn et al, 2000). This was using quality of life questionnaires amongst participants with low vision. This may now be challenged with on the cost basis by electronic and social media routes, but this would need to be verified by future work. However Wolffsohn et al found that the quality of life scores were consistently lower than if the questionnaires were undertaken either in person or over the telephone. From this the method of food diary/FFQ recording can also have an impact upon comparisons with other studies with postal completion having high internal consistency but lower scores and in person or telephone completion having higher scores, but with less internal consistency.

In future studies it would be sensible to adopt a more robust discussion prior to the start on the study by any participant, and provide more data on the minimal side effects, in any future studies of this nature. It may be that utilising a food frequency questionnaire at the initial and final reading stages, run by the study data collection team, would have reduced the non-response and drop out rate. If a food diary similar to the one used in this study were to be used again it would be useful to run the initial food diary for a week prior to attending for the initial measurement, creating less focus on the dietary intake and so reducing skewing of the food diary results from normal.

The use of food diary software with whole food intake input when compared to isolating L and Z intake would be beneficial for future studies. This will permit comparison of far more parameters than in this study and gives further information on the overall dietary intake. It may be useful in comparing information on BMI status also.

Summary

This section covered the food diaries recorded during the study and discusses the outcomes from these diaries. In the next section the areas that would enhance the results in future studies are reported along with an interpretation of the results and the conclusions drawn from the study.

Chapter 6 Discussion and conclusions

In Chapter 5 the food diaries recorded during this study and the outcomes produced were discussed. There was no significant change to the dietary intake of the cohort that returned the food diaries. In this chapter enhancements to study design for any future studies are reviewed. The clinical implications of the study are discussed and the conclusions drawn from the study are stated.

Although the H_O sets out that there would be no difference between the 6mg L and 10mg L concentrations, it would seem logical that a higher concentration of something would lead to more of it being taken up by the body, and if that concentration is sufficiently higher then the result would be expected to be significantly different. It may well be that in this study the 6 to 10mg difference was simply not sufficiently higher, but a greater difference (10 to 20mg) was also found to provide no significant difference in the LUTEGA study (Dawczynski et al, 2013). It would be of interest to be able to compare the lower concentration of this study (6mg) with the higher concentration of the LUTEGA study (20mg) to check on significance of that difference. It is worthy of comment that p=0.07 is not far from significance, and with factors mentioned below, including a simpler and more accurate objective measurement device, it piques the interest sufficiently to make this (6mg to 10mg) worth running again; building in the features and for the reasons mentioned in the section 'Future studies' below.

A general rule is that post hoc power calculations should be avoided (Levine and Ensom 2001). The reason why in this thesis a post hoc power calculation was undertaken was due to the fundamental shift in the base parameters used. The mean change in MPOD was initially 0.089, and in the final cohort 0.062. The standard deviation was initially 0.215 and in the final cohort 0.430. These changed the effect size from 0.414 to 0.145, thus the change from sample size of 38 to 296. One outcome from being aware of this is that for future studies it would be rational to attempt to reduce the variation in results, which from other areas of this discussion brings attention to instrument and study design once more.

It must be borne in mind that the final power (Post Hoc calculation) of the study was 23.57% instead of 80% which makes the acceptance of H_0 less comfortable statistically. At 80% the results would potentially be wrong in 20% of cases, here this figure rises to 76.43% of cases, which is high. This supports the desire to repeat the measurements, with other factors built in. It may be sensible to consider changing the 80% acceptance to 90%, further reducing the change of an incorrect result, but also increasing the likelihood of finding clinically significant results rather than simply statistically significant results.

This could be further enhanced by using a significance level of 0.01 rather than 0.05. The whole premise of the thesis was to find clinically significant results for primary care practitioners to work with. Both of the suggested changes would further increase the numbers needed to recruit.

A number of authors have conducted intervention studies with visual performance measurements as part of the battery of tests. In this study it was not the case, as it did not directly affect the H_0. In support of this study not running visual performance measures the results from two papers reviewing 6mg L concentrations found no increase in visual performance measures reviewed (Bartlett & Eperjesi, 2007; Bartlett & Eperjesi, 2008); whereas other studies have reported an improvement to visual performance with different concentrations of L (10mg to 20mg L BCVA (Dawczynski et al, 2013); 20mg L to 10mg L with 10mg MZ (Loughman et al, 2012); combined 8mg Z and 9mg L, 8mg Z or 9mg L with foveal and parafoveal VA (Richer, et al., 2011) and 12mg L with 'Daily task comparative analysis' (Nolan, et al., 2011)). In future studies it may be worthwhile considering bringing in some form of visual performance measurement without leading to elongated test battery times which can deter participants (Richer, et al., 2011). Measures such as BCVA or contrast sensitivity may be suitable to match this recommendation.

Competition at receptor sites for L and Z reported in a paper reviewed in the literature review (Chapter 2) was held as a possible reason for an ONS with equal amounts of L and Z producing smaller changes to MPOD (Richer, et al., 2011). In this study the iC ONS contained 6mg combined L and Z in a 5:1 ratio, whereas V contained L only. This concurs with the 'natural' state of L and Z in a 5:1 ratio and so does not present any competitive reduction in uptake of the carotenoids, therefore supporting the choice of ONS used within this study.

Having reviewed many studies and considered the methods used in this study it may be possible that a number of factors were confounding the comparisons. It would be wise to attempt to remove as many of these confounding factors as possible to gain greatest insight into which concentration really does create significant improvement, if any.

Although block stratification and food diaries were used in this study to reduce the potential effects of confounders, the literature review uncovered other areas that could in future studies be considered for limiting the comparability or confounding the results. Ethnicity (Xie et al, 2001) in this study all participants were White British, thus the results are only comparable to a similar group. Gastro-intestinal health (Hooper et al, 2002)

although in this study this may be accounted for in the food diaries, age banding and randomisation no direct questions on mal-absorption were asked to screen for block apportionment or as a potential exclusion criteria. Alcohol intake (Lieber, 1988), which was not specifically enquired into, but may have been controlled by randomisation.

A recent paper has set out a prediction model for AMD in the general population with the highest 'area under curve' or measureable effect of a phenomenon being the risk factors age, gender, 26 SNPs in AMD risk genes, smoking, body mass index and baseline AMD phenotype (Buitendijk, et al., 2013). This confirms work from 2006 that looked at couples (spouses) (Wenzel et al, 2007). Spouses who had concordant diet and BMI had similar serum levels of L and Z, but had different retinal MPOD measurements, suggesting that other factors, as mentioned, were also affecting their uptake into the retina. There were no couples (spouses) within this study cohort. Thus future studies may wish to consider the genetics and body mass index (BMI) of patients to assist with comparability and assessing how best to help each different group.

Higher BMI, if accounted for by body fat, will also potentially skew the uptake of MP by the retina. This is because L and Z are preferentially taken up by adipose tissue when compared to the retina, thus the higher the body fat % the lower the level of 'free' L and Z available for uptake by the retina (Mozaffarieh et al, 2003).

Thus a broader control of variables would include gender, smoking habit, age, diet, ethnicity, gastro-intestinal health, alcohol intake, relevant genetics and BMI. These additional controls would make recruitment more difficult as the questioning becomes more intrusive and enquiring into the participant's genetic make up. It does however make randomisation of allocation important.

Ethnicity is relevant in defining which groups the results are applicable to. In smaller studies it may be preferable to have single ethnic groups so as to define each groups characteristics in relation to the ONS studied. This does suggest that a study for each definable group would be beneficial. Smaller groups are unlikely to be representative of the larger population they are drawn from unless the study is designed to ensure that this is the case.

The volunteer population for this study varied substantially from the estimated population (Table B1.1) for Sheffield in 2010 (Central P B C consortium, 2010; Office for National Statistics, 2010; Sheffield City Council, 2011) with bias towards female over male, non-

smoking over smoking and with an older mean age. This may be due to health awareness in these groups being higher or more proactive (Foote et al, 2003). Health awareness may skew the results in favour of less significance, given that the subjects may already chose a diet richer in carotenoids. This is confirmed by the findings from AREDS 1 study report 22 (AREDS Research Group, 2007). Those in the highest quintile for nutrient absorption had slower progression of their AMD when compared to the lowest quintile. How this study population compares to a standard Optometric population for the same area is not stated.

Bioavailability is the proportion of a nutrient that is absorbed from the diet and used for normal bodily functions (Aggett, 2010). Macronutrients (carbohydrates, proteins and fats) usually have a very high bioavailability with more than 90% being ingested. Micronutrients, as are found here in these supplements, vary substantially. For micronutrients the first stage is releasing from the food and conversion into a chemical form (e.g. a micelle) that will enter the gut cells or pass between them – bio-accessibility (Holst & Williamson, 2008). The prime site for this is the small intestine.

As the L and Z are fat soluble and need to be carried to tissue by micelles the use of statins by the participant can also affect bioavailability. Statins reduce the number of micelles available in an attempt to control cholesterol levels.

Just as food form, being raw or cooked, has an effect on releasing the micronutrients, so does the carrier used in supplements. If there is some fat/oil available then the carotenoids under study here, being fat-soluble, will be more readily bio-accessible (van Het Hof et al, 2000). With the supplements in this study this would apply to the V ONS, but not the iC, potentially favouring the uptake of the former.

In nature L and Z can co-exist in either an esterified (diester) or a non-esterified form. Neither ONS in this study contains L in an ester form. This form may present problems with bioavailability due to the two fatty acid groups that must be cleaved off before the human body can use the L (Noy, 2000). This is backed up with reports about the efficacy of this hydrolysis of L esters in the human body, which is less than 5% (Breithaupt et al, 2002; Granado et al, 2002). This may also decline with age (Chung et al, 2004). However other studies do not support this with either no significant differences (Chung et al, 2004) or a greater response from L esters than from pure L (Bowen et al, 2002).

It is noteworthy that these last two studies commented only upon serum response to L form and not retinal response. The serum uptake is useful in making the L and Z available for use by the body, but as we are interested in its effect upon and in the retina, we are most concerned by the retinal uptake. Anything that restricts retinal uptake would work against the enhancement of MPOD and could provide another confounder. This justifies the use of un-esterified L in this study and the absence of serum level measures, which could easily dissuade participants from volunteering.

Qualified optometrists using slit lamp binocular indirect ophthalmoscopy and fundus photography undertook review for inclusion/exclusion in a primary care clinic. Since the study was initiated the use of ocular coherence tomography (OCT) has become more common in clinical practice. As this technology provides a greater degree of detail of the macula and its surrounding area, adding an OCT review into the initial process may help in discovering early AMD cases, which can reduce MPOD changes and provide further reasons for variation in macula response to ONS.

In both arms of the intervention 44 participants were lost to follow up and eight discontinued the intervention (52% total drop out). The 44 simply ceased communicating with the administrators and did not re-attend for the final reading. It is problematical to say why this occurred, but the drop out without response rate was high (44%). Reasons for this may become apparent from the smaller number (8%) who discontinued. These eight participants reported back that they disliked taking tablets/capsules especially large ones. Some had symptoms such as nose bleeds or headaches that they connected to taking the supplements, even though, after questioning there was no apparent causal connection. One person admitted to hating completing food diaries and someone else disliked knowing what they ate.

It is possible that the connection between time spent on the study by the participants and what they got back for taking time to do this was not seen to be properly rewarded. Richer et al describes a reward scheme for participants who received $25 per visit for travel expenses and $100 at the end of the study if they complete all visits (Richer, et al., 2011). They report a 90% attendance for two of the three visits with only nine of the 60 enrolled failing to complete the study (see Figure 6.1). This compares with 52% failure to complete the study for this cohort. Whilst providing funding does increase the costs of the research it may well be that it can reduce the enrolment numbers and therefore administrative costs to cover the expenses. Other reasons for this ‘high’ drop out rate could be the low level of contact with participants during the four month supplementation

period or the nature of the establishment being a primary eyecare practice not a recognised research establishment.

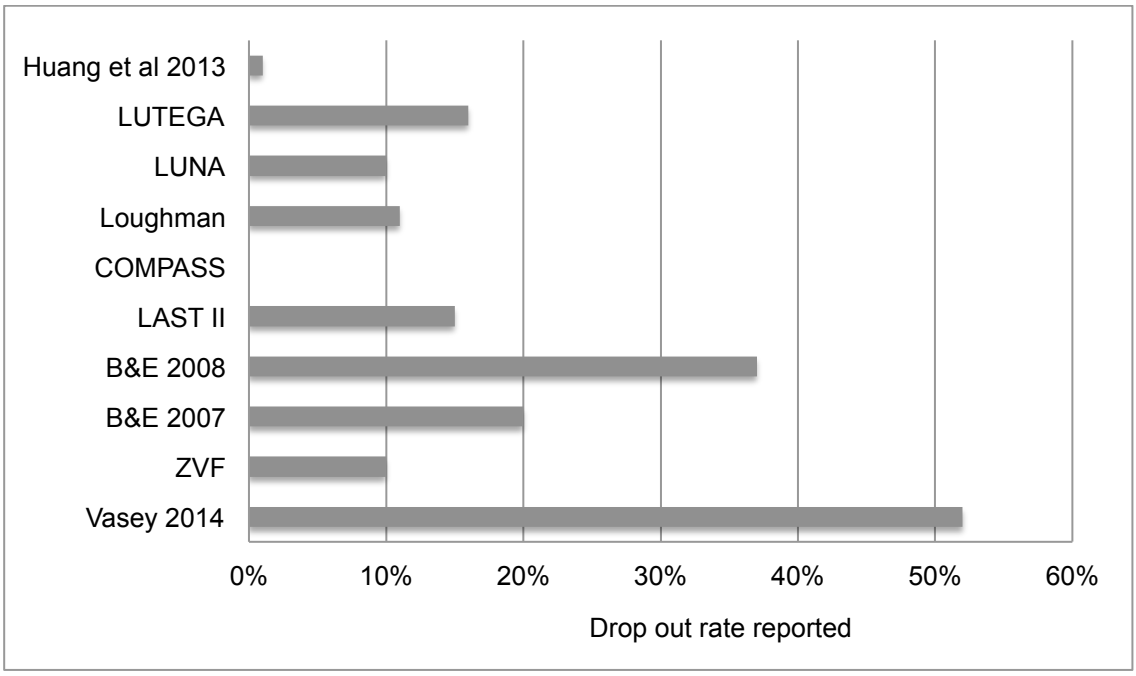


Figure 6.1 Total reported participant drop out rate for any reason by study

Concern over inter-study comparability due to the differential drop out rate may be reduced following on from work by Bell et al in 2013 (Bell et al, 2013). The majority of drop outs in this current study were clasified as ‘missing at random’ rather than ‘missing not at random’. ‘Missing not at random’ suggests a systematic problem with the drop outs which could bias results and so limit comparision between studies.

This concern can be further mitigated by comparision of the means for the whole recruited population prior to drop out (100) and the completers (42) at baseline. The mean and standard deviations for each eye and for completers and the full population were as shown in Table 6.1.

100 (Initial)	RE	LE	42 (Completers)	RE	LE
Mean	0.204	0.210	Mean	0.215	0.227
Standard deviation	0.131	0.129	Standard deviation	0.132	0.133

Table 6.1 Means and standard deviations for intial 100 baseline MPOD measure and 42 completers baseline MPOD measure for both RE and LE.

After statitiscal analysis using a comparison of two population means, this has a p value for REs of 0.326 and LEs of 0.243 which at the 95% confidence interval shows no statistically significant difference between the two populations.

It would be suitable to adopt a more robust discussion prior to the start on the study by any participant, and provide more data on the minimal side effects, in any future studies of this nature. It may be that utilising a food frequency questionnaire at the initial and final reading stages, run by the study data collection team, would have reduced the non-response rate.

From other studies a number of methods to check ONS compliance have been suggested. Within this study we simply asked at the final visit if they had taken the ONS. This relies upon trust and is subjective. Other studies have counted capsules at visits to check with compliance (Richer, et al., 2011), which is still open to abuse with capsules being disposed of to meet targets. Others have measured serum or skin carotenoid levels to check for compliance (Richer, et al., 2011; Loughman et al, 2012). The measurement of serum levels of L and Z can check compliance by ensuring serum levels are rising, if not then the participant will not be compliant. There is no evidence yet for serum non-responders to supplementation. Whilst this does not ensure that all tablets/capsules are taken it does point to some being taken, without which no serum/skin changes would be registered. Whichever method is chosen it does leave open the possibility that not all ONS tablets/capsules have been taken and so concentrations talked about within studies have actually been reduced by non-compliance. Due to the nature of this problem there is no surety that the compliance is evenly distributed or the degree to which it is skewed, within or between studies.

If the hypothesis that supplementation with L and Z does increase MPOD levels is accepted then there is a seeming anomaly with stable or negative MPOD levels (non-responders) that have been reported in other studies (Nolan et al, 2011; Richer et al, 2011). In this current study the non-response rate to supplementation was 50%. This compares with 23% in the COMPASS study and 27% in the ZVF study. As both eyes were measured in this study it was also apparent in 14 individuals that one eye did respond whilst the other was classified as a non-responder. This does bring into question the quality of measurements by the device used within this current study, or the understanding of non-response. As intra-participant differential response to L and Z supplementation has not been reported on elsewhere this may be worth investigating as it may lead to a better understanding of what factors control response.

From the work described earlier (Bartlett et al, 2010a) the poor repeatability and reproducibility of MPOD readings from the MacuScope left any changes below 0.58 as suspect. In no single case did any of the participants in this study change by as much as 0.58, thus the changes registered could be described as 'noise'. For future studies into this area an instrument with better repeatability (a measurement is repeatable if the original experimenter repeats the investigation using same method and equipment and obtains the same result) and reproducibility (a measurement is reproducible if the investigation is repeated by another person, or by using different equipment or techniques, and the same result is obtained) should be sought. The commercialisation of alternate methods described in Chapter 2 may allow for greater accuracy of change being recorded. The requirements for such an instrument are summed up well: Low cost clinical instrument capable of more simple and accurate evaluations with more rapid and more complete capture of the entire macular profile. (Richer et al, 2007)

L, Z and MZ have all shown to be taken up into the serum and to raise MPOD levels safely (Connolly et al, 2011), although it has been suggested that the presence of MZ could inhibit the uptake of the other two carotenoids (Thurnham et al, 2008). It has also been suggested that there is a group of people who are non-responders to L supplementation in terms of its conversion to MZ (Beatty et al, 1999). This could mean that high concentrations of L in supplements would not yield the rise in MPOD expected due to a problem with this conversion pathway. It was unknown if any such non-responders were within this study group. Future studies may want to incorporate MZ into a further cohort to assess its affect.

Since the start of this study the long-term study AREDS 2 (AREDS 2 Research Group, 2013) has reported initial findings. The principal findings of this study are:

- No additional benefit form adding Omega-3 PUFAs or L and Z to the original formulation.
- Those taking AREDS formulation without Beta-carotene but with L and Z had AMD progression risk reduced by 18% when compared to those taking the original AREDS formulation with beta-carotene but no L and Z.
- Participants with low dietary L and Z at baseline (≤0.823mg/day) taking AREDS formulation with L and Z were 25% less likely to develop advanced AMD compared with similar baseline diet participants who did not take the L and Z.
- No benefit was found for baseline diets above 1.030mg/day.

- Beta-carotene may compete with L and Z for uptake into the body.
- Beta-carotene appeared to increase risk of lung cancer in previous smokers.
- Reducing zinc content and removing beta-carotene from the original AREDS formulation had no effect on progression to advanced AMD.
- Secondary analysis showed that taking a combination of 500mg vitamin C, 400IU vitamin E, 25mg zinc, 2mg copper, 10mg L and 2mg Z reduced the risk of progression to advanced AMD in those who already showed signs of the disease.

The study did not show a preventative effect upon 'normal' eyes from L and Z supplementation, as this was not a design feature of the study.

The lack of significant effect of omega-3s found within AREDS 2 supports the choice of ONS used within this study, which did not contain any omega-3s. Although one other study (Dawczynski et al, 2013) found positive changes to MPOD and BCVA with L/Z/EPA/DHA combined there was no report within the study of how this compared to L and Z without EPA/DHA present. The reduced level of Zinc found within AREDS 2 compared with AREDS 1 was not found to have reduced the efficacy of the ONS and again correlates well with the Zinc levels found within V and iC used in this study. The absence of beta-carotene in AREDS 2 and the ONS used in this study is supported by the results of AREDS 2.

The Ocular Nutrition Society has stated its position on AREDS 2 (Position Statement on AREDS 2, 2013). In conversations at clinical conferences it is clear that some clinicians consider ONS as an 'alternative therapy' or without scientific base. This Ocular Nutrition Society statement helps overcome the view and advances research methodology queries on the application of AREDS 2 to 'normal' patients without AMD, listing 15 concerns why this may be appropriate to do:

1. Study design focussed on AMD progression not prevention or reversal
2. No primary positive results from L, Z, EPA or DHA, only apparent in secondary outcomes.
3. 'Well nourished' population not representative of United States population
4. AMD progression was based upon visual acuity and photographs only, not visual function tests.
5. Genetic evaluations only conducted on 25% of population and results are awaited for these.

6. MPOD was measured in only 25% of the population and results are awaited for these.
7. Placebo group participants had high levels of EPA and DHA in diets that may confound the results
8. In groups with low habitual L and Z intake, L and Z supplementation can be effective in reducing incidence and/or progression of cataract.
9. Zinc levels were set at twice the recommended amount and the oxide form used is not well absorbed
10. There is no true control group as all participants received a supplement.
11. L and Z are not a substitute for beta-carotene as they do not convert to vitamin A
12. Centrum was the ONS of choice (AREDS 1 67%, AREDS 2 89%) but did not contain L in AREDS 1
13. Time scale of five years was unnecessary to show L and Z results
14. Hispanic Americans made up only 2% of the study population, which is underweight for the United States population.
15. Participants with geographic atrophy faired worse in AREDS 2 compared with 1, this may point to other nutrient factors in geographic atrophy.

Future studies

There are several factors that could be built into any future studies. Checking three different concentrations of L at 6mg, 10mg and 20mg would be useful to allow complete analysis within the same cohort. These should be in the non-esterified form and in a 5:1 ratio if Z is included. Further review of the role of MZ would also be useful. Combinations of these concentrations including and excluding omega-3 PUFAs would also help.

The participants should better reflect the underlying population, with less health awareness, healthy diets and with no AMD or other macula or retinal disorder, ideally without cataract affecting image quality. Ocular coherence tomography is an imaging technique that is becoming more readily available in clinical practice. This technique provides a cross section of the retina, allowing the observer to review each layer within the scan area. The scan area provides both depth (layer by layer) and width (across the central macula area) observation. Macula assessment using this imaging technique can further enhance determining where the participant is on the spectrum of healthy eye to end stage AMD. This can further the understanding of how ONS can affect different stages of this spectrum. Introducing ocular coherence tomography would therefore enhance future studies.

It would be good to introduce some visual performance measure as a further outcome alongside MPOD. Given the risk of overdeveloping the bank of tests, BCVA or contrast sensitivity may be the best options for this.

Block stratification by, or at a least measurement of data with a mind to potential confounders would be good, including age, smoking habit, gender, diet (possibly via FFQ) ethnicity, gastrointestinal health, BMI, alcohol intake and relevant genetics. Being able to take a baseline micronutrient profile would also be useful in understanding who would benefit most from supplementation. This is achievable with food diaries.

Compliance should be checked using capsule counting or serum carotenoid levels, alongside encouragement to maintain the ONS via telephone, sms or social media. Compliance should also include a check that the ONS are being consumed with fat/oil present in the diet, aiding absorption. To encourage re-attendance a small stipend to cover travel may be worth considering.

The timescales used in the studies discussed varied from four months to 18 months, with plateauing of MPOD being found from one month to 12 months (ester form) would suggest that any future study would benefit from being planned to last at least six months and ideally 12 to ensure plateauing had occurred.

Consideration may be given to use of alternative measurement strategies for macular function, including auto-fluorescence, macular densitometry as adopted by Loughman et al (Loughman et al, 2012), or the instrument currently being developed at Cardiff University, the retinal densitometer (Cardiff University PhD studentship, 2012). Maculux (ebiga-VISION GmbH, Rungerstrasse 22-24, 10179 Berlin, Germany) is also a device that measures MPOD and has been tested against cadaver eyes to verify results obtained.

More than anything else, for this to be applicable to a clinical setting, with the ability in future to be able to provide an ONS to patients on a prescriptive basis the development of a low cost clinical instrument capable of simpler, more accurate and reproducible evaluations with rapid and complete capture of the entire macular profile. Without this, although the scientific knowledge base will grow, its application, quickly, simply and efficiently for the benefit of the patient will not become a reality.

Clinical implications

Given the results from this study the clinician cannot rely on providing a mildly higher concentration of L/Z to significantly expedite the take up of the nutrients nor the change to MPOD for the benefit of the subject.

More attention to advice on those groups who take less L in their diet, encouraging them to supplement, would seem to be appropriate, but who lies within this group may be difficult to ascertain in a short primary care consultation, without simpler and more accurate instrumentation. Awareness of supplements available, the form of L therein (ester or not) along with levels of L contained is vital for appropriate advice to be given (Bartlett & Eperjesi, 2006).

When considering how to measure MPOD in optometric practice, review and reliance upon evidence provided from the published literature is strongly urged. As new instruments are released and assessed by independent reviewers it is hoped that clinicians will be able to access more reliable and reproducible devices to screen and monitor at risk groups, ideally using objective rather than subjective measurement.

Conclusion

The use of an ocular supplement with 6 mg of L and Z compared with one with 10 mg of L, in this study, yielded no statistically significant difference. The result was close to significance with a one tailed test but it was clearly in the range of normal variation. Given the building in of the limitations mentioned above and the further development of repeatable and reproducible results, further studies would be beneficial to determine if the result of this study was repeatable or if a greater difference in concentration does create significantly different results for those taking the ONS.

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Summary

This section has provided detail of how to enhance future studies in this area along with the interpretation of the data and the conclusions drawn from the study.

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Appendices

A. *Supplements*
B. *Further results*
C. *Poster displayed in practice*
D. *Advertising study to potential participants*
E. *Consent form*
F. *Letter acknowledging enrolment and awaiting block stratified matching*
G. *Letter inviting participant onto study*
H. *Food diaries*
I. *Letter reminding participant to return food diaries after initial assessment*
J. *Letter inviting for final assessment and providing final food diary*
K. *Ethical approval paperwork*

Supplements

This section covers the micronutrients included within the ONS chosen for this study. The two ONS chosen for this study were commercially available, and had been so for a length of time to allow any adverse effects to become apparent, at the time of the study and provided a different concentration of L to test the effect of this upon MPOD. For each micronutrient information is provided about how it interacts within the human body with especial interest in how it affects the eye. Information on RDA and safety levels is provided where it is available.

Function of the ocular supplements used within this study

Two ONS were chosen to permit a direct comparison between the commonly available L concentrations (6mg and 10mg) at the time of the study. The constituent elements within the two ONS are shown in Table A1.1. In both ONS the L is un-esterified. The effects of the stated contents of the supplements used within this study are below. Details of these and other nutrients used within eye-related supplements are provided in various papers (Bartlett & Eperjesi, 2004; Bartlett & Eperjesi, 2005)

	ICaps	Vitalux Plus
Vitamin A	800ug	-
Vitamin C	125mg	60mg
Vitamin E	50mg	20mg
Vitamin B2 (Riboflavin)	1.4mg	-
Vitamin B3 (Niacin)	-	10mg
Zinc	20mg	10mg
Copper	1,000ug	0.25mg
Manganese	2mg	-
Selenium	55ug	-
Lutein	-	10mg
Lutein/Zeaxanthin	6mg	-

Table A1.1. Ingredients of the two ocular nutritional supplements used within this study

Lutein

Lutein has been described in the introduction. There is no recommended daily intake or upper limit levels set for L. Studies have shown no toxicity effects over six months (2.5-10mg/day) (Khachik et al, 2006), over 4 or 13 weeks (4-400 mg/kg of body weight) (Harikumar et al, 2008). There has been a suggestion that genotoxic effects may exist with the breakdown products of L, which may worsen oxidative stress (Kalariya et al, 2009)

Vitamin A

This is included in the ONS as Vitamin A is needed by the retina in the form of retinal, which combines with protein opsin to form rhodopsin the light-absorbing molecule that is necessary for both low-light (scotopic vision) and colour vision.

Vitamin A is held within the body in the form of retinol, which is fat soluble, and converted to and from retinal, it's visually active aldehyde form. Absorption of vitamin depends upon it's form and carrier; taking retinol as the comparator the equivalent availability post absorption of β-carotene dissolved in oil is 50% (1/2) and β-carotene found in normal dietary circumstances 8.33% (1/12). This is further regulated by the amount of retinol in the body already and so holds closest to these values in vitamin A deficient individuals. As can be seen from the carrier, lipids increase the uptake (Solomons & Orozco, 2003).

The recommended daily intake (United States Department for Agriculture, 2011) for adult (19 years plus) males is 900 micrograms per day (UL 3000 micrograms) and for adult females 700 micrograms per day (UL 3000 micrograms a day) unless pregnant (770 micrograms a day) or lactating (1300 micrograms a day).

The role vitamin A plays within the body include:
Vision, gene transcription, immune function, embryonic development and reproduction, bone metabolism, haematopoiesis, skin and cellular health and antioxidant activity.

Within the eye it is utilized in the retinal form 11-cis-retinal which binds to rhodopsin (Rods) and iodopsin (Cones). Light striking this isomerizes 11-cis-retinal to the all-'trans' form, which detaches from the opsin via photo-bleaching. This generates the nervous signal along the optic nerve. Recycling converts the all-'trans' form to 11-cis-retinal once more, which rebinds with the opsin. Some of the all-'trans' form is converted into a retinol form, which is transported to the retinal pigment epithelium for storage along with interphotoreceptor retinal binding protein, available for use when needed (Combs, 2012).

Deficiency can arise from a reduced intake of foodstuffs containing vitamin A (primary) or poor absorption due to concurrent life style or medical issues. Visual symptoms include reduced vision in low light levels (night blindness), drying of the conjunctiva (xerosis), Bitot's spots (keratin plaques) corneal surface erosion, roughening and eventual destruction (keratomalacia) which leads to total blindness.

Toxicity. Being a fat soluble vitamin, the excretion of vitamin A is more difficult than water soluble vitamins and so toxicity can arise as a problem. Acute toxicity occurs at 25,000 IU/kg of body weight with chronic levels from 4,000 IU/kg of body weight daily over 6-15 months. Liver toxicity can occur at 15,000 IU per day especially in cases with concurrent excess alcohol intake. Children can reach toxic levels at 1,500 IU/kg of body weight (Penniston & Tanumihardjo, 2006).

Effects of excessive vitamin A consumption: nausea, irritability, anorexia, vomiting, blurry vision, headaches, hair loss, muscle and abdominal pain and weakness, drowsiness and altered mental state. Chronically: hair loss, dry skin, drying of the mucous membranes, fever, insomnia, fatigue, weight loss, bone fractures, anaemia, and diarrhoea (Eledrisi, 2012). These toxicities only occur in the preformed retinoid state (such as from the liver) whereas the carotenoid forms, in the absence of chronic alcoholism give no such symptoms except for the unfortunate carotenodermia of orange-yellow skin discolouration (Takita et al, 2006).
Water-soluble versions have been synthesized, but have shown to yield more toxic effects than the fat-soluble state (Myhre et al, 2003).

Vitamin C

This is included in the ONS as it has an antioxidant effect along with the maintenance of blood vessels by its action on collagen (enzymatic co-factor).
Vitamin C is an antioxidant and pro-oxidant protecting the body against oxidative stress and a co-factor in at least eight enzymatic reactions, acting as an electron donor. It facilitates the immune system and acts as an antihistamine. The antioxidant role is facilitated by its oxidation and then reduction back via enzymes and glutathione.

Humans do not synthesise their own vitamin C and so must ingest it. The absorption is via active transport and simple diffusion. The uptake varies inversely with the vitamin C available within the body already. Excess amounts are rapidly excreted or stored in various structures around the body, including the retina (Hediger, 2002).
The recommended daily intake is 90mg/day for adult males (UL 2,000mg/day) and 75 mg/day for adult females (UL 2,000 mg/day) (United States Department for Agriculture, 2011).

The primary symptom of deficiency is scurvy. Smokers with lower levels of vitamin C have been shown to have a higher risk for lung-borne infections (Sargeant et al, 2000). Side effects from large concentrations include nausea, vomiting, diarrhoea, flushing of the

face, headache, fatigue and disturbed sleep. Vitamin C also enhances iron absorption. Kidney stones are a commonly held side effect of vitamin C, which as yet is unproven in the literature. It displays low toxicity.

Vitamin E

This is included in the ONS as it is an antioxidant.

This is a group of eight fat soluble compounds (α-, β- γ- and δ forms of tocopherols and tocotrienols) acting as antioxidants, stopping reactive oxygen species being formed during fat oxidation (Herrera, 2001).

Deficiency of vitamin E can lead to: Spinocerebellar ataxia, myopathies, peripheral neuropathy, ataxia, skeletal myopathy, retinopathy and impairment of the immune response.

Most studies have concentrated work on α-tocopherol, which can affect other forms of vitamin E. Little work by comparison has been carried out with the tocotrienols, and yet these show more potency as antioxidants and some neuroprotective roles (Lekli, et al., 2008).

The recommended daily intake (United States Department for Agriculture, 2011) for adult males and females is 15mg/day (lactating 19mg/day)(UL 1,000md/day).
Toxicity, up to 1,600 IU daily can be tolerated with minimal side effects acutely (Hathcock et al, 2005). Concern has been raised with long term high concentration supplementation with vitamin E which has been reported to lead to: Increased mortality, congestive heart failure, coagulopathies, impaired immunity and constitutional and gastrointestinal effects (Miller et al, 2005; Bjelakovic et al, 2007).

This vitamin can act as an anticlotting agent, with its effect as an anticoagulant being described as very modest in the α-tocopherol form and potent in the quinone form (Dowd & Zheng, 1995; Cox et al, 1980). This warranted screening out those participants who were already on anti-platelet and anticoagulant medication, as the vitamin E would enhance their effect, potentially to the detriment of the participant.

One group of authors has also suggested that there may be a rise in AMD risk with vitamin E supplementation (Olson et al, 2011).

Zinc

Inclusion of Zinc in an ONS is because zinc is believed to interact with taurine and vitamin A (enhancing its absorption), modify photoreceptor plasma membranes, regulate the light-rhodopsin reaction, modulate synaptic transmission and serve as an antioxidant (Grahn et al, 2001).

Zinc is not stored within the body so a steady intake in our diet is beneficial (Rink & Gabriel, 2000). It is utilized in cellular metabolism: catalyst with enzymes, immune function, protein synthesis, wound healing, DNA synthesis and cell division.
Recommended daily intake for adult males is 11mg/day (UL 40mg/day) and for female adults 8 mg/day (UL 40mg/day) (pregnant 11mg/day and lactating 12mg/day) (United States Department for Agriculture, 2011).

Deficiency of Zinc is characterised by growth retardation, loss of appetite and impaired immune function. In more severe cases hair loss, diarrhoea, delayed sexual maturation, impotence, hypo-gonadism in males, eye and skin lesions, weight loss, delayed wound healing, taste abnormalities and mental lethargy.
Studies have found that zinc, used in combination with antioxidants, does show reduced risk of advancement of advanced macular degeneration, but not for early forms of the disease (AREDS Research Group, 2001).

Toxicity in its acute phase can yield nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea and headaches. Chronic effects include low copper status, altered iron function, reduced immune function and reduced levels of high-density lipoproteins (Hooper et al, 1980). Copper reduction has been noted with zinc intake levels of 60mg/day for up to 10 weeks hence the inclusion of copper in the formulation. Zinc also interacts with quinolone antibiotics reducing absorption, (Lomaestro & Bailie, 1995) penacillamine (Brewer et al, 1993) and thiazide diuretics (increased zinc excretion) (Wester, 1980).

Vitamin B2

Vitamin B2 is included in ONS as it is an antioxidant and supports cellular energy production. It is water-soluble and is dispersed throughout the body. Excess amounts are excreted in urine.

Recommended daily intake for adult male is 1.3 mg/day and female 1.1 mg/day with no upper limits described (United States Department for Agriculture, 2011).

Deficiency causes ariboflavinosis, which includes cheilosis, sunlight hypersensitivity, angular cheilitis, glossitis, seborrhoeic dermatitis, pharyngitis, hyperaemia and oedema of the pharyngeal and oral mucosa. There are no studies pointing to in vivo toxicity with vitamin B2

Vitamin B3

Vitamin B3 is included in ONS as it supports cellular energy production, helps the body with fat and protein usage and assists in maintaining good circulation within the vascular system.

Also a water-soluble vitamin with similar dispersal and excretion patterns.
Recommended daily intake for adult males is 16mg/day and for females 14 mg/day (UL 35 mg/day) (United States Department for Agriculture, 2011).
Deficiency, when combined with tryptophan deficiency, leads to pellagra, including aggression, dermatitis, insomnia, weakness, mental confusion and diarrhoea.
Toxicity can lead to nausea, vomiting, liver toxicity, glucose intolerance, vaso-dilation and yellowing of the eyes and skin.

Selenium, copper and manganese

Selenium is included in the ONS as it facilitates vitamin A and E absorption and enhances antioxidant activity. Copper is depleted when Zinc levels increase and as an essential micronutrient inclusion in the ONS ensures it remains available in viable quantities. Manganese is required as a co-factor for many proteins and in antioxidant activity. It also supports the absorption of vitamin B.

Safety

Nutritional supplements are not without their potential side effects as described above. This has been reviewed in some detail by the Expert Group on Vitamins and Minerals (EGVM) in 2003, which set out safe upper limits for eight supplements and guidance on 22 others. The recognition that human studies into vitamins and minerals, especially in the most vulnerable groups (children and older people) by this group has added impetus to the work underway by EURRECA, attempting to harmonise advice across Europe.

Most of the current advice recognises that the supplements are at greatest risk of side effects if taken in high concentrations and for long periods of time. Abdominal pain and diarrhoea are reported (vitamin C >1,000mg/day and calcium>1,500mg/day) along with loss of feeling in the arms and legs (vitamin B6 >10mg/day). Some may have irreversible

harmful effects if taken in these high concentrations over a long period of time (β-carotene, vitamin B3, zinc, manganese and phosphorus).

Bioavailability will play a role in the relative levels of concentrations that have the side effects and how rapidly these begin.

Ocular nutritional supplements have been specifically reviewed (Bartlett & Eperjesi, 2005) with the main conclusions avoid vitamin A when pregnant, with liver disease and high alcohol intake; this vitamin also being related to low bone mineral density. β-carotene carries an increased risk of lung cancer in smokers, whilst vitamin E and Gingko Bilboa display anticoagulant and antiplatelet effects, thus detrimental to those with vascular disorders.

Lutein has not been found to have any side effects at a level of 26mg per day (le Marchand, et al., 1995), well above the levels utilized in this study.

Advice should be provided by any practitioner offering supplements, and an awareness of these potential side effects should be created. Re-direction to their medical practitioner may be wise in some cases.

Summary

This section covered the micronutrients included within the ocular supplements chosen for this study (ICaps and Vitalux plus). For each micronutrient information has been provided about how it interacts within the human body with especial interest in how it affects the eye. Information on RDA and safety levels has been provided when it is available. In the next section the methodology of experiment 1 is set out allowing for an *a priori* power calculation.

Further results

The recruited population was study 1 n=14 and study 2 n=42. The two study population figures are compared with the Sheffield population figures in Table B1.1. The average population age was 56 years.

		Percentage of populations		
		Study 1	Study 2	Sheffield
Gender[1]	Male	42.8	64.0	50.2
	Female	57.2	36.0	49.8
Smoking status[2]	Smokers	7.1	5.0	22.8
	Non-smokers	92.9	95.0	77.2
Age[3]	16-19	0.0	0.0	7.0
	20-29	0.0	0.0	19.7
	30-39	0.0	3.0	12.8
	40-49	21.4	28.0	13.6
	50-59	28.6	34.0	10.7
	60+	50.0	35.0	20.6

Table B1.1. Study population figures compared with Sheffield city population figures. 1 (Office for National Statistics, 2010), 2 (Central P B C consortium, 2010) 3 (Sheffield City Council, 2011)

Dietary supplement study in this clinic

We are looking for volunteers to take part in our study to investigate the effect of dietary supplements on the eye, specifically the level of pigment in the retina.

You do not have to have had any problems with your eyes to take part in this study.

If you would like to participate or find out more information about this interesting research then please ask a member of staff or alternatively contact:

Gilbert Vasey BSc MSc MCOptom FCA MIoD
Specsavers Opticians, Crystal Peaks
0114 251 3111

Advertising study to potential participants

Volunteers sought for dietary supplement research

We are looking for volunteers for a research project on the effect of dietary supplements on the eye. The project involves a simple measurement at the start of the research, taking commercially available dietary supplements for 4 months and then a final measurement at the end of the 4 months. If you are interested in volunteering then please let any of our team know and they will be able to provide you with further details.

Researchers Lead Gilbert Vasey, Administration Jennifer McGrath, Measurements Carly Brookes.

Consent form

Research workers and role responsible

Mr Gilbert Vasey, Director/Optometrist, researcher

Miss Jennifer Denny, Manager/ Dispensing Optician, randomisation and database

Miss Carly Brookes, Exam Lane Assistant, Measurements

Project Title

The effects of dietary supplements containing differing concentrations of lutein on the macular pigment optical density

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

The purpose of the study is to determine the effect of different amounts of lutein in dietary supplements on macular pigment levels after a four month period. Higher levels of macular pigment have been shown to reduce the risk of developing age-related macular degeneration, a condition which leads to distortion of central vision and can eventually lead to a complete loss of central vision. Two groups of participants will be randomly selected from volunteers and given one of two ocular dietary supplements (similar to vitamin tablets) that are commercially available. The goal of the study is to assist clinicians in deciding upon which ocular dietary supplements to recommend to patients.

What are the benefits of the study?

The benefits of the study are:

Personal: You will be provided with an ocular dietary supplement without charge. These supplements have been shown to have no adverse effects.

Science: The knowledge bank of science will be enhanced by further understanding the uptake of active ingredients in ocular dietary supplements and their proportional effects upon the eye.

Why have I been chosen?

You have been chosen because you are free from any apparent disease at the centre of the back of your eye (macular disorder), are capable of providing informed consent and will be available for the duration of the project.

What will happen to me if I take part?

By volunteering to participate you will be giving anybody in the research team named above, consent to analyse your results.

You will need to have a measurement of macular pigment level at the start of the study. This involves attending the practice and having the measurement taken. This is carried out with a device known as a MacuScope. The device shows you a blue light which appears to flicker. As the technician adjusts the intensity of the light the flicker will slow and then stop. This measurement is taken twice for each eye and provides us with an immediate reading which we can share with you. This tells us if you have high, average or low macular pigment optical density.

The ocular dietary supplement plan can then be started, consisting of one or two tablets daily for four months. At the end of this period you will need to re-attend the practice and have a second reading using the MacuScope taken.

You will need to maintain a food diary. Once for a week at the start of the study and again for a week at the end of the study.

You will not be required to carry out any further tasks.

The ocular dietary supplement you will be provided with is selected for you on a randomised basis, allowing for age, gender and smoking status. It will be a low or high lutein supplement.

There have been no reported adverse effects from taking these supplements. If however you feel that you are suffering an adverse effect then please cease taking the supplements immediately and contact the practice on 0114 251 3111 or in person for further advice.

The anticipated effect of the two different doses is that they will have similar effects upon your macular pigment levels.

Are there any potential risks in talking part in the study?

The ocular dietary supplements we are using in this study are widely available and used by many people every day. The risk of intolerance to these supplements is very small. The instrument used to measure the macular pigment level is in regular use within our and other practices and has CE certification. There have been no recorded adverse events from its use.

Do I have to take part?

No, you do not have to participate if you do not wish to do so. You are free to withdraw at any time from the project. No sanctions will be taken against anyone who refuses to participate in or withdraws from this project.

Expenses and payments:

There are no expenses or payments for participation in this project.

Will my taking part in this study be kept confidential?

Yes, your participation in the study will be fully confidential. There will be no way to link any research data to any individual participant. The results will be analysed and put through statistical tests for significance. The results will be used to guide future recommendations to clinicians.

Data will be stored initially in electronic format on a standalone computer which is kept within a locked room with limited access. After the study the data will be printed off, and then deleted from the computer. The hard copy will be stored in line with Aston University rules for research studies and eventually disposed of by shredding.

Privacy and confidentiality will be protected to the extent permissible by law. We cannot, however, guarantee privacy or confidentiality. None of the identifiable data will be forwarded or used for third party related activity. (After the study no mailing or telephone calls to you will occur due to participation in this study.)

What will happen to the results of the research study?

We aim to publish the results of this project in academic peer reviewed journals and also to disseminate our findings at research conferences. If you would like to know our findings please leave a contact email or address at the end of the consent form. There will not be any reference to an individual's performance in any publication or presentation.

Who is organising and funding the research ?

The project is being organized by Mr Gilbert Vasey. There is no funding for this research project.

Who has reviewed the study?

The research has been submitted for approval by Aston University's Ethics Committee.

Who do I contact if something goes wrong or I need further information

Please feel free to contact Mr G T Vasey (gtvasey@btinternet.com 0114 251 3111)

Who do I contact if I wish to make a complaint about the way in which the research is conducted

If you have any concerns about the way in which the study has been conducted, then you should contact Secretary of the University Research Ethics Committee on j.g.walter@aston.ac.uk or telephone 0121 204 4665.

VOLUNTEER CONSENT FORM

Title of Project: The effects of dietary supplements containing differing concentrations of lutein on macular pigment optical density

Name of Chief Researcher: Mr Gilbert Vasey

		Tick Box
1	I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
3	I agree to take part in the above study.	

Please provide you full name, signature and date the form below

Mr/Mrs/Ms/Miss_____Signature_____

__

Date _____/_____/_____

Would you like to know our findings? Yes No

For the purposes of randomisation please provide the following data:
Please enter how old you are currently
Age _____ years

Please circle the appropriate answer for gender and if you smoke, at all, currently

Gender Male Female

Do you smoke Yes No

Once you have been randomised into a study group you will be contacted by the research administrator who will ask you to attend the practice to have a measurement taken and to be provided with your dietary supplements and your food diary. After four months we will contact you again asking you to attend for a final reading and submission of your food diary. You will not be informed of which ocular dietary supplement dose group you are part of during the study. This helps prevent bias of the results by accident. So that we can contact you please provide your contact details below:

Number and Street_____

Area _____

City/Town_____

County_____

_

Postcode_____

_

Telephone_(_____)_____

_

E-mail_____

DD/MM/YYYY

Dear

Lutein dose study

Thank you for returning your consent form.

As part of our randomisation process we have built in a matching process to reduce other confounding causes of changes to uptake of the dietary supplements. For this purpose I have placed you in a block of participants who have similar characteristics for gender, smoking status and age.

Once I have found someone with similar characteristics I can enter you both in the study. Please do bear with me during this matching process.

As soon as I have a match I will be in contact with you.
Once again thank you for your interest in taking part in this study.

Yours sincerely

Jennifer McGrath
Research administrator

DD/MM/YYYY

Dear

Lutein dose study

Our randomisation process has now allowed me to enter you into the study.

Would you please contact the practice on 0114 251 3111 or attend in person to make an appointment for the following:

1. Macula protective pigment density measurement using the MacuScope
2. Receive your food diary
3. Receive your supplements for the four month period.

When booking the appointment please state that this is for “the research study on macular pigment”.

Once again thank you for your interest in taking part in this study.

Yours sincerely

Jennifer McGrath
Research administrator

Instructions on how to fill in your food diary

Please fill out this food diary every day for one week. Every time you eat or drink something write it down in the diary provided under the correct day.

Try and describe the food as accurately as possible:

For example:
One small or large bowl of cornflakes with skimmed milk
Two slices of toast thinly or thickly spread with butter
Wholemeal, white or brown bread
Skimmed or semi-skimmed milk
Large, medium or small banana

Try to give rough estimates of the food and drink consumed:

For example:
One small cup of tea or one large cup of coffee
Two or three chocolate biscuits
Two or three tablespoons of baked beans

Try to be as accurate as possible (it would be great if you could include weights!).

Remember to include all foods and drinks consumed at home and at other places such as restaurants and friend's houses etc.

Try to fill in the diary as you eat, instead of leaving it till the end of the day. This ensures that you won't forget what you have eaten.

Please also make a note of any vitamins and other supplements that you may have taken giving details of dose and supplier where available (other than the supplements we have provided you with).

Repeated for days three through to seven.

Day 1	
Breakfast:	Supper:
Lunch:	Snacks:

Day 2	
Breakfast:	Supper:
Lunch:	Snacks:

DD/MM/YYYY

Dear

Lutein dose study

Thank you for attending for the initial measurement of macula pigment density.

By now you should have begun taking you supplements and have hopefully completed you food diary. If you haven't yet completed it could I encourage you to do so as it is an important check in the study.

If you have any queries during the four month trial period would you please contact me here at the practice.

Yours sincerely

Jennifer McGrath
Research administrator

DD/MM/YYYY

Dear

Lutein dose study

It is now approaching four months since you had the original reading taken for the macular pigment. As part of the protocol of the study we now need to take a second and final reading.

Would you please contact the practice on 0114 251 3111 or attend in person to make an appointment for the following:

1. Macula protective pigment density measurement using the MacuScope
2. Receive information on age related macular degeneration

When booking the appointment please state that this is for “the research study on macular pigment”.

Can I take this opportunity to remind you that you will also need to complete the final week food diary before attending the appointment. I enclose this diary herewith.
When you attend the appointment please bring with you the two food diaries (initial week and final week).

Once again thank you for taking part in this study.

Yours sincerely

Jennifer McGrath
Research administrator

Ethics approval documentation

Aston University
Life & Health Sciences

Response from AOREC

Project title: ***The effects of dietary supplements containing differing concentrations of lutein on the macular pigment optical density***

Reference Number: Vasey OD **Researchers:** Gil Vasey and Frank Eperjesi

I am pleased to inform you that the Audiology / Optometry Research Ethics Committee has approved the above named project.

The details of the investigation will be placed on file. You should notify The Committee of any difficulties experienced by the volunteer subjects, and any significant changes which may be planned for this project in the future.

Yours sincerely

AOREC

20th November 2009