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THE EFFECT OF NUTRITIONAL SUPPLEMENTATION ON VISUAL FUNCTION

HANNAH ELIZABETH BARTLETT

Doctor of Philosophy

ASTON UNIVERSITY

9th June 2005

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Age-related macular disease (ARMD) is the leading cause of irreversible visual loss in the developed world. Information about risk factors for ARMD is limited and there are no treatment options for most people.

The oxidative stress hypothesis for development of ARMD has prompted interest in the role of antioxidant supplementation in the prevention of onset or progression of the condition. The human body has several defence mechanisms against oxidative damage, including antioxidant enzymes and antioxidant nutrients.

The role of nutritional supplementation in ocular health is of interest to eye care practitioners and ARMD patients. Practitioners have expressed a need for clearer guidance regarding the recommendation of supplements to their patients.

Randomised controlled trials (RCTs) are considered to be the gold standard when investigating the effect of an intervention. They involve random assignment of participants into treatment and placebo groups. The advantage of trials of this type is the ability to reduce the influence of confounding variables by random assignment of the treatment (intervention), and the ability to reduce bias or the possibility that any observed effect is due to other factors.

The aim of this research was to determine the effect of a lutein-based nutritional supplement on measures of visual function in normal and ARMD-affected eyes.

Thirty participants were recruited to the ARMD cohort (aged between 55 and 82 years, mean \pm SD: 69.2 \pm 7.8) and 46 were recruited into the normal cohort (aged between 22 and 73 years, mean \pm SD: 50.0 \pm 15.9). Outcome measures were distance (DVA) and near (NVA) visual acuity, contrast sensitivity (CS), photostress recovery time measured with the Eger Macular Stressometer (EMS), central visual function assessed with the Macular Mapping test (MMT), and fundus photography. Reliability studies were carried out for the EMS and the MMT. A change of 14 s is required to indicate a clinically significant change in EMS time, and a change of 14 MMT points is required to indicate a clinically significant change in MMT score.

Sample sizes were sufficient for the trial to have 80 % power to detect a significant clinical effect at the 5 % significance level for all outcome measures in the normal cohort, and for CS in the ARMD cohort. The study demonstrated that a nutritional supplement containing 6mg lutein, 750 μ g vitamin A, 250 mg vitamin C, 34 mg vitamin E, 10 mg zinc, and 0.5 mg copper had no effect on the outcome measures over nine or 18 months in normal or ARMD affected participants.

The finding that nine months of antioxidant supplementation, in this case, has no significant effect on CS in ARMD-affected participants adds to the literature, and contrasts with previous RCTs, the AREDS and the LAST. This project has added to the debate about the use of nutritional supplementation prior to the onset of ARMD.

When asked for nutritional advice with respect to ARMD, practitioners should be guided by the results of RCTs. Patients should be advised to consult their GP before commencing a supplementation regime if they are taking prescribed medication. As optometrists, our aim should be to advise patients of the risk factors for ARMD, and to promote the importance of maintaining a healthy, well-balanced diet.

Keywords: age-related macular disease, randomised controlled trial, antioxidant, lutein.

To mum and dad

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Chapter 1: Background

1.1 Definition of age-related macular disease

Age-related macular disease is a degenerative disorder of the central area of the retina (the macula) often associated with visual impairment. It is more frequent over the age of 50 years. The macula is the part of the retina, centred on the foveola in which the ganglion cell layer is more than one cell in thickness. It has an approximate diameter of 5.5 mm^[1]. The inner macula is defined as the area within a circle, centred on the foveola, of diameter 3000 μm . The outer macular is defined as an area between the inner macular and a circle with a diameter of 6000 μm ^[2].

1.2 Classification of age-related macular disease

Classification of age-related macular disease is required for studies investigating the condition in order to make reliable comparisons between findings. In an attempt to standardise the terminology, the International Classification and Grading System for Age-Related Maculopathy (ARM), and Age-Related Macular Degeneration (AMD) has been developed^[2].

ARM is most often clinically apparent over the age of 50 years and is characterised by:

- Discrete white/yellow spots identified as 'drusen', which are external to the neuroretina or retinal pigment epithelium (RPE). They may be soft and distinct, or soft and indistinct. Hard drusen do not characterise ARM.
- Areas of hyperpigmentation (in the outer retina or choroid) associated with drusen.
- Areas of hypopigmentation of the RPE, without any visibility of the choroidal vessels, associated with drusen.

AMD is the late stage of ARM and includes both 'wet' (also called neovascular) and 'dry' (also called geographic atrophy [GA]) forms.

GA is characterised by any sharply delineated roughly round or oval area of hypopigmentation or apparent absence of the RPE in which choroidal vessels are more visible than in surrounding areas. It must be at least 175 μm in diameter.

Neovascular AMD (also known as 'disciform AMD' or 'exudative AMD') is characterised by any of the following:

- RPE detachment(s), which may be associated with neurosensory retinal detachment, associated with other forms of ARM
- Sub-retinal or sub-RPE neovascular membranes
- Epiretinal, intraretinal, subretinal, or sub-pigment epithelial scar/glial tissue or fibrin-like deposits

- Subretinal haemorrhages not associated with other retinal vascular disease
- Hard exudates (lipids) within the macular area, not related to any other retinal vascular disease.

The term age-related macular disease (ARMD) will be used throughout this thesis, where appropriate, to encompass ARM and AMD.

1.3 Prevalence of ARMD

ARMD is the leading cause of irreversible visual loss in the developed world ^[3-8]. In the UK, half of those registered as blind or partially sighted every year (approximately 30,000) have the condition ^[9]. This pattern in the registered population is reflected in the rest of Europe, North America and Australia ^[4, 10-15].

There are estimated to be nearly one million visually impaired people in Britain, 90% of whom are over 65 years of age ^[16]. ARMD is the most frequently occurring condition that results in permanent vision loss in this age group ^[5, 17]. The proportion of blindness attributable to the disease is expected to increase during the 21st century as a result of the aging population ^[16, 18]. People over the age of 65 represent the fastest growing segment of the US population, and between 2000 and 2020 this group is expected to increase by 53%. This age group will then make up 16.5% of the entire US population ^[19]. It has been more recently predicted that 2.95 million people in the United States will have AMD in 2020 ^[15].

Information about risk factors for ARMD is limited and there are no treatment options for most people. Treatments, such as laser photocoagulation ^[20, 21] and photodynamic therapy can delay the progression of visual loss in the small proportion with neovascular AMD ^[22], but are unlikely to significantly reduce related blind and partial sight registrations.

1.4 Risk factors for ARMD

A detailed review of the risk factors associated with ARMD can be found in the appendix ^[23] (p. 94). The main factor in the development of this condition is age ^[4-8, 24-27], but smoking ^[28-36] and genetic predisposition ^[34, 37-44] have also been consistently linked with its development. Proposed modifiable risk factors are shown in table 1.1 and proposed non-modifiable risk factors are shown in table 1.2. ARMD is a multi-factorial condition, and it is likely that in those who are genetically predisposed, the condition manifests itself according to exposure to other risk factors.

Table 1.1: A summary of studies investigating the relationship between modifiable risk factors and ARMD.

Modifiable risk factor	Evidence	
	Positive association	No association reported
Smoking	[28-36]	[25, 45, 46]
Alcohol	[47, 48]	[33, 49-52]
Socioeconomic factors	[5, 33, 53]	[25, 34, 54]
Nutrition	[33, 55-59]	[60-63]
Body mass index	[45, 53, 64, 65]	
Dietary fat intake	[33, 66]	[5]
Cardiovascular disease	[5, 34, 64, 67, 68]	[33, 65]
Hypertension	[64, 69, 70]	[45, 64, 67, 68]
Statins	[71, 72]	[73, 74]
Aspirin	[75]	[45, 75]
Type II diabetes	[76]	[25, 34, 49, 50]
Sunlight exposure	[77-79]	[33, 34, 45, 46]
Parity (more than one child)	[80]	

Table 1.2: A summary of the studies investigating the relationship between non-modifiable risk factors and ARMD.

Non-modifiable risk factor	Evidence	
	Positive association	No association reported
Increasing age	[4-8, 24-27]	
Genetic predisposition	[34, 37-44]	
Female gender	[25, 81, 82]	[5, 27, 45, 83]
White ethnicity	[53, 83-85]	[5]
Blue iris colour	[86]	[33, 46, 87, 88]
Type I diabetes	[76]	
Hyperopic refractive error	[5, 33, 34, 50, 89, 90]	
Cataract	[46, 91]	
Cataract surgery	[5, 45, 92, 93]	[94]
Weak handgrip strength	[25, 34]	
Optic disc appearance		[95]
Increased birth weight	[96]	
Early menopause (following removal of ovaries)	[97]	

1.5 Grading of ARMD

Image analysis, change detection, and image comparison are essential for monitoring the progress of disease. Photographs are commonly used as an outcome measure in ocular clinical trials, and so it is important to be able to quantify features for comparison. This is particularly relevant in the development of treatment and prevention strategies via controlled trials. Many studies have employed the use of grading scales or photographic standards for comparison by observers for qualitative or semi-quantitative assessment of fundus photographs^[98-102]. The three main ARMD grading scales are the International Classification and Grading System^[2] the Wisconsin Grading System^[102], and the Age-Related Eye Disease ARMD classification system^[103]. These are briefly described below.

The International Classification and Grading System for Age-related Maculopathy and Age-related Macular Degeneration^[2].

Three circles are centred on the foveola, of diameters corresponding to 1000 μm , 3000 μm , and 6000 μm . Lesions are graded within each of the central, inner, or outer circles. A further five open circles printed on clear plastic can be used to estimate the size of drusen, area involved by drusen, and area involved by increased or decreased pigmentation, or by AMD. The diameters of these circles correspond to 63 μm (C_0), 125 μm (C_1), 175 μm (C_2), 250 μm (C_3), and 500 μm (C_4). The photographs are viewed on a fluorescent viewing box furnishing light with a Kelvin rating of 6200 °. The photographs are viewed with a total magnification of x 15.

The predominant drusen type is the most common type of drusen present within the outer circle. Large drusen ($> 63 \mu\text{m}$) are counted separately, but small drusen are not. The area covered by the drusen can be estimated within each of the three circles in the grid and expressed as a percentage of the area within the specific subfields defined by the grid.

GA is graded according to presence, location, and area covered by it. The minimum area involvement by GA is that of the circle corresponding to 175 μm diameter, because it is difficult to detect choroidal vessels and determine the edges of the GA in smaller areas.

Neovascular AMD may be made up of non-rhegmatogenous retinal detachments or serous RPE detachments. These may be difficult to distinguish from each other and both are graded as neovascular AMD. They are graded according to presence, location, and area covered by the lesion.

The Wisconsin Age-related Maculopathy Grading System^[102]

A grid of three circles and four radial lines, concentric with the centre of the macula, is placed over a photograph. The photographs are mounted on clear plastic sheets, and placed on a fluorescent viewing box using light with a Kelvin rating of approximately 6200°. This wavelength was chosen because light with a lower Kelvin rating has a more yellow hue, and therefore is

less likely to allow identification of subtle drusen. The slides are examined stereoscopically with a total of x 15 magnification.

The circles have radii corresponding to 500, 1500, and 3000 μm on the fundus. The grid defines nine subsections of fundus; some characteristics are graded over the whole area, and some according to subsection. Three sets of open circles printed on clear plastic are used to estimate the size of drusen, area involved by drusen, and areas of pigmentation.

The grading system is divided into three areas; characteristics of drusen, other lesion typical of AMD, and other abnormalities.

The AREDS ARMD classification system

The Wisconsin and International classification and grading systems are similar and are designed for use with stereoscopic analogue photographs viewed using a fluorescent light box. The AREDS was a randomised controlled trial (RCT) designed to evaluate the effect of a high-dose vitamin C, E, beta-carotene, and zinc supplement on the progression of cataract^[104] and ARMD^[58]. The main ARMD outcomes in this study were the development of GA that involves the centre of the macula, or the development of the neovascular form of the disease. The AREDS investigators defined four categories of ARMD, into which participants were allocated at baseline (see table 1.3).

Table 1.3: The AREDS ARMD categories.

	First eye ^a			Second eye
AMD category	Drusen size ^b	Drusen area ^b	Pigment abnormalities ^c	
1	None or small (< 63 μm)	< 125 μm diameter circle (\approx 5 – 15 small drusen)	None	Same as first
2	Small (< 63 μm) or Intermediate (≥ 63 , > 125 μm) None required if pigment abnormalities present	≥ 125 μm diameter circle At least one druse	Absent or present, but GA absent	Same as first or category 1
3a	Intermediate (≥ 63 , < 125 μm) or Large (≥ 125 μm) None required if non-central GA present	≥ 360 μm diameter circle if soft indistinct drusen are present (≈ 20 intermediate drusen. ≥ 656 μm diameter circle if soft indistinct drusen are absent. At least one druse	Absent or present but GA absent	Same as first or category 1 or 2
3b	First eye same category as 3a			Visual acuity (VA) < 20/32 due to AMD; or unocular disqualifying disorder present
4a	First eye same category as 1, 2, or 3a			Advanced AMD ^d
4b	First eye same category as 1, 2, or 3a			VA < 20/32 due to AMD, but advanced AMD not present

^a Must have VA $\geq 20/32$, no advanced AMD, and no disqualifying lesion.

^b Drusen and GA are assessed within two disc diameters of the centre of the macula.

^c Pigment abnormalities within one disc diameter of the centre of the macula.

^d GA involving centre of macula or signs of choroidal neovascularisation (presence beneath the RPE or sensory retina of fluid, blood, or fibrovascular or fibrous tissue).

Use of digital images in grading

The advantages of non-mydratic digital cameras compared to film-based cameras are lower cost, ease of use, good resolution, ability to manipulate the image, provision of immediate feedback regarding the quality of the image for the photographer and the presence of abnormalities for the patient, and lack of dilation. Disadvantages include lack of stereopsis, relative decrease in colour contrast compared to film images in eyes with very red fundi, and a higher frequency of ungradable photographs. Several studies have compared digital

photographs and stereoscopic digital photographs with regard to their use in grading of abnormalities.

Digital stereo images were compared with 35-mm colour transparencies with regard to the quality and reliability of grading age-related macular disease in the context of a multicenter European epidemiologic study (the EUREYE Study) ^[105]. The International Classification and Grading System ^[2] was used to grade 137 digital images and 35-mm slides. The agreement in grading scores between imaging scores was expressed in absolute percentages and was calculated using the weighted kappa (κ) statistic. Interpretation of the κ statistic employed the following categories ^[106]: < 0.20, poor; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, good; > 0.81, very good agreement.

The weighted κ value for between-technique agreement ranged from 0.41 for number of drusen < 63 μm to 0.79 for drusen type and total area occupied by drusen. The κ values for atrophic and neovascular end-stage ARMD were 0.87 and 0.94 respectively. The between-technique agreement on stages of ARMD was approximately 0.76. The agreement between graders was similar for both techniques of imaging. The investigators concluded that digital imaging is reliable for the purpose of grading ARMD using this grading system in epidemiological studies.

A more recent study compared 35-mm stereoscopic slide transparencies with digitised non-stereoscopic images for grading abnormalities in ARM and AMD ^[107]. This group also used the International Classification and Grading System ^[2]. For small hard and intermediate soft drusen, κ values ranged between 0.56 and 0.72, and for the three macular subfields they ranged between 0.31 and 0.64. The κ values ranged from 0.00 to 0.27 for the presence of hyperpigmentation, from 0.80 to 0.82 for the presence of GA, and from 0.81 to 0.88 for the presence of choroidal neovascularisation (CNV). The κ value was 0.78 for the area covered by GA, and 0.83 for the area covered by CNV. The group concluded that digitised non-stereoscopic colour images are useful for grading ARM and AMD.

In 2004, Klein *et al.*, found that gradings resulting from high resolution digital images were comparable with those resulting from film-based images ^[108]. The images were taken using a 45 ° digital camera and standard 30 ° fundus camera and graded using the Wisconsin grading system ^[102]. Exact agreement between gradings of digital and stereoscopic film images taken through pharmacologically dilated pupils was 91 % ($\kappa = 0.85$), between digital images taken through dark-adapted pupils and film images taken through pharmacologically dilated pupils was 80 % ($\kappa = 0.69$), and between digital images captured through dark adapted and pharmacologically dilated pupils was 86 % ($\kappa = 0.78$).

The results of these studies support the use of both the Wisconsin grading system and the International Classification system for grading digital images of ARMD affected fundii. The

AREDS ARMD classification system has been used for grading of fundus images as part of a RCT investigating the effect of lutein and lutein combined with other antioxidant nutrients on the progression of AMD [59]. In this trial, the photographs were rated by a single investigator, masked to image date or intervention group.

1.6 Aetiology of ARMD

The exact aetiology of ARMD is not known, but several hypotheses have been proposed:

Age-related deterioration of Bruch's membrane

The conductivity of Bruch's membrane declines with age [109-111], the consequential impedance to diffusion through the membrane compromises metabolic exchange between the choroid and retina [112] and disrupts photoreceptor function [113]. The progressive increase in lipid content of Bruch's membrane throughout life is exaggerated in the macula compared to the periphery [114]. Histological studies of eyes with AMD have identified clinically invisible deposits of amorphous material between the RPE and its basement membrane (basal laminar deposit or drusen [115]) and between the basement membrane of the RPE and the remainder of Bruch's membrane (basal linear deposit) [116-119]. There is evidence that basal laminar deposit is derived from the damaged RPE [120] and that it may interfere with diffusion of solutes between the RPE and choriocapillaris. Basal laminar deposit acts as a substrate for vascular growth [121].

Vascular insufficiency

The age-related decrease in foveal choriocapillaris blood flow is further attenuated in AMD patients [122, 123]. Narrowing of capillary lumen, atrophy of the capillaries, and a loss of cellularity of the capillaries results in a condition of 'zone hypoxia'. Zone hypoxia results in a compromised RPE, and deposition [124]. The normal diffusion of substances and gasses across the RPE-Bruch's membrane complex may also be hindered by a disturbance of the choroidal circulation. This could inhibit the removal of waste materials and disrupt the supply of metabolites and gasses to the neural retina [125]. Deterioration of the RPE may result from this build up of waste products, or from ischaemia [126].

Genetics

The occurrence of AMD in families and populations provides evidence for a genetic basis to the condition. Several studies have shown that there is an increased risk of the disease with a positive family history [34, 127]. A population-based study suggested an overall concordance of 37 % in monozygotic twins compared with 19 % for dizygotic twins for ARM [128]. A link between environment and genetics has been found via a significant association between manganese superoxide dismutase gene polymorphism with AMD [129]. This enzyme has a role in oxidative stress. Genes for other macular dystrophies such as Stargardt's macular dystrophy and Best's vitelliform macular dystrophy have been mapped to specific chromosomes [130-133]. The gene in Stargardt's disease is caused by mutations in the ABCR gene at chromosome 1p21 [134]. It has

been suggested that ABCR is a susceptibility gene for AMD based on the finding that a greater percentage of non-neovascular AMD patients had mutations in the exons of this locus compared to controls ^[135].

Oxidation hypothesis

It is generally thought that oxidative damage is responsible for aging and that this process has an important role in the pathogenesis of ARMD ^[136], as well as diseases such as diabetes, chronic obstructive airway disease, Parkinson's disease, and cervical cancer ^[137, 138]. Oxidation refers to the removal of electrons. Reactive oxygen intermediates (ROI) is a term used to describe free radicals, hydrogen peroxide, or singlet oxygen. Free radicals are molecules that contain one or more unpaired electrons in their outer orbits ^[139]; singlet oxygen and hydrogen peroxide contain their full complement of electrons but in a reactive or unstable state. Aging, irradiation, inflammation, air pollution, cigarette smoke, and raised partial pressure of oxygen are all known to increase the production of ROI ^[140, 141]. Free radicals extract electrons from other molecules in order to achieve stability. These molecules are rendered unstable by this interaction, and a cytotoxic oxidative chain results. Carbohydrates, membrane lipids, proteins, and nucleic acids may all be damaged by ROI.

The outer segments of the photoreceptors contain high concentrations of polyunsaturated fatty acids (PUFAs), which are prone to oxidative damage. This region is also exposed to a relatively high oxygen tension. Peroxidised PUFAs are thought to act as the main precursors to lipofuscin damage ^[142]. Lipofuscin is the name given to a group of autofluorescent lipid/protein aggregates ^[143] that accumulate within the RPE throughout life ^[144].

The first sign of decline in function of the retinal outer layers is the appearance of lipofuscin in the RPE ^[145]. The main substrate for its formation in the RPE is the undegradable end-products resulting from the phagocytosis of photoreceptor outer segments. The lipofuscin formed in the RPE is continuously exposed to visible light (400 – 700 nm) and high oxygen tensions (70 mm Hg). These are ideal conditions for the formation of ROI and therefore damage to cellular proteins and lipid membranes ^[142].

1.7 Role of antioxidant supplementation in ARMD

The oxidative stress hypothesis for development of ARMD has prompted interest in the role of antioxidant supplementation in the prevention of onset or progression of the condition. The body has several defence mechanisms against the production of ROI. The first involves antioxidant enzymes such as catalase and peroxidase ^[146]. Other micronutrients such as selenium, zinc, manganese, and copper facilitate these antioxidant enzymes ^[146, 147]. The second involves antioxidant nutrients such as vitamin E (alpha-tocopherol) ^[148-152], beta-carotene (pro-vitamin A) ^[153] and vitamin C (ascorbate) ^[146, 154-157]. Other antioxidants believed to play a part in maintenance of ocular health include the carotenoids lutein and zeaxanthin ^[158].

Further defence mechanisms include antioxidant compounds such as metallathionein, melanin, and glutathione, and DNA repair. Compartmentalisation is another defence mechanism and this involves the separation of ROI from cellular components that are susceptible to oxidative damage^[146]. Insufficient intake of dietary antioxidant vitamins and minerals can decrease the efficiency of the body's natural antioxidant systems and may allow cellular damage by ROI^[141, 159].

The role of nutritional supplementation in ocular health is of interest to eye care practitioners. Many have expressed a need for clearer guidance regarding the recommendation of supplements to their patients^[160, 161]. The increase in advertising and marketing of nutritional formulations has, in turn, increased awareness of the potential benefits of these supplements within the general population.

1.8 Function of ocular nutritional supplements

Vitamins A, C, and E, the carotenoids beta-carotene and lutein, the trace element zinc, and the herb *Ginkgo biloba*, have all been investigated with regard to their role in prevention of onset or progression of ARMD. Their functions are discussed below, and more comprehensive discussion of nutrients considered to be beneficial for general ocular health can be found in the appendix^[162-164] (p. 111-140).

Vitamin A

Vitamin A is fat-soluble and exists in three different oxidative states: alcohol (retinol), acid (retinoic acid), and aldehyde (retinal). It normally exists within the body as retinol. The functions of vitamin A can be classified into four areas; reproduction of cells, reproductive function, growth and development of embryo and foetus, and vision^[165-169].

Vitamin A has a role in the production of rhodopsin, the photopigment found in rod outer segments. Rhodopsin is composed of a protein called scotopsin, and a carotenoid pigment called 11-cis-retinal. Vitamin A is present in the cytoplasm of the photoreceptors and the retinal pigment epithelium as all-trans-retinol, which is converted to 11-cis-retinal via two enzymatic steps.^[170]

The molecular mechanisms of other retinol functions are not well understood, however, it has a role in development of proteins,^[171] in normal cell membrane function, and mucus secretion.^[172] Antioxidant activity of retinol protects rod cell phospholipids from oxidation.^[173] Vitamin A deficiency is associated with reduced night vision, dry skin, and susceptibility to infection.

Quantification of vitamin A can be confusing, as three different units are used. Micrograms (μg), retinol equivalents (RE), and international units (IU). They can be interchanged as follows; 1RE = $1\mu\text{g}$ retinol, 1 RE = $6\mu\text{g}$ beta-carotene, 1 RE = 3.333 IU vitamin A.

Vitamin C

Vitamin C (ascorbate) is water-soluble and has several biological functions:

1. It is a reducing agent, forming part of the body's antioxidant defences against ROI and free radicals ^[174]
2. As an antioxidant it protects low density lipoprotein (LDL) cholesterol against oxidation, which is thought to be a precursor to heart disease ^[175]
3. It reduces the stiffness of arteries and platelet aggregation, which may protect against heart disease ^[176]
4. Natural antihistamine ^[177]
5. Improves nitric oxide activity, which is required for dilation of blood vessels and is potentially important in lowering blood pressure. ^[178]
6. Reduction of the activity of the enzyme, aldose reductase. This enzyme is responsible for the accumulation of sorbitol in the eyes, kidneys, and nerves of diabetics, which results in diabetes-associated deterioration of these parts of the body ^[179].

Vitamin C deficiency, in severe cases, is called scurvy. Early signs of vitamin C deficiency include bleeding gums, fatigue, and easy bruising.

Vitamin E (alpha-tocopherol)

Alpha-tocopherol is the most effective antioxidant of the vitamin E group, and protects against lipid peroxidation ^[148]. It protects cell membranes and LDL cholesterol, ^[180] and has indirect effects on blood cell regulation, connective tissue growth, inflammation, and genetic control of cell division ^[181]. Units used to quantify vitamin E are IU and α -tocopherol equivalents, where 1mg α -tocopherol equivalents = 1.5 IU.

Beta-carotene (pro-vitamin A)

Beta-carotene, a carotenoid, is fat soluble and has the following functions:

1. Quenches singlet oxygen and prevents the formation of free radicals (note that the natural *cis* form acts as an antioxidant, whereas the synthetic *trans* form exhibits pro-oxidant behaviour) ^[182]
2. Quenches free radicals
3. Enhances some aspects of immune function.

Lutein and zeaxanthin

Lutein and zeaxanthin are the only carotenoids found in human serum that are also found in the retina and macula ^[183-185] prompting them to be termed the macular pigment (MP). Dissection of human retinae has demonstrated that zeaxanthin dominates in the macula centre whereas lutein is more abundant in the medial and peripheral macula ^[186]. This suggests a possible

protective role of lutein for rod photoreceptors ^[187] and of zeaxanthin for the central cone photoreceptors ^[188].

MP may prevent light-initiated oxidative damage to the retina and therefore protect against subsequent age-related deterioration ^[189]. The absorbance spectrum of MP peaks at 460 nm and it is purported to act as a broadband filter, reducing the sensitivity of the macular region to short wavelength light which is most damaging in the 440 to 460 nm range ^[190, 191]. Zeaxanthin is reported to be a superior photo-protector during prolonged light exposure; the shorter time-scale of protective efficacy of lutein has been attributed to oxidative damage of the carotenoid itself ^[192].

MP also acts as a scavenger of ROI. The relatively high concentration of MP in the inner retinal layers ^[193] is very likely to indicate a photoprotective role, while the presence of MP in the photoreceptor outer segments ^[194] is suggestive of a ROI-quenching function. Lutein and zeaxanthin have been found in higher concentration in the rod outer segments of the perifoveal retina than the peripheral retina, again lending support to their proposed protective role in ARMD ^[195].

Zinc

Zinc is an essential component of over 200 enzymes, including antioxidant enzymes such as superoxide dismutases. ^[141] Zinc deficiency has been linked with anorexia nervosa ^[196] and poor wound healing. ^[197, 198] There is limited evidence for a role of zinc in male infertility, ^[199] immune function in the elderly ^[200], and reduction of opportune infection in AIDS patients ^[201].

Ginkgo biloba

Ginkgo biloba is the world's oldest living species of tree, and *Ginkgo biloba* extract (GBE) is one of the most frequently recommended products for cognitive disorders. It has the following functions:

1. Reduction of platelet aggregation and reduction of the development of free radicals ^[202, 203]
2. Increasing vasodilation and reduction of blood viscosity ^[204]
3. Quenching free radicals ^[205, 206]
4. A role in neurotransmitter metabolism ^[207]
5. It acts as an antioxidant ^[208].

1.9 RCTs investigating the role of nutritional supplementation in ARMD

RCTs are considered to be the gold standard when investigating the effect of an intervention ^[209, 210]. They involve random assignment of participants into treatment and placebo groups. The advantage of trials of this type is the ability to reduce, by masking, the influence of confounding variables by random assignment of the treatment (intervention), and the ability to

reduce bias or the possibility that any observed effect is due to other factors. The term 'double-masked' or 'double-blinded' refers to the fact that neither investigator nor participants know who is in the treatment or placebo group. In RCTs designed to investigate the effect of nutritional supplements, this is usually achieved by coding of the tablet containers. At the end of the trial period the code is broken and the gathered data analysed.

In summary, any RCT will involve the following steps ^[211]:

1. Sample selection from the population
2. Baseline variables measured
3. Participants randomised
4. Interventions applied (one will be a placebo)
5. Follow up of the cohort
6. Outcome variables measured
7. Results analysed.

Several RCTs have been carried out to investigate the role of nutritional supplementation in preventing the onset or progression of ARMD. There is a detailed review of these in the appendix ^[212] (p. 141), and a shorter discussion below. For a summary, see table 1.4.

The Age-Related Eye Disease Study (AREDS)

Investigators concluded that dietary supplementation with high doses of vitamin C (500 mg), vitamin E (273 mg), beta-carotene (15 mg), and zinc (80 mg) for an average of 6.3 years significantly reduced risk of progression of AMD in people with extensive intermediate drusen, large drusen, or non-central geographic atrophy (GA) in one or both eyes, or VA <20/32 attributable to AMD in one eye in those taking a combination of antioxidants plus zinc, and a suggestive reduction in risk for those taking zinc alone ^[58].

Alpha-tocopherol, beta-carotene study (ATBC)

This trial was originally designed to investigate the role of beta-carotene (BC) and vitamin E (alpha-tocopherol, AT) in the prevention of lung cancer in over 29,000 smoking males. At the end of the trial an ophthalmological examination carried out on a random sample of 941 male participants aged 65 years or over, determined that long-term supplementation of AT or BC does not affect the prevalence of ARM in smoking males ^[62].

Vitamin E, cataract, and age-related maculopathy trial (VECAT)

The trial concluded that daily supplementation with vitamin E (d- α -tocopherol, 500 IU) does not prevent development or progression of ARM ^[213].

Zinc in ARMD

Investigators concluded that with two years of daily 200 mg zinc sulphate supplementation the decrease in mean visual acuity in the zinc treated group was less than that of the placebo group [214]. In other words, zinc reduced progression of AMD.

One way of comparing the reliability of RCTs is to calculate the ability of the trial to detect a difference between treatment means. An approximate formula for this calculation is:

$$R = 2C\sqrt{2}\sqrt{f}$$

Where 'R' is the percentage difference detectable in an experiment, 'C' coefficient of variation, and 'f' is the number of participants in each group. Calculation of the R value for this trial shows that it was unlikely to detect any difference between treatments smaller than 72% and that the results should be treated with caution [212]. The investigators suggested that potential sources of bias include the use of subjects from a relatively small geographical area, and high soil and water mineral contents in this area.

Zinc and the second eye in age-related macular disease

Investigators concluded that 200 mg daily zinc sulphate supplementation had no short-term effect on the course of AMD in patients with an exudative form of the disease in one eye [61]. The calculated R value suggests that the trial was likely to be able to detect a treatment effect greater than 16%, which is a much greater degree of precision than the 'Zinc in ARMD' study.

Visaline® in age-related macular disease

Results showed no significant effect on measured parameters between the intervention (1.5 mg buphenine HCl, 10 mg beta-carotene, 10 mg tocopherol acetate, and 50 mg ascorbic acid) and placebo groups in patients with non-exudative AMD. The fact that no treatment effect was determined is unsurprising considering the small sample size and the calculated R value for the study of 89% [212].

The Lutein Antioxidant Supplementation Trial (LAST)

The Lutein and Antioxidant Supplement Trial (LAST) was a 12-month RCT designed to evaluate the effect of lutein alone or lutein combined with additional carotenoids and antioxidants/minerals on MP optical density and objective visual outcome measures in 90 subjects with ARMD. Glare recovery and contrast sensitivity significantly improved with both interventions, although it is worth noting that 95.6 % of the study population was male [59].

Ginkgo biloba in AMD

One RCT found an improvement in VA in AMD patients who supplemented with GBE [215], although it has been suggested that these results should be treated with caution [216].

Table 1.4: Summary of nutrition in ARMD RCT results.

RCT (publication year)	Overall result	Nutrients included	Amount (mg)
AREDS (2001)	Positive for AMD	Vitamin C	500
		Vitamin E	273
		Beta-carotene	15
		Zinc	80
		Copper	2
ATBC (1998)	No effect	Vitamin E	50
		Beta-carotene	20
VECAT (2002)	No effect	Vitamin E	335
Zinc in ARMD (1988)	Positive effect (treat with caution ^[212])	Zinc	200
Zinc and the second eye in AMD (1996)	No effect	Zinc	200
Visaline [®] (1995)	No effect	Vitamin C	200
		Vitamin E	40
		Beta-carotene	40
		Buphenine*	1.5
LAST (2004)	Positive effect	Lutein	10
		Antioxidants (for full list see paper ^[59])	
<i>Ginkgo biloba</i> in AMD (1986)	Positive effect (treat with caution ^[216])	GBE	160
* Buphenine is a beta-adrenergic stimulant. It increases blood flow, mainly by acting directly on the arteries and arterioles of skeletal muscle.			

1.10 Epidemiological studies investigating the effect of nutrition on ARMD

Vitamin A

Subjects who consumed foods rich in vitamin A at least once per day had a 40% reduced risk for AMD than those who ate the foods less than once per week [odds ratio (OR), 0.59; CI, 0.37-0.99] ^[5]. However, this protective effect has not been demonstrated by other studies ^[49, 217, 218].

Vitamin C

A non-significant protective effect of high oral vitamin C intake against ARMD has been reported ^[218]; similarly, the Baltimore Longitudinal Study of Aging (BLSA) found a non-significant protective effect of the highest quintile of plasma vitamin C levels compared with the lowest quintile (OR, 0.55) ^[219]. The first National Health and Nutrition Examination Survey (NHANES I),

however, found that a diet high in food containing vitamin C was negatively associated with AMD ^[5]. The Blue Mountains Eye Study (BMES) found no protective effect ^[217].

Vitamin E

AMD patients have shown statistically lower serum levels of vitamin E compared with age matched normals ^[220]. Similarly, The BLSA found that higher blood levels of vitamin E were protective, but found no evidence to support the protective effect of vitamin E supplementation ^[219]. The Eye Disease Case Control Study group (EDCCS) found no significant effect of vitamin E supplementation on AMD, although the serum used for assessment may not correlate with retinal levels ^[221]. An inverse relationship between carotenoid, vitamin E and zinc intake and ARM has been determined ^[218].

The POLA study investigated the relationship between plasma α -tocopherol and AMD. There was a significant relationship between lipid standardized plasma α -tocopherol and ARM ($p = 0.04$) and AMD ($p = 0.003$). The risk reduction for AMD for those in the highest quintile versus the lowest quintile was 82% ^[222].

Lutein and zeaxanthin

Serum concentrations of lutein and zeaxanthin and MP density are reported to be responsive to dietary modifications ^[223-225]. A cross-sectional study reported that people with plasma concentrations of lutein in the lowest third of the distribution have a significant OR for risk of ARMD of 2.0 (95 % CI: 1.0 - 4.1) compared with those in the highest third after adjustment for other risk factors ^[226]. There were no significant trends between plasma concentrations of lutein or lutein plus zeaxanthin.

A study of retinal levels of lutein and zeaxanthin in donor eyes found an 82% lower risk of AMD in retinae among the 25% with highest lutein and zeaxanthin levels compared to the 25% with the lowest levels ^[227]. A 70% reduced risk of AMD has been demonstrated with high ($> 0.67 \mu\text{mol/L}$) versus low ($0.25 \mu\text{mol/L}$) lutein/zeaxanthin plasma levels ^[221]. Measurement of macular pigment optical density (MPOD) in healthy eyes showed an age-related decline, and healthy eyes considered to be at risk for AMD had significantly less MP than healthy eyes not at risk ^[228]. This evidence suggests that there is an increased risk of AMD with lower plasma and retinal levels of lutein and zeaxanthin.

The Beaver Dam Eye Study reported no protective effect against AMD of dietary intake of lutein and zeaxanthin ^[218].

1.11 Intervention studies investigating the effect of nutritional supplementation on ARMD

Lutein and zeaxanthin

Lutein supplementation at achievable dietary levels increased and maintained serum levels, and this was associated with an improvement in glare recovery and VA ^[229]. A pilot study found that short term intervention including 15mg lutein, was associated with statistically significant changes in macular focal electroretinogram parameters, suggestive of an improvement in retinal function in ARM ^[230].

A 35% increase in lutein serum levels and a 20% increase in MPOD was demonstrated in a study supplementing 11 subjects daily with 11mg of lutein from 60g of spinach and 150g of corn/maize ^[223]. Supplementation with 10mg/day of lutein esters for 12 weeks was shown to increase serum lutein levels by five times and MPOD by approximately 20% ^[231]. Serum levels of lutein doubled over 24 months of taking 15 mg lutein three times weekly ^[229].

Zinc

Investigators found no protective effect of zinc supplementation against development of AMD over an 8-10 year period ^[232].

1.12 Safety of ocular nutritional supplements

The Food Standards Agency published a report on safe upper limits for vitamins and minerals in May 2003. Media interpretation of its contents raised fears that people are overdosing on vitamin and mineral supplements and could face irreversible harmful effects. The report was compiled by the Expert Group on Vitamins and Minerals (EVM), an independent expert advisory committee. The EVM assessed 34 substances, recommending safe upper limits for eight and guidance for 22. The Group noted that there was a lack of human studies into adverse effects on vitamins and minerals, especially for children and older people. The EVM was not asked to consider beneficial effects of vitamins and minerals.

Perusal of the report itself shows that the amounts of vitamins and minerals that most people take are not thought to be harmful. The most important points of the report are summarised below:

1. Some substances may have irreversible harmful effects if taken at high doses for long periods. These include beta-carotene, nicotinic acid, zinc, manganese, and phosphorus
2. Levels of vitamin C above 1000mg/day may cause abdominal pain and diarrhoea
3. Levels of calcium above 1500mg/day, and iron above 17mg/day may cause abdominal pain and diarrhoea in some people

4. Levels of vitamin B6 above 10mg/day taken over long periods may lead to loss of feeling in the arms and legs.

Various factors influence the likelihood of experiencing an adverse reaction:

Water/fat solubility

Water-soluble nutrients exist in aqueous solution, for example, vitamin C. Fat-soluble nutrients exist in membranes or lipoproteins, for example, vitamin E and beta-carotene. There is a higher risk of toxicity with fat-soluble nutrients.

Absorption efficiency

For example, beta-carotene absorption decreases in efficiency as dose increases.

Control of nutrient metabolism

If there is tight metabolic control, the chance of toxicity is reduced.

Pro-oxidant behaviour

If a nutrient exhibits oxidising behaviour under certain conditions such as high concentration, the possibility of oxidative stress is increased.

A review of the possible adverse reactions and contraindications of ocular nutritional supplements can be found in the appendix ^[233] (p. 122). The main conclusions of this review were that practitioners should be particularly aware of potential relationships between vitamin A and reduced bone mineral density, beta-carotene and an increased risk of lung cancer in smokers, and the anticoagulant and anti-platelet effects of vitamin E and GBE respectively ^[234]. Vitamin A supplements should be avoided by women who may become pregnant, in those with liver disease and those who drink heavily.

When discussing ocular nutritional supplements with patients, practitioners should be aware of the contraindications and the potential for adverse reactions. Those contraindicated from certain supplements, or identified as at risk of adverse reaction, should be advised to discuss supplementation with their medical practitioner.

1.13 Research rationale

The aim of this research is to determine the effect of a lutein-based nutritional supplement on measures of visual function in normal and ARMD-affected eyes. A RCT has been carried out to investigate this research question, and to attempt to make an original and significant contribution to the literature in this area.

Review of the literature provides evidence for a role of oxidative damage in the pathogenesis of ARMD. RCTs investigating the effect of antioxidant supplementation on ARMD have found conflicting results. The AREDS investigators, however, reported a 25 % reduced risk of

progression of AMD in those taking a combination of zinc plus antioxidants with intermediate or large drusen, non-central GA, or advanced AMD in the second eye.

Lutein and zeaxanthin were not included in the AREDS formulation because they were not readily available for manufacture in nutritional supplements during the planning stages. Evidence supporting a protective role of these oxygenated xanthophylls in the macular is widely reported. They are particularly relevant to human ocular health and are the only carotenoids present in the lens ^[235] and retina ^[236].

It has been suggested that they play a similar role in humans as in plants, as antioxidants and screeners of high-energy blue light ^[237]. There is evidence for selective deposition of lutein in the retina, increase of retinal and serum levels of lutein with supplementation, and an increased risk of ARMD with reduced retinal lutein levels. Xanthophylls have been shown to have superior antioxidant properties than beta-carotene, and have a lower tendency for pro-oxidant behaviour ^[238]. Furthermore, beta-carotene has been linked to an increased risk of lung cancer in smoking males ^[239].

The LAST recently reported improvements in glare recovery, contrast sensitivity, and distance and near visual acuity with lutein plus antioxidant supplementation in AMD patients ^[59]. The results of the LAST study had not been reported at the start of this trial. It looked at the effect of lutein and lutein/antioxidant supplementation in a cohort consisting of a majority of men, and did not assess the effect of supplementation in normals. The intervention in the LAST contained 10 mg lutein.

The current paucity of treatment modalities for this condition has prompted research into the development of prevention strategies. A positive effect of the supplementation on normals may be indicative of its potential role in prevention or delaying the onset of ARMD. This may be of particular importance for those with a positive family history, or exposure to other risk factors. A positive effect in ARMD affected eyes may suggest a role of nutritional supplementation in prevention of progression of the disease, or even in reversal of symptoms.

1.14 The CONSORT statement

The original CONSORT (Consolidated Standards of Reporting Trials) statement was developed to help authors improve the reporting of trials. Reporting of this RCT will adhere to the guidelines set out in the revised CONSORT statement ^[240]. The checklist of items to include when reporting a randomised trial is shown below:

PAPER SECTION and topic	Item	Descriptor	Thesis page
TITLE & ABSTRACT	1	How participants were allocated to interventions.	2
INTRODUCTION			
Background	2	Scientific background and explanation of rationale.	10-28
METHODS			
Participants	3	Eligibility criteria for participants and the settings and location where data were collected.	32-33
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	34-37
Objectives	5	Specific objectives and hypotheses.	32
Outcomes	6	Clearly defined primary and secondary outcome measures, and, when applicable, any methods used to enhance the quality of measurements.	35-37
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analysis and stopping rules.	33-34
Randomisation:			
Sequence generation	8	Method used to generate the random allocation sequence, including details of any restriction (e.g., blocking, stratification).	35
Allocation concealment	9	Method used to implement the random allocation sequence (e.g., numbered containers), clarifying whether the sequence was concealed until interventions were assigned.	33,35
Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	33-37
Blinding (masking)	11	Whether or not participants, those administering the intervention, and those assessing the outcomes were blinded to group assignment. If done, how the successful blinding was evaluated.	37 55
Statistical methods	12	Statistical methods used to compare groups for primary outcomes. Methods for additional analyses, such as subgroup analyses and adjusted analyses.	37

RESULTS			
Participant flow	13	Flow of participants through each stage. Specifically, for each group report the number of participants randomly assigned, receiving intended treatment, completing the study protocol, and analysed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	54
Recruitment	14	Dates defining the periods of recruitment and follow up.	37
Baseline data	15	Baseline demographic and clinical characteristics of each group.	55-60
Numbers analysed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by 'intention-to-treat'. State the results in absolute numbers when feasible.	54
Outcomes and Estimation.	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision.	61-68
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analysis and adjusted analysis, indicating those pre-specified and those exploratory.	61-68
Adverse events	19	All important adverse events or side effects in each intervention group.	68
DISCUSSION			
Interpretation	20	Interpretation of the results, taking into account the study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	69-74
Generalisability	21	Generalisability (external validity) of the trial findings.	69-74
Overall evidence	22	General interpretation of the results in the context of current evidence.	69-74

The title and abstract section of the CONSORT checklist is addressed by the thesis summary. This chapter addresses the introduction section of the checklist. The methods section of the checklist is addressed in chapter two, the results section in chapter four, and the discussion section in chapter five.

1.15 Summary

The aim of this thesis is to investigate the effect of nutritional supplementation on measures of visual function in normal and ARMD-affected eyes. Literature pertaining to the proposed role of nutrition ARMD has been reviewed, and a research rationale has been put forward. In the next chapter, the protocol for the RCT will be described.

Chapter 2: Randomised controlled trial design

The aim of this thesis is to investigate the effect of nutritional supplementation on measures of visual function in normal and ARMD-affected eyes. In the previous chapter, evidence for and against the proposed use of nutritional supplementation in the prevention of onset and progression of ARMD was reviewed. The research rationale was put forward, and the choice of a RCT design explained. In this chapter, the protocol for the RCT is described. The protocol was published in BMC Nutrition Journal and the manuscript can be found in the appendix ^[241] (p. 158). This chapter addresses the methods section of the CONSORT checklist.

2.1 Research objectives

The aim of this research is to determine the effect of a lutein-based nutritional supplement on measures of visual function in normal and ARMD-affected eyes. A randomised controlled trial (RCT) has been designed to investigate this research question, and to attempt to make an original and significant contribution to the literature in this area. Reporting of this trial adheres to the Consolidated Standards of Reporting Trials (CONSORT) statement ^[240].

2.2 Recruitment

The study required recruitment of people with and without ARMD. Recruitment methods employed included:

1. Sending information to Birmingham optometrists, ophthalmologists, and a specialist centre for rehabilitation of people with sight loss in the West Midlands
2. An editorial in the Birmingham Evening Mail
3. Recruitment e-mails sent to the Royal National Institute for the Blind (RNIB) and all staff and students at Aston University and Aston Science Park
4. A project website has also been developed at www.aston.ac.uk/lhs/research/nri/opo/amd
5. Direct recruitment from a specialist rehabilitation centre for people with sight loss.
6. Article published in the optical press
7. Article published in the Macular Disease Society newsletter
8. Letters sent to hospital optometrists in the Midlands
9. Letters sent to ophthalmologists in Nottingham and Warwick
10. HB interviewed by Midlands Today television news programme
11. HB interviewed for the Food Programme on BBC Radio 4
12. The trial was registered for an International Standard Randomised Controlled Trial Number (ISRCTN 78467674).

2.3 Research centres

The main research centre was the Neurosciences Research Institute, Aston University, Birmingham, B4 7ET, UK. A secondary research centre was Whitakers of Saltaire Opticians, 59

Bingley Road, Gordon Terrace, Saltaire, Shipley, BD18 4SB, UK. Data collection took place in standard consulting rooms at both centres. Enrolment, randomisation, and data collection were carried out by HB. HB and FE were masked to group assignment. HB is a research optometrist and FE is an optometrist and lecturer at Aston University, Birmingham, UK.

2.4 Inclusion/exclusion criteria

For inclusion participants had to 1) provide written informed consent, 2) be available to attend one of the research centres, 3) present with no ocular pathology in one eye, or no ocular pathology other than dry ARMD in at least one eye. A cataract grading system consisting of grades one, two and three for each of cortical, nuclear, and posterior subcapsular cataracts was designed for use with a direct ophthalmoscope. This in-house system was used to avoid the need for dilation necessary with other cataract grading scales. Participants presenting with lens opacities precluding fundus photography were excluded. Throughout the trial period, progression of any type of cataract to the successive grade will required the participant to withdraw.

Exclusion criteria include type I and II diabetes because vitamin E has been shown to affect glucose tolerance ^[242-246] and diabetic retinopathy may confound the results. Also, zinc may increase glycosylation in diabetics ^[247]. Those taking anti-platelet or anti-coagulant medication were excluded because of possible interaction with vitamin E ^[234, 239, 248, 249], as were those who use nutritional supplements that potentially raise vitamin and mineral intake above safe limits. The most recent guidelines for upper limits of nutritional supplementation are set out in the UK Food Standards Agency report ^[250]. Neovascular AMD and other ocular disease that could potentially interfere with the results were excluded.

2.5 Masking

The study formulation and placebo tablets were produced by Quest Vitamins Ltd, Aston Science Park, Birmingham, B7 4AP, and are identical in external and internal appearance, and taste. The manufacturer allocated distinguishing symbols, μ and λ . The tablets were packaged in identical, sealed, white containers; the only difference being the symbol on the label. Investigators and participants did not know which symbol represented the placebo tablets, and which represented the active formulation.

2.6 Sample size calculation

From baseline data collection the group sizes required in order to have 80% power at the 5% significance level for distance visual acuity (DVA), near visual acuity (NVA), contrast sensitivity (CS), the Macular Mapping test (MMT), and the Eger Macular Stressometer (EMS) were calculated. The baseline data was used for calculation of standard deviation. For the ARMD cohort, group sizes for AREDS photograph grading were also calculated. The mean and

standard deviation values were calculated from the baseline data taken from 45 normal and 30 ARMD affected participants. These values are shown in tables 2.1 (normal) and 2.2 (ARMD) and suggest that a total of 26 normal, and 188 ARMD participants were required.

Table 2.1: Sample size calculations for the normal group.

	DVA (logMAR)	NVA (logMAR)	CS (log units)	MMT (MMT points)	EMS (s)
Mean	-0.10	-0.05	1.60	97.6	10.8
Standard deviation (S)	0.09	0.08	0.11	4.1	3.9
Effect size (E)	0.10	0.10	0.30	14	14
E/S	1.11	1.25	2.72	3.4	3.6
(E/S) ²	1.23	1.56	7.40	11.65	12.9
Sample size = 16/(E/S) ² (2 sided)	13	10	2	1	1

Table 2.2: Sample size calculations for the ARMD affected group.

	DVA (logMAR)	NVA (logMAR)	CS (log units)	MMT (MMT points)	EMS (s)	AREDS grade
Mean	0.16	0.22	1.36	82.7	18.4	2.86
Standard deviation (S)	0.24	0.21	0.22	23.4	11.4	1.12
Effect size (E)	0.10	0.10	0.3	14	14	1
E/S	0.42	0.48	1.36	0.6	1.23	0.89
(E/S) ²	0.17	0.23	1.9	0.36	1.51	0.8
Sample size = 16/(E/S) ² (2 sided)	94	70	9	45	11	20

2.7 Intervention

The nutrients suitable for inclusion in an ocular nutritional supplement have been reviewed and the manuscript published ^[162]. This review can be found in the appendix (p. 111).

The study formulation contained the following:

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

The placebo tablets contained an inert cellulose matrix.

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

2.8 Randomisation

The random allocation of participants to study groups forms the basis for testing the significance of differences between the groups in measured outcomes. It facilitates the equal distribution of baseline characteristics except for chance variation. It is important that the randomisation procedure truly allocates randomly and that neither intentional nor unintentional factors can influence the process. An advantage of this trial was that only one investigator was involved in the randomisation process. As we only had two groups, computer generated random numbers were employed with odd and even being the randomisation categories. The random number generator function in Microsoft Excel was used to allocate participants to μ and λ groups. Odd numbers were allocated to the μ group.

2.9 Baseline data

On application, participants completed a health questionnaire, a food frequency questionnaire, and a food diary. The health questionnaire provided information about general health, medication, nutritional supplementation, smoking history, ocular health, and time spent living abroad. The food questionnaire and diary requested information about diet for analysis using Foodbase 2000 software (The Institute of Brain Chemistry and Human Nutrition, London N7 8DB). This software has been used in a prospective cross-sectional study of diet and nutritional supplement use in people with and without ARMD [251]. This study has been published and can be found in the appendix (p. 165).

Follow-up food diaries and food frequency questionnaires were provided for completion at the end of the trial period. Participants were encouraged not to change their diet for the duration of the trial. The issue of additional supplementation was discussed at the first visit. Participants were not expected to stop taking additional supplements, but were encouraged to provide details of these supplements and also not to change their supplementation regime for the duration of their involvement in the trial. The health questionnaire was reviewed at each follow-up visit. Participants were also asked to report any side-effects at each follow-up visit and were provided with a contact telephone number at the start of the trial.

2.10 Outcome measures

The investigation of several measures of visual function was required because ARMD can produce varying signs and symptoms.

Visual acuity

Refractive error was neutralised with lenses at each visit and the resulting trial lenses were used for all vision tests. Distance and near VA was measured using EDTRS logMAR charts. LogMAR charts have 5 letters and 0.1 logMAR progression per line. The advantage of using these charts is that they provide an equal-interval scale, and there are five letters per line.

Standard Snellen charts do not provide a linear scale and have a decreasing number of letters per line as the letter size increases.

It has been reported that logMAR charts are the most accurate tool for measuring VA in practice and in clinical research studies ^[252]. The 95 % confidence limit for a change in VA using these charts is five letters or one line (0.1 logMAR) ^[253]. This value has been used as the effect size in the power calculations for this trial.

Contrast sensitivity

Contrast sensitivity (CS) is measured using a Pelli-Robson chart (Clement Clarke International, Edinburgh Way, Harlow, Essex, CM20 2TT, UK) and provides additional information about vision. The Pelli-Robson chart determines the contrast required to read large letters and is designed to test mid- to low-spatial frequencies. Some people may have normal visual acuity, but reduced contrast sensitivity at low spatial frequencies, particularly if they suffer from ocular pathologies such as ARMD. Pelli-Robson CS scores have been reported to be repeatable to within ± 0.15 log units (three letters) ^[254]. This suggests that a significant change in CS score using the Pelli-Robson chart is 0.30 log units (six letters).

Macular Mapping Test

The MMT (The Smith-Kettlewell Research Institute, 2318 Fillmore Street, San Francisco, CA, 94115, USA) was developed to map visual defects caused by macular disease. It was developed by MacKeben and Colenbrander ^[255] and differs from conventional field analysis in that the stimuli are single letters rather than spots of light. This is a novel piece of equipment and each participant was given a practice run to eliminate learning effects. At the end of the test a single figure score is presented.

Photostress recovery time

Eger Macular Stressometer (EMS) (Gulden Ophthalmics, Elkins Park, PA 19027) is used to assess photostress recovery time (PSRT). This is the time taken for the regeneration of photopigments in bleached photoreceptors to a level that allows resolution of, for example, a letter at near. Resynthesis of the photopigments is dependent upon the integrity of the photoreceptors and RPE ^[256]; it follows that the PSRT may be extended in those with diseases affecting these structures.

Fundus photography

Fundus photographs of the macular were taken using the Topcon non-mydratic TRC-NW5S retinal camera (Topcon House, Bone Lane, Kennet Side, Newbury, Berkshire RG14 2PX, UK). The AREDS ARMD classification system was used to grade all images. For grading purposes the images were coded such that the grader was masked to intervention group and image date.

One investigator (HB) graded all images. In order to assess the reproducibility of the grading system, two optometrists independently graded a subset of coded images (see chapter 3).

2.11 Follow up

Data collection took place at baseline, nine, and 18 months and started in December 2002. Recruitment was planned to be complete by September 2003 to allow all participants 18 months of follow-up, finishing in March 2005. However, the low number of participants by September 2003 prompted the decision to continue recruitment for a further nine months and have those participants enrolled for nine months only.

2.12 Data collection procedure

Allocation of interventions was implemented by HB. HB and FE were masked to the intervention codes, which were retained by the manufacturer until completion of data collection and analysis. All measurements were taken in a standard test room. The background luminance of the chart was 220 cd/m^2 , which is within the $80 - 320 \text{ cd/m}^2$ recommended range ^[257]. It has been reported that when chart luminance is within the moderate photopic range, doubling the chart luminance only alters the VA score by just under 0.02 log units ^[257]. The data collection routine was set out as follows:

1. Trial eye selected (initial visit only). If both eyes were eligible for inclusion, the right eye was used
2. Forty-five degree fundus photograph taken
3. Discussion about general and ocular health, medication and nutritional supplementation, and changes to these during follow-up assessments
4. Assessment of distance vision using the EDTRS chart at 3 m
5. Refraction and assessment of distance VA (DVA) using the EDTRS chart at 3 m
6. Contrast sensitivity using the Pelli-Robson chart at 1 m
7. Near addition (where appropriate) and assessment of near VA (NVA) using the Logarithmic near Visual Acuity Chart '2000' (Precision Vision, 994 First Street, La Salle, IL 61301, US)
8. MMT
9. EMS.

2.13 Analyses

For each outcome measure the change between baseline, nine month, and 18 month values was calculated. An independent samples t-test was used to determine whether the means of these values differ at the 5% significance level between the placebo and antioxidant formulation results for ARMD-affected and normal participants. If the data was not normally distributed, a Mann-Whitney U test was used. A mixed between-within subjects ANOVA was used to assess the effect of the between-subjects variable (group), and the within-subjects variable (time), on the outcome measures (dependent variable). This analysis tests whether there are main effects

for each of the independent variables and whether there is significant interaction between the two.

2.14: Ethical approval

The study has been approved by the Aston University Human Sciences Ethical Committee (code 02/M). The tenets of the Declaration of Helsinki were followed ^[258].

2.15 Summary

RCTs are considered to be the gold standard as far as clinical research is concerned. This chapter described the protocol for the use of an RCT to investigate the role of a lutein-based nutritional supplement in prevention of onset or progression of ARMD. The outcome measures for this RCT are DVA, NVA, CS, MMT, EMS, and fundus photography. The change in logMAR VA and CS needed to constitute a clinically significant change have been reported. However, this information is not available for the MMT or EMS. The next chapter describes reliability studies that have been carried out to assess the change in MMT and EMS score required to indicate a clinically significant change. The results of these studies were used in the calculation of required sample sizes. Chapter three will also describe reproducibility analysis of the AREDS system that was used to grade fundus images.

Chapter 3: Reliability studies

The previous chapter described the protocol for the RCT that will form the main part of this thesis. The need for reliability data to determine the change in each outcome measure that indicates a significant change between participant visits was highlighted. The main use of this information is in the calculation of required sample sizes. Review of the literature provided reliability data for logMAR VA and CS. It was however, necessary to conduct reliability studies for the EMS and MMT. It was also necessary to independently assess the reproducibility of the AREDS ARMD classification system.

3.1 Reliability, normative data, and the effect of ARMD on the Eger Macular Stressometer photostress recovery time

This study has been published ^[259] is included in the appendix (p. 172). It was also presented at the American Academy of Optometry Conference, Hawaii, April 2004. A brief summary of the manuscript follows:

3.1.1 Purpose

To assess repeatability and reproducibility, to determine normative data, and to investigate the effect of ARMD, compared with normals, on photostress recovery time (PSRT) measured using the Eger Macular Stressometer (EMS).

3.2.1 Method

The study population comprised of 49 healthy eyes of 49 participants. Four EMS measurements were taken in two sessions separated by one hour by two practitioners, with reversal of order in the second session. EMS readings were also taken from 17 ARM and 12 AMD affected eyes.

3.1.3 Results

EMS readings are repeatable to within ± 7 seconds. There is a statistically significant difference between controls and ARM affected eyes ($t = 2.169$, $p = 0.045$), and AMD affected eyes ($t = 2.817$, $p = 0.016$). The EMS is highly specific, and demonstrates sensitivity of 29 % for ARM, and 50 % for AMD. There was a significant linear relationship between EMS score and age for those aged < 50 years ($F = 6.76$, $p = 0.015$), but not for those aged ≥ 50 years ($F = 0.29$, $p = 0.60$). VA explained just 1.2 % of the variance in EMS scores in the ARMD cohort.

3.1.4 Conclusions

The EMS may be a useful screening test for ARM, however, direct illumination of the macular of greater intensity and longer duration may yield less variable results.

3.2: Reliability, normative data, and the effect of ARMD on the Macular Mapping test

This study was presented as a paper at the American Academy of Optometry Conference in Florida, December 2004. It is currently under peer review at BMC Ophthalmology.

3:2.1 Background

Exudative AMD and/or GA are the forms of ARMD that result in the most severe visual loss, and their prevalence in the US population over 40 years of age has been estimated at 1.47% [95% confidence interval (CI), 1.38% - 1.55%]^[15]. The likelihood of visual deterioration in those with exudative AMD may be reduced with laser photocoagulation and photodynamic therapy^[260-263], but the success rate deteriorates with increasing latency of diagnosis as lesions extend towards the foveal avascular zone. Consequently, early diagnosis is crucial, and at risk patients are often advised to use an Amsler grid at home for detection of scotomas and metamorphopsia^[264-266].

The Amsler grid has been used in clinical practice since the 1940s^[267] for detecting and monitoring retinal conditions such as ARMD^[268-270]. It has been shown, however, that a report of distortion of the Amsler grid can come from perceived lines filling-in across scotomas, or equally from non-scotomatous retinal impairments, and the clinician has no way of knowing which. Seventy-seven percent of standard and 87 % of threshold scotomas that are 6° or less in diameter are not detected by Amsler grid testing^[267]. The poor performance of the Amsler grid may be related to the inability to properly maintain fixation while testing the peripheral visual field^[271], crowding effects caused by peripheral presentation of multiple lines^[272], inability to assess factors such as quality of examination performance, and low compliance to perform the Amsler grid at home^[271]. Despite the shortcomings of this test, the Amsler has been used as a measure of visual change in a RCT investigating the effect of nutritional supplementation in atrophic AMD^[59].

The MMT was developed for quick, inexpensive assessment of residual vision in patients with maculopathies, and identification of regions of intact retina suitable for reading using 'eccentric viewing'^[273]. The test involves presentation of letters on a background, designed so that the results relate directly to the ability to read. The software permits selection of 16 different contrast levels for both the background and the letters to allow low-contrast and reverse-contrast assessment. The MMT addresses the issue of gaze stabilization by using peripheral landmarks that cover a large area, in the form of radial spokes pointing at the centre of the screen^[274].

Although the MMT was designed primarily for quick assessment of residual vision in patients with maculopathies, the peripheral fixation aid, along with the use of letter targets may make it a useful tool for monitoring progression of macular disease.

The objective of this study was to assess reliability (repeatability and reproducibility), to obtain normative data, and to assess the effect of ARMD on MMT scores.

3:2.2 Method

SUBJECTS

Thirty-one normally sighted participants were recruited from staff, students, and patients of the Division of Optometry and the Neurosciences Research Institute, Aston University, Birmingham, UK. This research adhered to the tenets of the Declaration of Helsinki. All participants gave informed consent to take part in the study, which was approved by the Institutional Human Ethics Committee. Participants varied in age from 23 to 77 years (mean \pm SD, 50.3 \pm 19.0 years). It has been shown that visual function starts to decline at about the age of 50 years^[275], and so for analysis the participants were divided into two subgroups; 15 participants aged < 50 years, from 23 to 46 (32.4 \pm 7.5 years), and 16 participants aged > 50 years, from 51 to 77 (67.1 \pm 8.9 years). There was no gender difference between groups. LogMAR VA ranged from -0.1 to 0.1 (0.0 \pm 0.05 logMAR) for the younger group and from -0.2 to 0.1 (0.0 \pm 0.10 logMAR) for the older group. There was no significant difference in VA between these groups ($t = 0.54$; $p = 0.59$).

Exclusion criteria were: best-corrected logMAR VA of worse than 0.1 logMAR (VA was measured under standard testing conditions using a logMAR chart, retro-illuminated to a luminance of 130 cdm^{-2} ^[276] and each letter seen was scored as 0.02 log units, with guessing encouraged); retinal disease detected using a direct ophthalmoscope; glaucoma; lenticular opacities greater than grade one on the in-house cataract opacity grading system; prescribed medication associated with changes in retinal function.

The ARMD-affected eyes were classified according to the International Classification and Grading System^[2].

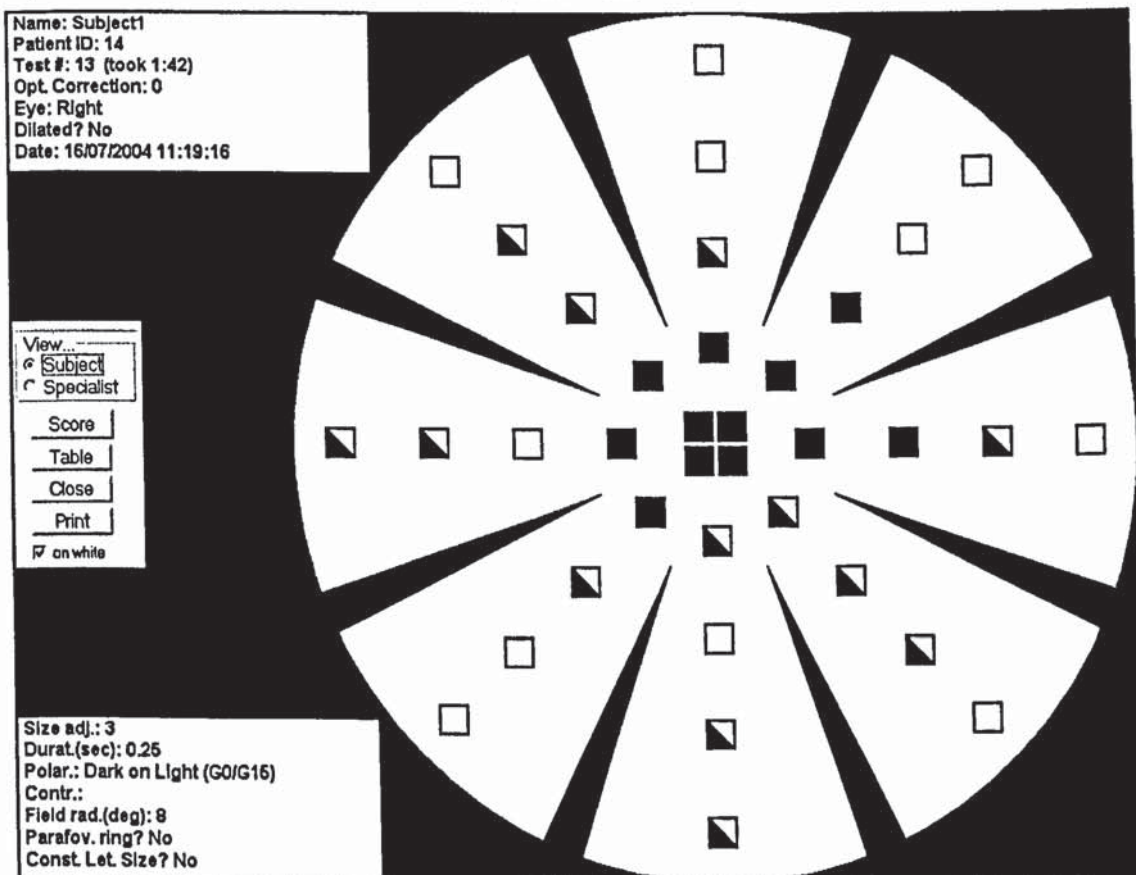
The MMT was carried out on 17 ARM affected eyes of 17 participants aged from 55 to 82 (69.4 \pm 7.7 years) and 12 AMD affected eyes of 12 participants aged from 65 to 78 (71.8 \pm 4.3 years). VA ranged from -0.08 to 0.2 (0.04 \pm 0.09 logMAR) for the ARM group, and from 0.2 to 0.76 (0.50 \pm 0.21) for the AMD group. The eyes included did not have lenticular opacities greater than grade 1 on the cataract grading system, and were not affected by any other ocular condition.

MATERIALS

The MMT is a software program used in conjunction with a desktop or laptop computer, designed specifically to map visual defects due to macular disease. The test screen displays a characteristic background pattern throughout the test, which resembled a 'wagon wheel'. Eight

spokes point inwards, but do not reach the centre of the circular display area. This pattern provides the patient with sufficient peripheral landmarks to indicate the location of the centre of the circular display area ^[277], even if the centre is not directly visible to the patient (see figure 3.1).

Figure 3.1: Subject view of the MMT results. Black squares indicate letters that were not seen, half-shaded squares indicate those that were seen but not correctly identified, and white squares indicate letters that were correctly identified.



When loaded for the first time, the program prompts the user to calibrate the system. It is necessary to measure the diameter of the wagon wheel, to check the horizontal/vertical proportion of the monitor, and to set the monitor to maximum contrast and brightness. The diameter of the wagon wheel on the screen allows the system to suggest a correct viewing distance, such that the wagon wheel has the correct angular extent of 18° diameter. The viewing distance for our system was 76.2 cm.

The targets comprise of the Sloan letters ^[278] with a change in the width-to-height ratio from 5 x 5 to 4 x 5 for better legibility. Serifs are also added to the D to reduce the probability of confusion with O. Letter sizes vary according to eccentricity, starting at 5 pixels high, and increments of 5 pixels to 45 each. Thirty-two standard locations ensure that the letters do not 'collide' with the spokes of the wagon wheel. There are an additional four central locations.

PROCEDURE

All participants were seated comfortably, with their head supported using a chin rest, and the eye not being tested occluded. In order to ensure control of direction of gaze, each participant was asked, 'Do you have a sense of where the centre of the wagon wheel is?'. All participants responded positively, and were then instructed to direct their eye to the centre and to keep it there as still as possible. Participants with AMD were also advised that the centre of the wagon wheel may seem to disappear. As the background illuminance was low (6 lux), and our participants did not have lens opacities greater than grade one of the RCT cataract grading system, we were not concerned about glare, and therefore selected the black letters on a white background presentation mode. The following explanation of the test was given to every participant; 'Black letters will appear one at a time anywhere on the white wagon wheel. It is important that you do not move your eye to try and look at them. When you see a letter, say the name of it out loud. If you are not sure what the letter is, have a guess'.

The same testing room was used for each test with background lighting switched off. When both eyes matched the inclusion criteria, the right eye was tested, and when only one eye was suitable for inclusion, this eye was tested. The prescription providing best-corrected VA at the test distance was placed into a trial frame, with the eye not being tested occluded. Full aperture trial lenses were used.

When each participant was ready the examiner pressed the mouse button and a letter appeared in an unpredictable location in the visual field, remaining visible for 234 msec. The participant told the examiner which letter they perceived and the examiner entered this response into the computer for scoring. This prompts display of the next letter, and so on. There are three categories of response; 1) target not detected, 2) target detected, but not recognized (including incorrect responses), 3) target correctly recognized. The final score is calculated using the following system; target not detected = 0, target detected but not recognized = 1, target detected = 2. The score is displayed at the end of the test and the participant is able to view a 'map' of the areas seen and unseen.

Data were collected by two optometrists, HB and LD, during two sessions separated by one hour from the same subjects. A trial run was completed for each subject by HB in the first session and LD in the second session to encourage test familiarity and reduce learning effects. In session 1 the first experimental test was carried out by HB, and the second by LD, and in session two this order was reversed. The study was designed to assess reproducibility (HB1-LD1, LD2-HB2), and repeatability (HB1-HB2, LD1-LD2),

Data analysis employed the independent-samples t-test for comparing ages and the chi squared test to compare proportion of males by group. MMT scores are non-continuous data, and so nonparametric statistical tests were used for analysis. The Mann-Whitney U test was

used to compare MMT scores between groups, and Spearman's rank order correlation to explore the impact of age and VA on MMT score. When assessing the effect of ARMD on MMT score, power analysis shows that the group sizes were sufficient to have an 80% chance of detecting a difference in means of 5% at the 5% level of significance using the independent samples t-test.

3.2.3 Results

RELIABILITY

Reproducibility was determined by comparing HB1-LD1 and LD2-HB2, and repeatability was determined by comparing HB1-HB2 and LD1-LD2. The differences between data sets for two of the comparisons (HB1-HB2 and HB1-LD1) were normally distributed and these were used for analysis. See figures 3.2 and 3.3 for Bland and Altman plots ^[279] of test-retest data of the HB1-HB2 and HB1-LD1 comparisons respectively.

Figure 3.2: Difference in MMT score between HB1 and HB2 compared with the mean (n = 31 normal eyes). The mean bias is represented by the solid line and the 95 % confidence limits are represented by the dotted lines

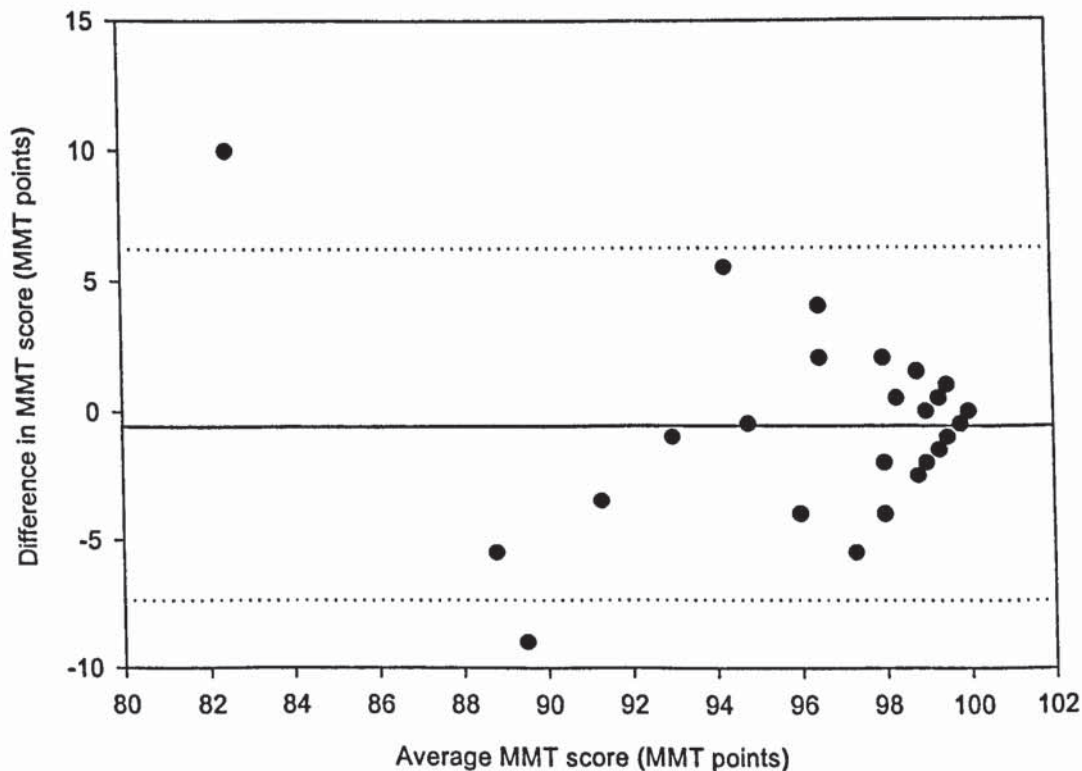
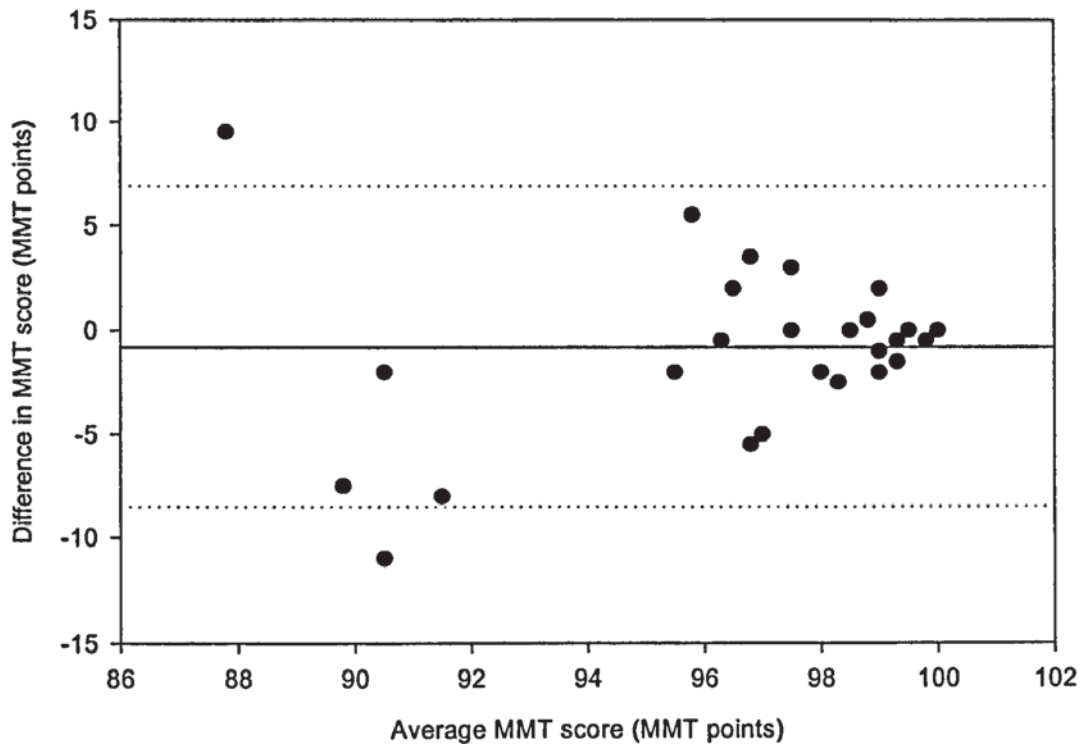


Figure 3.3: Difference in MMT score between HB1 and LD1 compared with the mean (n = 31 normal eyes). The mean bias is represented by the solid line and the 95 % confidence limits are represented by the dotted lines.



Accurate analysis of test-retest data can be achieved using the coefficient of repeatability ^[279, 280]. This gives the 95% confidence limits for the amount of difference between two sets of results. It is calculated as 1.96 multiplied by the standard deviation of the mean differences between the two sets of data. The coefficient of repeatability was ± 6.70 for the HB1-HB2 comparison and ± 7.24 for the HB1-LD1 comparison.

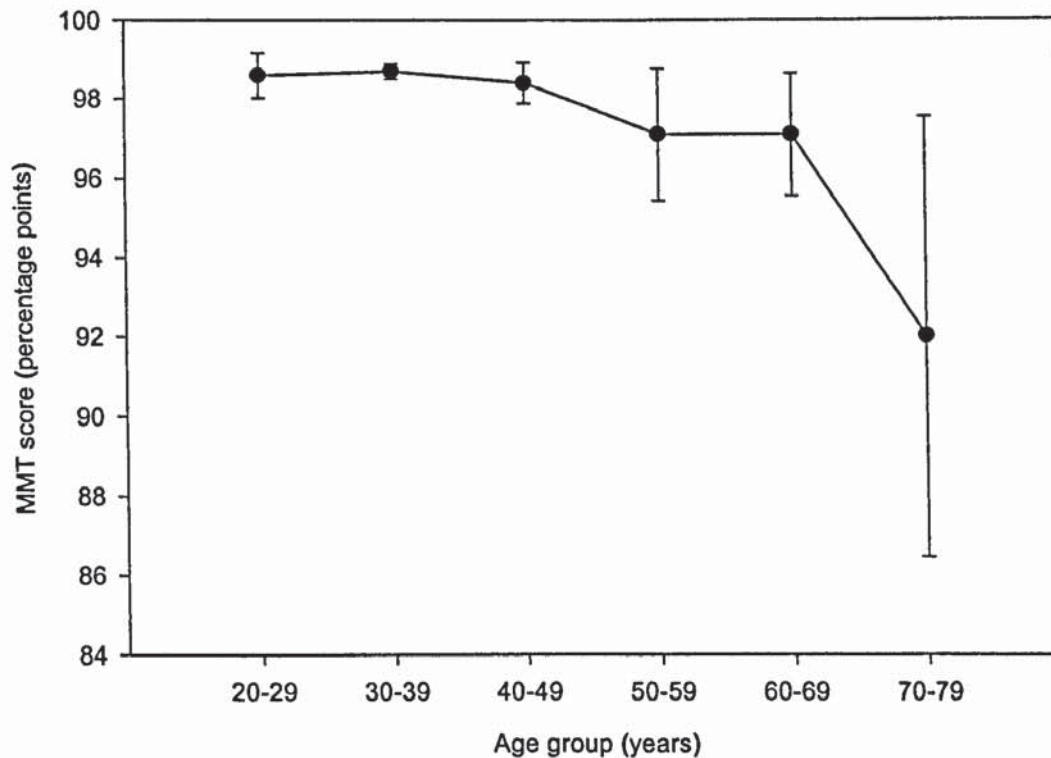
NORMATIVE DATA

Normal limits for MMT score for each age group were determined by calculating the 95% confidence limits (see table 3.1). The age-grouped mean values are represented graphically, in figure 3.4.

Table 3.1: Normal limits [mean - (SD x 1.96)] for MMT score by age-group.

Age-group (years)	n	Mean MMT score (MMT points)	SD	Lower 95% CI	Clinical lower limit (%)
20-29	6	98.6	0.58	97.46	97.5
30-39	7	98.7	0.20	98.3	98.5
40-49	3	98.4	0.53	97.36	97.5
50-59	4	97.1	1.67	93.83	94.0
60-69	4	97.1	1.54	94.08	94.0
70-79	8	92.0	5.54	81.14	81.0

Figure 3.4: MMT score plotted against age-group.



In order to explore the relationship between age and MMT score, a Spearman's rank order correlation was carried out. There was a strong negative correlation between the two variables with increasing age associated with lower MMT scores ($r = -0.56$, $n = 31$, $p = 0.001$). The non-parametric alternative to the one-way between groups ANOVA is the Kruskal-Wallis test. This was carried out to determine whether there was a difference in MMT score across the six age-groups. There was no significant difference ($X^2_{(5,26)} = 8.61$, $p = 0.13$), although this analysis may be hindered by the small group sizes.

To explore the reported decline in visual function from the age of 50 years, Spearman's rank order correlations were repeated separately for the under 50 and over 50 groups. There was no significant relationship between age and MMT score for the under 50 group ($r = -0.18$, $n = 16$, $p = 0.50$) (see figure 3.5). There was a strong negative correlation between the two variables for the over 50 group ($r = -0.59$, $n = 16$, $p = 0.016$) (see figure 3.6).

Figure 3.5: MMT scores plotted against age for subjects aged < 50 years. There is no significant relationship between the two variables ($r = -0.08$, $n = 15$, $p = 0.79$).

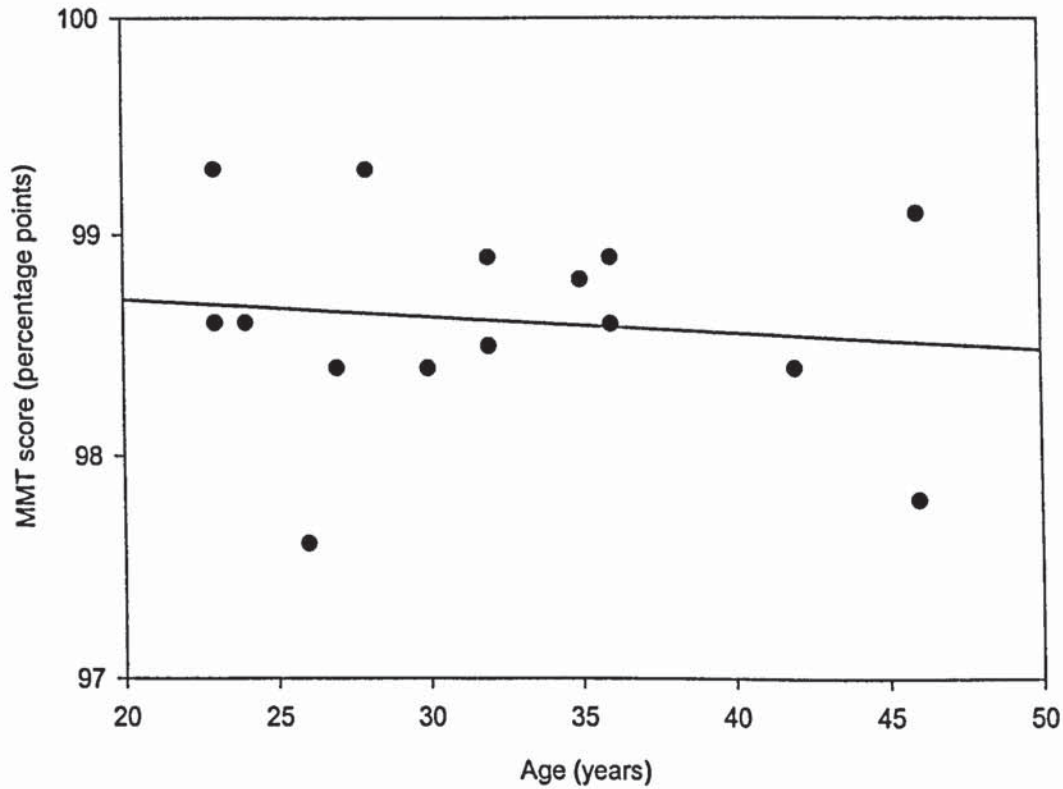
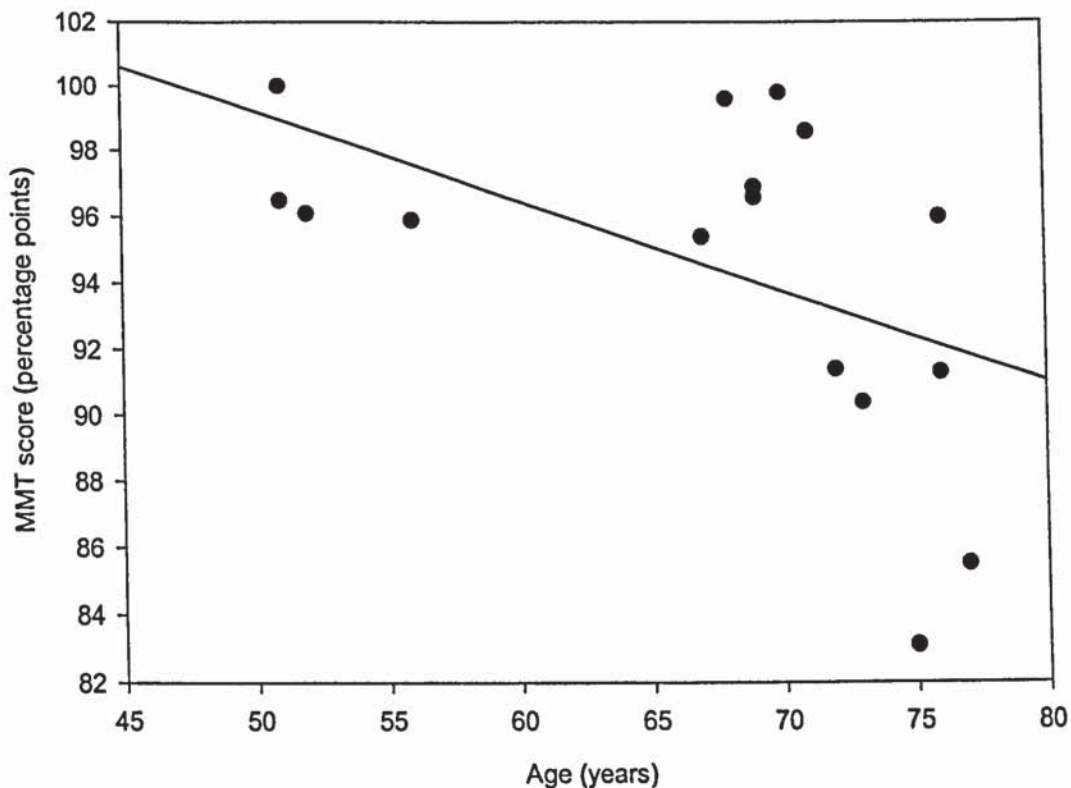


Figure 3.6: MMT score plotted against age for subject aged > 50 years. There is strong negative correlation between the two variables ($r = -0.59$, $n = 16$, $p = 0.016$).



Normal limits have been calculated for the under 50 and over 50 groups. The clinical lower confidence interval for the under 50 group was found to be 97.5 points (98.6 ± 0.46 %) and for the over 50 group 76.0 points (94.6 ± 9.4). These values are significantly different ($z = -2.67$; $p = 0.008$).

To determine whether there is a relationship between MMT score and VA, a Spearman's rank order correlation was carried out. There is strong negative correlation between the two variables ($r = -0.83$, $p < 0.001$). This would indicate that the MMT did not detect a reduction in visual function in the ARMD cohort that would not have been detected with VA measures alone.

EFFECT OF ARM

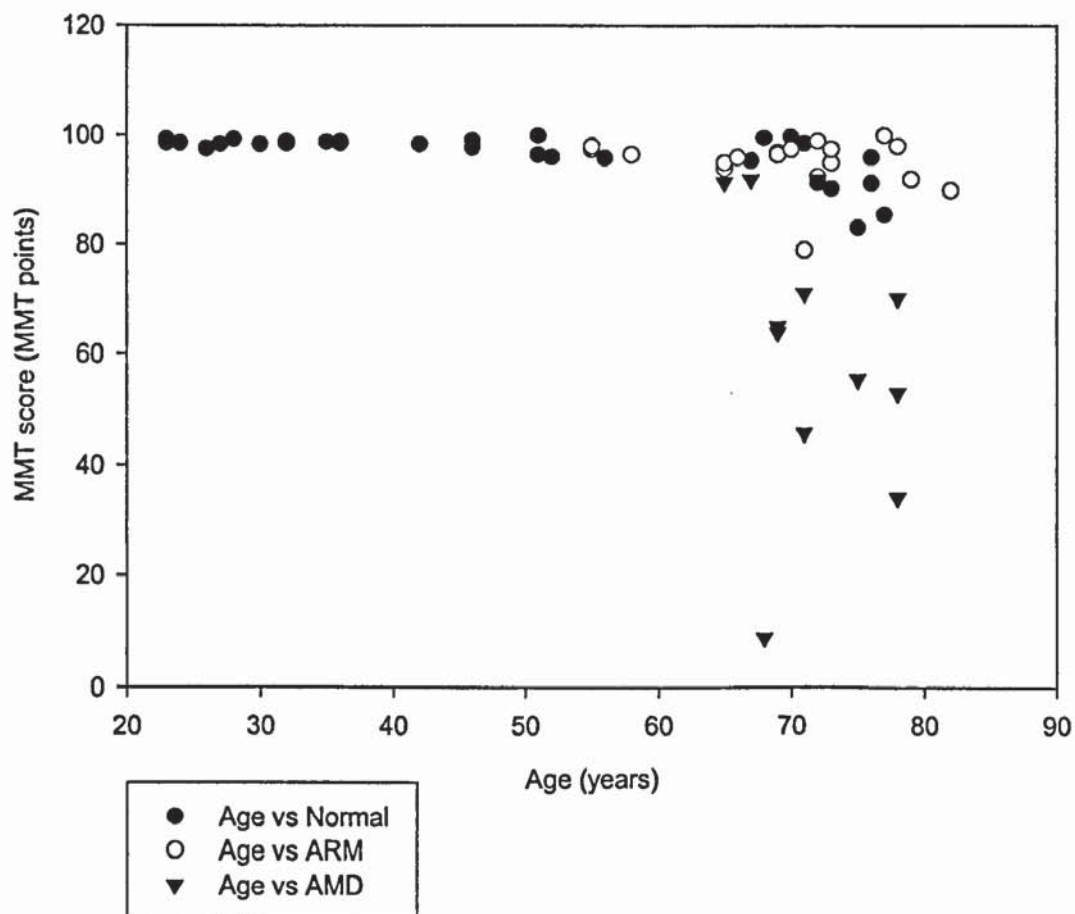
The over 50 and ARM groups were matched for age ($t = -0.78$; $p = 0.44$) and gender [$\chi^2_{(1)} = 0.50$, $p = 0.58$]. The mean MMT score was 94.5 points (94.6 ± 4.8) for the over 50 group and 95.0 points (94.9 ± 4.7) for the ARM group, and there was no significant difference between the groups ($z = -0.22$, $p = 0.83$). Power analysis shows that the group sizes were sufficient to have an 80% chance of detecting a difference in means of 14 points at the 5% level of significance using the unpaired t-test.

EFFECT OF AMD

The over 50 and AMD groups were matched for age ($t = -1.61$, $p = 0.12$) and gender [$\chi^2_{(1)} = 0.62$, $p = 0.82$]. The mean MMT score was 62.0 points (61.9 ± 23.8) for the AMD group. The difference in scores between the over 50 and the AMD group is statistically significant ($z = 3.76$, $p < 0.001$).

Figure 3.7 shows the effect of age on MMT score for the normal, ARM and AMD groups.

Figure 3.7: First MMT scores taken from normal, ARM and AMD participants plotted against age.



3.2.4 DISCUSSION

The primary objective of this study was to determine the reliability of the MMT. The developers of the MMT assessed its reliability by performing the test twice on 20 eyes. The conditions were made harder between the first and second run by decreasing the size of the letters by 5 pixels, and 92 % of all tested locations in the participants behaved within expectation. It was concluded that the test procedure reflects the functional topography with reasonable accuracy and reliability [273]. This independent reliability data has been used to determine the decrease in MMT score that is needed to indicate a clinical change between tests. The MMT scores are

reliable to within ± 7.0 points, indicating that the MMT score has to change by more than 14 points for the change to be clinically significant.

Secondary objectives were to obtain normative data, and to compare the effect of ARM and AMD on MMT scores. A Spearman's rank order coefficient was carried out to explore the relationship between age and MMT score. Age explains only 3.4 % of the variance in the MMT scores for those aged under 50 years, compared with 34.9 % of the variance for the over 50 group. There was a significant increase in MMT score with age for those aged over 50 years. These results complement work that suggests that visual function starts to decline at the age of 50 years ^[275], and also suggest that a larger study should be carried out to determine more specific age-related normal values for the over 50 group.

Normative data may be used to determine the lower limits of normal for age groups. Our raw data shows that for patients aged < 50 years, an MMT score of less than 97.5 points may indicate pathological change. This critical value for patients aged > 50 years is 76.0 points. This data was used to determine the sensitivity and specificity of the MMT. The MMT is highly specific, correctly identifying 100 % of normals for both the under 50 (95 % CI: 151 - 49) and over 50 (95 % CI: 149 - 51) groups. It is highly sensitive for the detection of AMD (75 %, 95% CI: 124 - 76), but has no sensitivity for ARM (0 %).

Comparisons between ARM-affected eyes and age- and gender-matched controls yielded no significant difference, although this part of the study was powered to detect a difference between groups. There was significant reduction in MMT score between AMD-affected eyes and age- and gender-matched controls. The results show that the MMT is not suitable as a screening instrument for ARM.

3.2.5 CONCLUSIONS

Although the reliability data indicates variability, the MMT has the advantage over, for example, the Amsler grid in that it uses a letter target, has a peripheral fixation aid, and it provides a numerical score. The MMT may be of use in initially determining the size of and monitoring any change in central scotomas. However, because it does not appear to detect a reduction in visual function in ARMD patients that would not have been detected with VA measures alone, it is unlikely to be of use in clinical research.

3.3 Reproducibility of the AREDS ARMD classification system

3.3.1 Purpose

In order to assess the reproducibility of this classification system, it is useful to compare two sets of results that should be the same. In other words, determine whether two observers using the same method of grading obtain the same results. The following classification guide was described by the AREDS investigators^[53] and was used by both observers:

Category 1:

No drusen or nonextensive small drusen only in both eyes (no distinction is made between hard or soft drusen, so the grading was based on size alone).

Category 2:

Extensive small drusen, nonextensive intermediate drusen, or pigment abnormalities in at least one eye.

Category 3:

Large drusen, extensive intermediate drusen, or noncentral GA in at least one eye.

Category 4:

Advanced AMD, or visual acuity less than 20/32 attributable to lesions of nonadvanced ARMD, such as large drusen in the fovea, in only one eye.

Definitions

Drusen size

Small drusen < 1/24 disc diameter

Intermediate drusen $\geq 1/24$ DD but < 1/12 DD

Large drusen $\geq 1/12$ DD

Drusen extent

Small drusen considered to be extensive when their cumulative area within 2 DD of the macular centre had a diameter of at least 1/12 DD.

Intermediate drusen were considered to be extensive when soft indistinct drusen were present and the total area occupied by the drusen was equivalent to the area that would be occupied by 20 drusen each having a diameter of 100 μm . If no soft indistinct drusen were present, intermediate drusen were considered to be extensive when they occupied an area of at least 1/5 DD.

Advanced ARMD

Defined by the presence of at least one of the following features: geographic atrophy, RPE detachment, CNV, or scars of confluent photocoagulation for CNV.

3.3.2 Method

Two optometrists (FE and HB) independently graded 35 images taken from 34 ARMD affected individuals using the Topcon non -mydriatic TRC-NW5S retinal camera. The AREDS classification scale was used and the grading was completed within one day. The images were coded numerically. All images were in TIFF format and were viewed at the same resolution (768 x 576 pixels) on a digital flat screen monitor with a resolution of 1024 x 768 pixels.

3.3.3 Results

The observed frequencies are shown in table 3.2. The bold figures along the diagonal show the observed frequencies of agreement; the corresponding expected frequencies are in brackets.

Table 3.2: Observed (and expected) frequencies of AREDS classification.

		HB				
FE	Grade	1	2	3	4	Total
	1	6 (1.2)	1	0	0	7
	2	0	5 (1.4)	2	0	7
	3	0	1	6 (2.1)	2	9
	4	0	0	0	12 (4.8)	12
	Total	6	7	8	14	35

The kappa statistic is calculated as 0.77. A kappa value of one implies perfect agreement, and $\kappa = 0$ suggests that the agreement is no better than that which would be obtained by chance. The suggested scale for judging the agreement implied by intermediate values is as follows:

Poor	$\kappa \leq 0.20$
Fair	$0.21 \leq \kappa \leq 0.40$
Moderate	$0.41 \leq \kappa \leq 0.60$
Substantial	$0.61 \leq \kappa \leq 0.80$
Good	$\kappa > 0.80$

3.3.3 Conclusions

There appears to be substantial agreement between the two optometrist graders using the AREDS classification system.

3.4 Summary

EMS scores are repeatable to within ± 7 s and MMT scores are repeatable to within ± 7 MMT points. There is substantial agreement between two optometrist graders using the AREDS ARMD classification system. The reliability data has been used in the calculation of sample sizes for the RCT. The grading study supports the use of the AREDS ARMD classification system in the grading of fundus photographs. The next chapter presents the results of the RCT investigating the effect of nutritional supplementation on visual function.

Chapter 4: Randomised controlled trial results

The previous chapters have addressed the scientific background, research rationale, and methods, for an RCT investigating the effect of nutritional supplementation on measures of visual function in normal and ARMD-affected eyes. The results are presented in this chapter, and the results section of the CONSORT checklist is addressed. The intervention groups will be referred to as V (vitamin) and P (placebo).

4.1 Enrolment, duration, and compliance

Out of the 107 people that completed an enrolment pack, 14 did not meet the inclusion criteria or decided not to enrol. The remaining 93 individuals were randomised into the treatment or placebo group, as described in section 2.8; a breakdown is shown in table 4.1.

Table 4.1: Summary of enrolment and follow-up figures.

	Normal		ARMD	
	P	V	P	V
Enrolled	29	29	13	22
Discontinued before first follow-up	4	8	3	2
Attended one follow-up (nine months)	25	21	10	20
Attended two follow-ups (nine and 18 months)	15	14	8	13

Of those participants that discontinued before the first follow-up, eight had health problems, one became pregnant, eight admitted poor compliance or couldn't take the tablets, and one moved out of the area. Statistical analysis does not include those participants that discontinued before the first follow-up. Compliance was assessed by counting remaining tablets at the follow-up visits (see table 4.2).

Table 4.2: Summary of trial duration (mean \pm SD) and compliance (% \pm SD).

	Normal				ARMD			
	9P	18P	9V	18V	9P	18P	9V	18V
Mean trial duration (months)	9.3 \pm 0.8	18.9 \pm 1.8	9.3 \pm 0.9	18.7 \pm 1.8	8.8 \pm 0.4	17.7 \pm 0.4	9.2 \pm 0.5	18.3 \pm 0.6
Mean compliance (% tablets taken)	94.7 \pm 3.3	93.0 \pm 3.7	92.3 \pm 3.4	92.9 \pm 4.0	93.7 \pm 4.4	94.3 \pm 3.6	94.4 \pm 3.9	95.1 \pm 3.2

There was no significant difference in compliance between groups, except for the nine-month comparison within the normal cohort ($z = -2.195$, $p = 0.028$).

Out of those participants taking the placebo tablet, 11 % correctly guessed which tablet they were taking, and 13 % incorrectly guessed. Out of those taking nutritional supplement, 15 % guessed correctly which tablet they were taking, and 12% incorrectly guessed. The remaining participants did not know which group they were randomised to. This suggests that masking was successful.

4.2 Baseline characteristics

The normal cohort ranged in age from 22 to 73 years (mean \pm SD: 50.0 \pm 15.9) and the ARMD cohort ranged in age from 55 to 82 years (mean \pm SD: 69.2 \pm 7.8). Seventy-four percent of the normal cohort and 53 % of the ARMD cohort were female. These values include only those participants who attended for at least one follow-up. Within these cohorts, there was no significant difference in age between groups following randomisation. There was no significant difference in gender between groups, except for the normal 18 month comparison ($X^2 = 4.71$, $p = 0.03$), where the P group contained two males and 13 females and the V group contained seven males and seven females.

All participants were Caucasian. There was no significant difference in eye colour or baseline DVA, NVA, CS, MMT or EMS scores between groups in either cohort. In the ARMD cohort, there was no significant difference in baseline AREDS category between the 18 month groups, but there was a difference for the 9 month groups ($z = - 2.11$, $p = 0.04$). The break-down of grading is shown in table 4.3.

Table 4.3: Summary of baseline AREDS grading by percentage for the nine month ARMD groups.

	Grade 1	Grade 2	Grade 3	Grade 4
9P	40	20	30	10
9V	15	30	20	35

Tables 4.4, 4.5, 4.6, and 4.7 summarise the differences between groups for the normal and ARMD cohorts respectively, for the remaining parametric baseline characteristics.

Table 4.4: Summary of the differences in baseline characteristics between the nine months groups within the normal cohort using the independent samples t-test.

*Pack-years are calculated as the number of packs smoked per day multiplied by the number of years smoked. This calculation was based on 20 cigarettes per pack.

^Dietary lutein was estimated from food frequency questionnaires.

Variable	9P (mean \pm sd) n = 25	9V (mean \pm sd) n = 21	t	P
Age	50.12 \pm 15.1	49.8 \pm 16.4	-0.07	0.95
Smoking history (pack-years)*	4.9 \pm 6.6	3.5 \pm 7.6	-0.47	0.65
Years abroad	0.3 \pm 1.04	1.3 \pm 3.6	-1.35	0.19
Dietary lutein (mg/day)^	14.1 \pm 7.6	10.7 \pm 4.8	1.73	0.09
Dietary vitamin C (mg/day)	139.6 \pm 105.7	112.1 \pm 53.6	1.11	0.30
Dietary vitamin E (mg/day)	6.1 \pm 3.0	6.1 \pm 2.7	-0.01	0.99
Dietary retinol equivalents (μ g/day)	670.8 \pm 249.2	656.9 \pm 250.6	0.18	0.86
Dietary zinc (mg/day)	7.4 \pm 2.1	8.1 \pm 2.4	-1.03	0.31

Table 4.5: Summary of the differences in baseline characteristics between the 18 months groups within the normal cohort using the independent samples t-test.

Variable	18P (mean \pm sd) n = 15	18V (mean \pm sd) n = 14	t	p
Age	48.3 \pm 15.8	46.7 \pm 16.0	0.25	0.80
Smoking history (pack-years)*	2.1 \pm 6.6	4.1 \pm 6.0	-0.84	0.41
Years abroad	0.13 \pm 0.5	0.6 \pm 1.6	-1.04	0.31
Dietary lutein (mg/day)	13.4 \pm 6.5	9.8 \pm 4.7	1.61	0.12
Dietary vitamin C (mg/day)	154.4 \pm 118.3	114.3 \pm 56.1	1.14	0.28
Dietary vitamin E (mg/day)	6.2 \pm 3.4	6.0 \pm 2.5	0.15	0.88
Dietary retinol equivalents (μ g/day)	672.9 \pm 276.5	628.9 \pm 217.3	0.46	0.65
Dietary zinc (mg/day)	7.7 \pm 2.6	7.5 \pm 1.8	0.26	0.80

Table 4.6: Summary of the differences in baseline characteristics between the nine months groups within the ARMD cohort using the independent samples t-test.

Variable	9P (mean \pm SD) n = 10	9V (mean \pm SD) n = 20	t	p
Age (years)	66.7 \pm 7.7	70.5 \pm 7.6	-1.25	0.23
Smoking history (pack years)	7.3 \pm 10.5	13.8 \pm 18.8	-0.90	0.33
Years abroad	0.2 \pm 0.6	0	1.00	0.35
Dietary lutein (mg/day)	11.8 \pm 7.1	10.0 \pm 4.4	0.80	0.43
Dietary vitamin C (mg/day)	161.1 \pm 71.0	88.0 \pm 53.7	3.04	0.005
Dietary vitamin E (mg/day)	7.4 \pm 3.6	6.6 \pm 3.6	0.57	0.57
Dietary retinol equivalents (μ g/day)	842.4 \pm 410.4	679.0 \pm 268.6	1.12	0.27
Dietary zinc (mg/day)	9.9 \pm 2.7	9.3 \pm 2.4	0.58	0.57

Table 4.7: Summary of the differences in baseline characteristics between the 18 months groups within the ARMD cohort using the independent samples t-test.

	18P (mean \pm sd) n = 8	18V (mean \pm sd) n = 13	t	p
Age (years)	66.0 \pm 7.5	70.0 \pm 5.8	1.30	0.21
Smoking history (pack years)	5.6 \pm 8.7	14.0 \pm 19.0	-1.11	0.21
Years abroad	0.6 \pm 1.1	0	1.49	0.18
Dietary lutein (mg/day)	12.9 \pm 6.0	12.0 \pm 4.6	0.38	0.71
Dietary vitamin C (mg/day)	153.0 \pm 73.1	103.4 \pm 69.6	1.48	0.16
Dietary vitamin E (mg/day)	7.5 \pm 4.0	6.6 \pm 4.3	0.43	0.67
Dietary retinol equivalents (μ g/day)	891.6 \pm 443.5	740.2 \pm 297.6	0.89	0.39
Dietary zinc (mg/day)	9.6 \pm 2.9	9.9 \pm 2.7	-0.25	0.80

There was no significant difference between groups for any of the baseline characteristics except for in the ARMD cohort, where the nine month P group consumed significantly more vitamin C than the nine month V group. These conclusions remain the same even when a p value of 0.01 is adopted to correct for the large number of t-tests carried out.

All participants were asked to fill out end-of-trial food diaries and food frequency questionnaires in order to assess any change in dietary habits over the trial period. The forms were sent out on

completion of data collection in March 2005. This means that there was at least nine months between baseline and final dietary analysis for all participants. Within the normal cohort, 80 % of the food diaries and food frequency questionnaires were returned by the nine and 18 month P groups, 86 % were returned by the nine month V group, and 79 % were returned by the 18 month V group. Within the ARMD cohort, 80 % were returned by the nine months P group, 75 % by the 18 month P group, 90 % by the nine month V group, and 77 % by the 18 month V group.

There was no change in dietary lutein, vitamin C, vitamin E, or vitamin A in any of the groups. The normal nine month P group had a significant change in mean zinc intake from (mean \pm SD) 6.82 ± 2.15 mg to 10.26 ± 4.39 mg ($t = -3.54$, $df = 24$, $p = 0.002$). The ARMD nine month V group had a significant change in mean zinc intake from 9.17 ± 2.44 mg to 11.41 ± 3.64 mg ($t = -2.912$, $df = 19$, $p = 0.04$). There was no significant change in dietary zinc over time for any other group. There was no change in ocular health, apart from changes associated with ARMD, in either cohort. No participants within the normal cohort developed ARMD-related ocular changes.

Participants were asked to provide details of additional nutritional supplementation at baseline. Not all participants were able to specify the amounts of nutrients within their supplements. As a result, chi-squared analysis rather than the independent samples t-test has been carried out to investigate any differences in supplementation between groups. The results for the normal cohort are shown in table 4.8, and for the ARMD cohort are shown in table 4.9. No differences were found between groups for either cohort.

Table 4.8: Summary of the differences in additional nutritional supplementation by group in the normal cohort using the chi-squared test.

Supplement	9P	9V	X ²	P	18P	18V	X ²	p
Multivitamin	7	6	0.004	0.95	4	4	0.01	0.99
Vitamin C	2	0	1.74	0.08	0	0		
Vitamin E	0	2	1.74	0.19	0	0		
Vitamin D	0	1	0.64	0.27	0	1	0.60	0.29
Vitamin B12	0	0			0	0		
Lutein	3	2	0.08	0.78	1	2	0.55	0.44
Zinc	0	0			0	0		
Selenium	1	0	0.86	0.35	0	0		
Calcium	1	1	0.06	0.90	1	1	0.002	0.97
Cod liver oil	2	2	0.004	0.83	1	3	1.41	0.24
GLA	1	0	0.86	0.35	1	0	0.96	0.33
Ginkgo biloba	0	0			0	0		
Omega 3 EFA	1	0	0.86	0.35	0	0		
Glucosamine	0	2	1.74	0.19	0	1	0.60	0.29
Folic acid	1	0	0.86	0.35	0	0		
Starflower	1	0	0.86	0.35	1	0	0.96	0.33
Garlic	1	1	0.06	0.90	0	0		
Evening Primrose	0	0			0	0		
Royal Jelly	0	0			0	0		
Ginseng	0	0			0	0		
Flaxseed	0	1	0.64	0.27	0	1	0.60	0.29
Eye-Vites	0	0			0	0		
Retinace	0	0			0	0		
Retinex	1	0	0.86	0.35	1	0	0.96	0.33
I-Caps	0	0			0	0		
Boots Healthy Eyes	0	0			0	0		
VisionAce	0	0			0	0		

Table 4.9: Summary of the differences in additional nutritional supplementation by group in the ARMD cohort using the chi-squared test.

Supplement	9P	9V	X ²	P	18P	18V	X ²	p
Multivitamin	5	5	1.96	0.16	4	5	0.30	0.59
Vitamin C	0	0			0	1	0.64	0.42
Vitamin E	0	0			0	0		
Vitamin D	0	0			0	0		
Vitamin B12	0	1	0.51	0.48	0	1	0.64	0.42
Lutein	4	5	0.71	0.40	4	5	0.83	0.37
Zinc	1	2	0	1	0	2	1.50	0.22
Selenium	0	2	1.08	0.30	0	2	1.50	0.22
Calcium	0	0			0	0		
Cod liver oil	3	5	0.07	0.80	2	3	0.01	0.92
GLA	0	0			0	0		
Ginkgo biloba	0	1	0.51	0.48	0	1	0.64	0.42
Omega 3 EFA	0	1	0.51	0.48	0	1	0.64	0.42
Glucosamine	0	1	0.51	0.48	0	2	1.50	0.22
Folic acid	0	1	0.51	0.48	0	1	0.64	0.42
Starflower	0	0			0	0		
Garlic	1	2	0	1	1	2	1.35	0.71
Evening Primrose	0	0			0	1	0.64	0.42
Royal Jelly	0	0			0	1	0.64	0.42
Ginseng	0	0			0	0		
Flaxseed	0	0			0	1	0.64	0.42
Eye-Vites	1	0	2.09	0.15	1	0	1.71	0.19
Retinace	0	1	0.51	0.48	0	1	0.64	0.42
Retinex	0	1	0.51	0.48	0	1	0.64	0.42
I-Caps	1	1	0.26	0.61	1	1	0.14	0.71
Boots Healthy Eyes	0	0			0	0		
VisionAce	1	2	0	1	0	2	1.50	0.21

In the normal cohort, 73.9 % of the nine month group and 69.0 % of the 18 month group were female, 10.9 % of the nine month group and 13.8 % of the 18 month group were smokers, and 19.6 % of the nine month group and 17.2 % of the 18 month group had previously smoked. In the ARMD cohort, 53.3 % of the nine month group and 57.1 % of the 18 month group were female, 3.3 % of the nine month and 0 % of the 18 month group were smokers, and 53.3 % of the nine month and 57.1 % of the 18 months group had previously smoked.

4.3 Results

For each outcome measure, the difference between baseline and follow-up was calculated. Each data set was checked for normality of distribution using SPSS software. This function provides the Kolmogorov-Smirnov statistic, which assesses the normality of distribution of the data. A non-significant result indicates normality. When the data set was normally distributed, an independent samples t-test was used for analysis. When the data set was not normally distributed, the non-parametric Mann-Whitney U test was used for analysis. There were no significant differences between groups at nine or 18 months for either cohort. Fundus photographs were graded as AREDS grade one for all images taken from the normal cohort. For the ARMD cohort, the fundus image grading data is included in the tables. Results for the normal cohort are shown in tables 4.10 and 4.11 and those for the ARMD cohort are shown in tables 4.12 and 4.13.

Table 4.10: Summary of the change in each outcome measure over nine months in the normal cohort.

Outcome measure	9P (mean ± sd) n = 25	9V (mean ± sd) n = 21	Test statistic	p
DVA	0.00 ± 0.04	0.00 ± 0.06	z = - 0.11	0.99
NVA	-0.04 ± 0.05	0.01 ± 0.08	z = - 1.50	0.13
CS	0.22 ± 0.56	0.07 ± 0.33	z = - 1.68	0.09
MMT	-0.72 ± 2.01	-0.15 ± 4.87	z = - 1.79	0.74
EMS	0.92 ± 6.32	0.10 ± 8.10	z = - 0.10	0.92

Table 4.11: Summary of the change in each outcome measure over 18 months in the normal cohort

Outcome measure	18P (mean ± sd) n = 15	18V (mean ± sd) n = 14	Test statistic	p
DVA	-0.01 ± 0.07	-0.01 ± 0.05	z = - 0.11	0.91
NVA	-0.01 ± 0.09	0.00 ± 0.07	z = - 0.20	0.84
CS	0.11 ± 0.13	0.05 ± 0.13	z = - 1.85	0.07
MMT	-0.30 ± 1.46	1.82 ± 4.25	z = - 1.30	0.19
EMS	0.53 ± 3.96	2.86 ± 3.38	t = - 1.63	0.11

Table 4.12: Summary of the change in each outcome measure over nine months in the ARMD cohort.

Outcome measure	9P (mean ± sd) n = 10	9V (mean ± sd) n = 20	Test statistic	p
DVA	- 0.02 ± 0.07	0.01 ± 0.07	t = - 0.92	0.33
NVA	- 0.07 ± 0.09	- 0.02 ± 0.11	t = - 1.35	0.19
CS	0.07 ± 0.07	0.01 ± 0.25	z = - 0.83	0.41
MMT	- 0.4 ± 7.7	- 5.9 ± 14.4	t = 1.09	0.29
EMS	- 3.40 ± 6.86	3.95 ± 18.54	t = - 1.17	0.25
Photograph grade	0.3 ± 0.1	0.1 ± 0.3	z = - 0.04	0.98

Table 4.13: Summary of the change in each outcome measure over 18 months in the ARMD cohort.

Outcome measure	18P (mean ± sd) n = 8	18V (mean ± sd) n = 13	Test statistic	p
DVA	- 0.05 ± 0.06	0.08 ± 0.21	z = - 1.39	0.17
NVA	- 0.10 ± 0.13	0.02 ± 0.21	t = - 1.37	0.19
CS	0.09 ± 0.27	- 0.01 ± 0.23	t = 0.81	0.43
MMT	- 1.44 ± 4.75	- 8.42 ± 25.10	z = - 0.11	0.91
EMS	- 5.88 ± 9.74	10.00 16.00	z = - 0.65	0.51
Photograph grade	0.5 ± 0.5	0.3 ± 0.5	z = -0.86	0.50

For those participants who attended two follow-up visits, a mixed between-within subjects ANOVA was carried out. This facilitated analysis of the effect of the between-subjects variable (group), and the within-subjects variable (time), on the outcome measures (dependent variable). This analysis tests whether there are main effects for each of the independent variables and whether there is significant interaction between the two. In this study it shows whether there is a change in each outcome measure over time (main effect for time), if there is a difference in the effect on each outcome measure between the two interventions (main effect for group), and also whether there is the same change in scores over time for the two groups (interaction effect). There is no non-parametric alternative to the mixed between-within subjects ANOVA, and so the analysis has not been carried out for the MMT scores or fundus photograph grading. The ANOVA results for the normal cohort are shown in table 4.14 and those for the ARMD cohort are shown in table 4.15.

Table 4.14: Mixed between-with subjects ANOVA results for the normal cohort.

Outcome measure	Main effect: time		Main effect: group		Interaction effect	
	F _(1,27)	P	F _(1,27)	p	F _(1,27)	p
DVA	1.352	0.267	1.632	0.212	2.521	0.266
NVA	1.101	0.340	0.275	0.604	2.702	0.076
CS	3.693	0.033	0.745	0.396	2.235	0.117
EMS	4.733	0.013	0.695	0.412	0.926	0.370

Table 4.15: Mixed between-with subjects ANOVA results for the ARMD cohort.

Outcome measure	Main effect: time		Main effect: group		Interaction effect	
	F _(1,19)	p	F _(1,19)	p	F _(1,19)	p
DVA	0.328	0.723	1.753	0.202	1.047	0.362
NVA	1.127	0.335	2.652	0.121	1.032	0.367
CS	0.396	0.676	1.564	0.227	1.130	0.334
EMS	3.943	0.029	2.497	0.131	3.870	0.030

In the normal cohort, there was a significant change in CS and EMS score over time, but there was no difference between the two groups, and no difference in the change in these outcome measures over time between the two groups. Eta squared describes the effects size. The following guidelines can be used for interpretation: 0.01 = small effect, 0.06 = moderate effect, 0.14 = large effect. The effect size for the change over time was moderate for CS (Eta squared = 0.119) and large for the EMS (Eta squared = 0.149). In the ARMD cohort, there was a significant change in EMS over time (Eta squared = 0.179), and also a significant difference in the change in EMS score over time between the two groups (Eta squared = 0.177). Group allocation itself had no significant effect on EMS score in the ARMD cohort. Figures 4.1 to 4.4 illustrate the mean change in DVA, NVA, CS, and EMS with time for each group.

Figure 4.1: Change in DVA with time for the ARMD and normal 18 month cohorts.

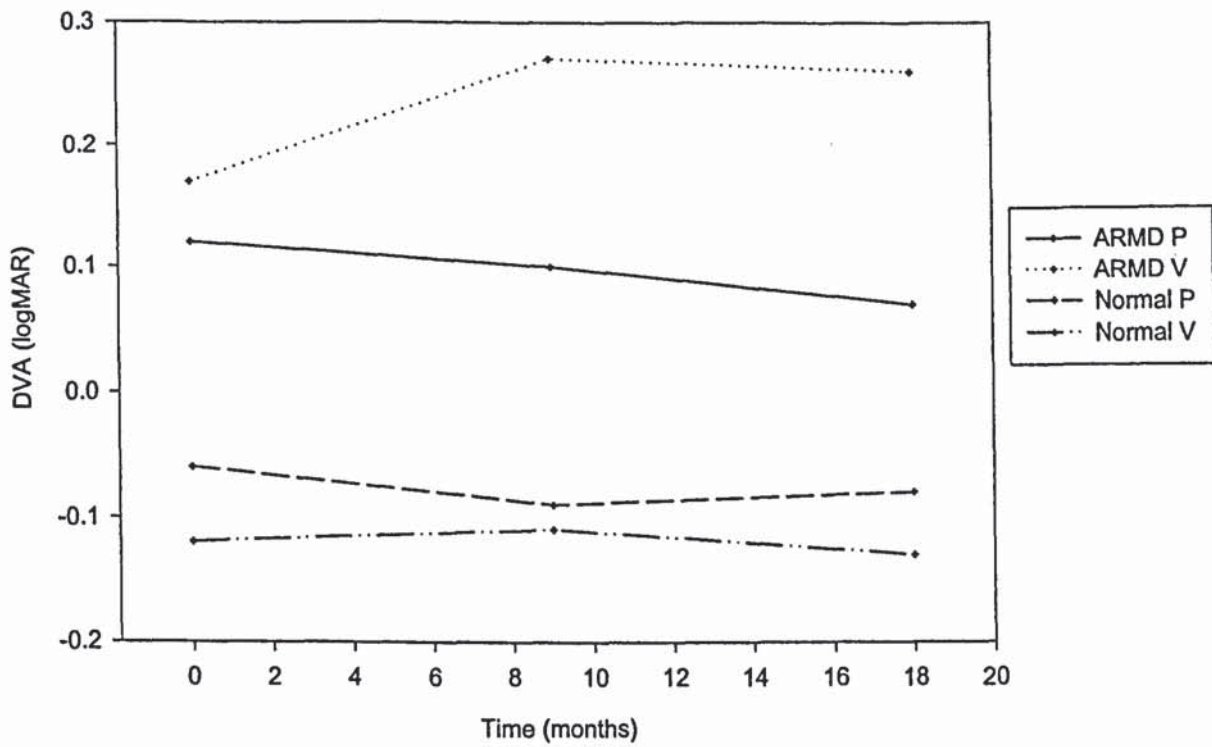


Figure 4.2: Change in NVA with time for the ARMD and normal 18 month groups.

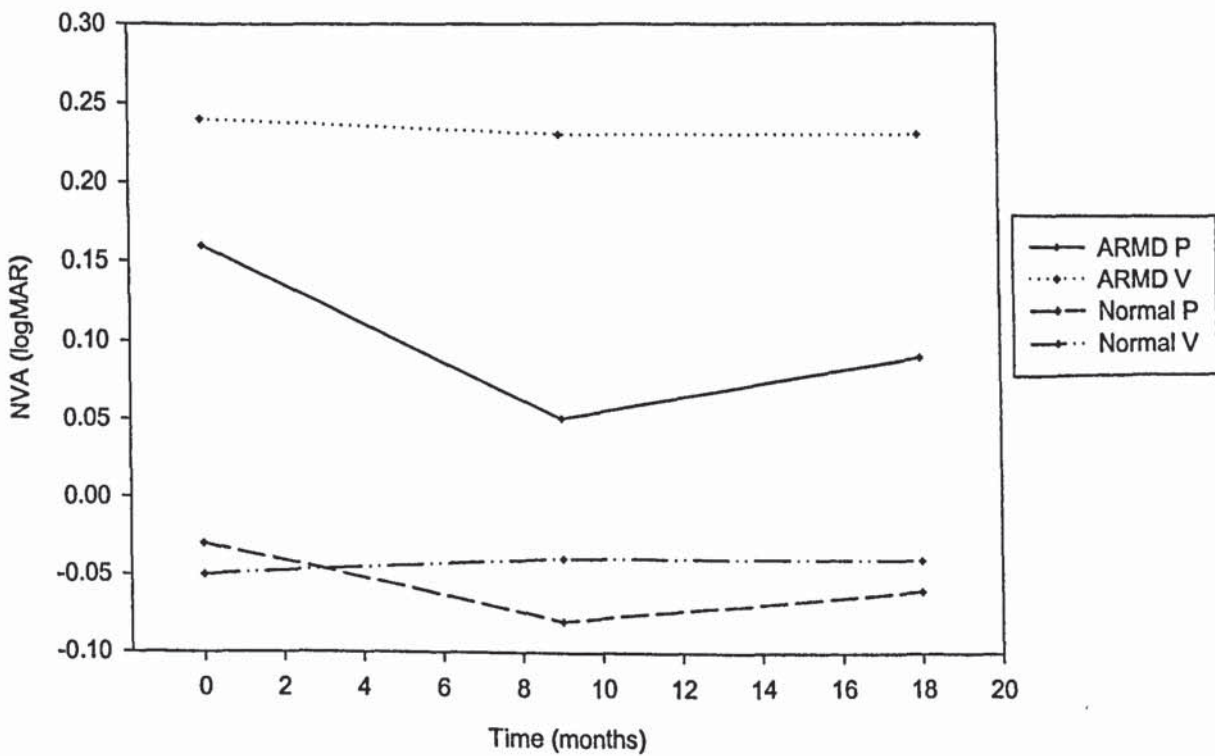


Figure 4.3: Change in CS with time for ARMD and normal 18 month groups.

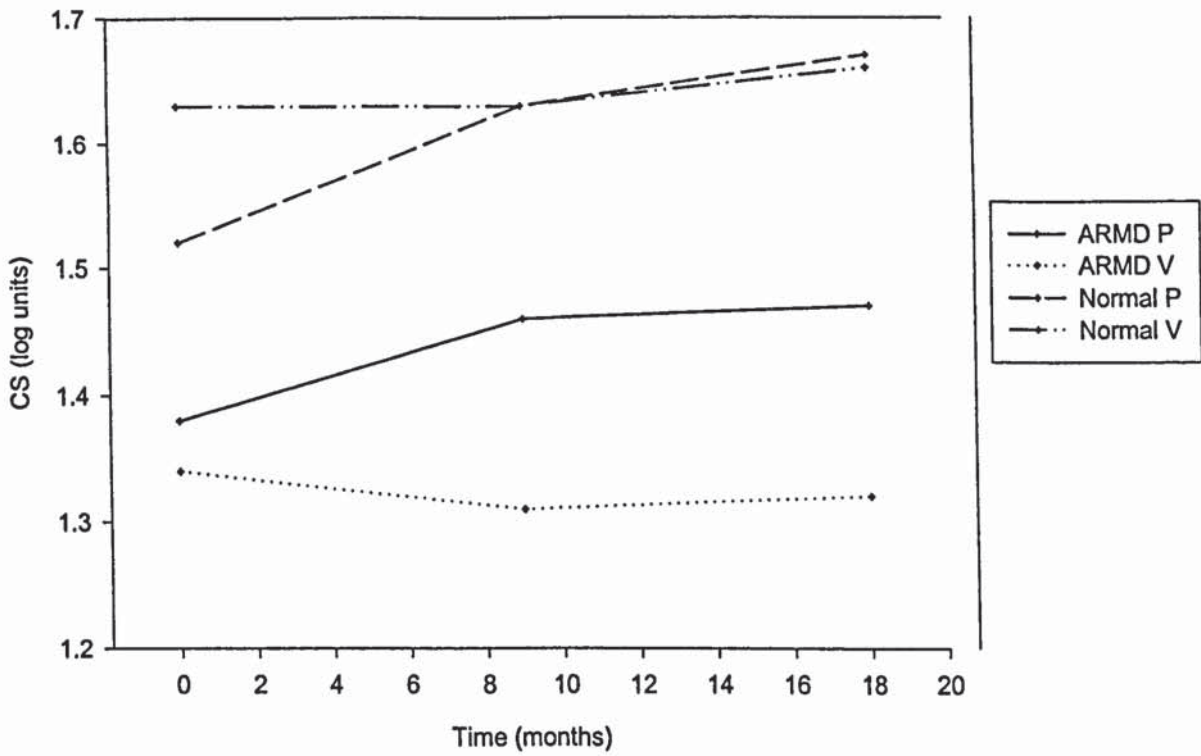
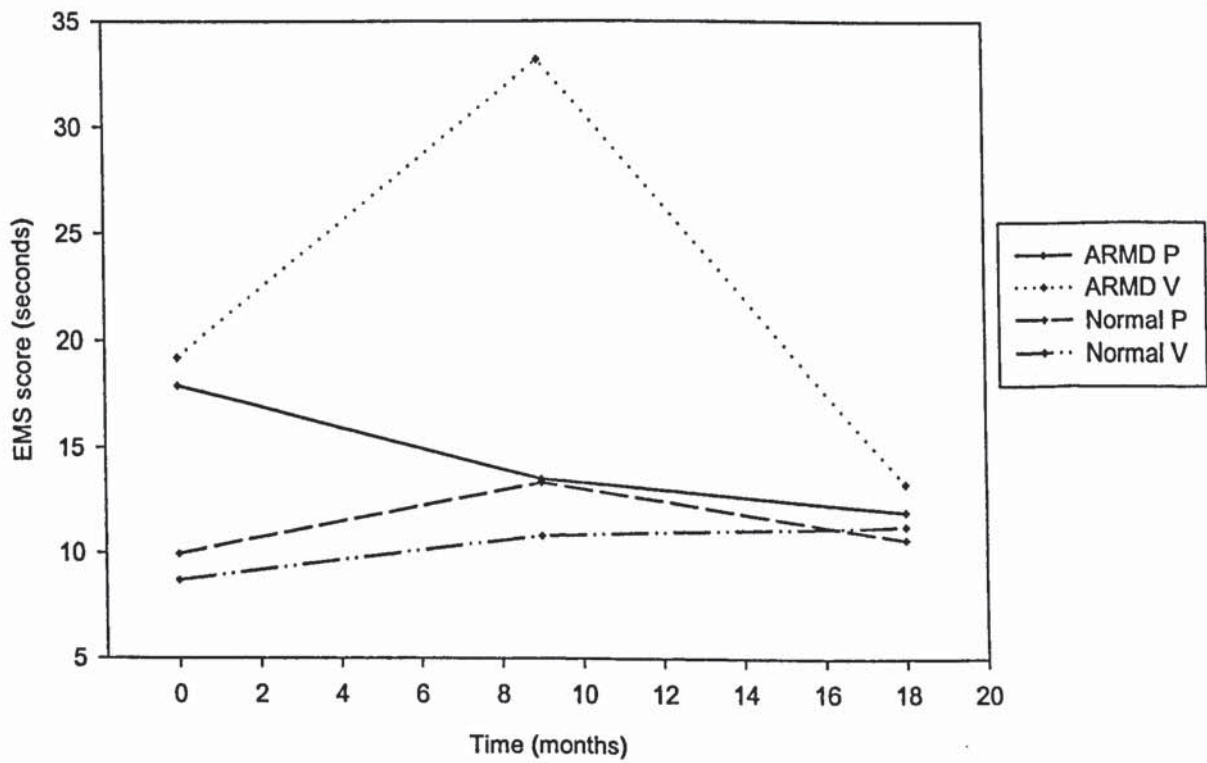
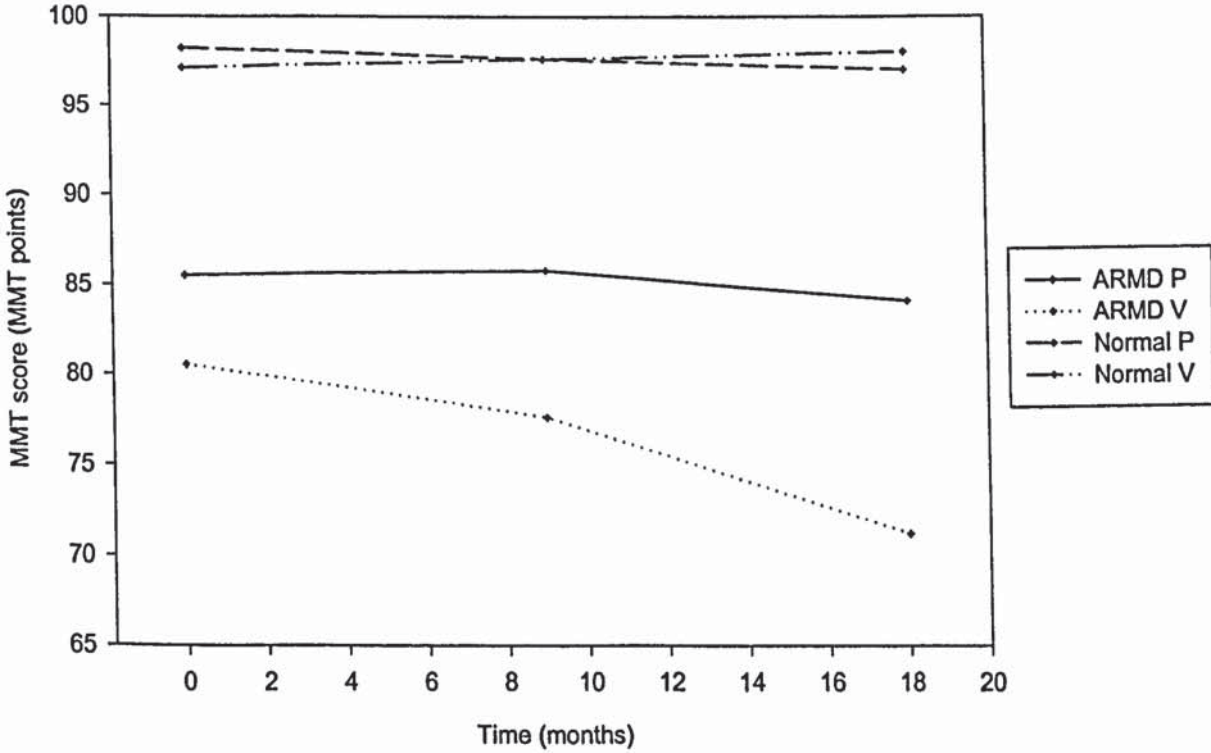


Figure 4.4: Change in EMS score with time for the ARMD and normal 18 month groups.



In order to investigate the effect of time on MMT scores and photograph grading, Friedman tests were carried out for both of the 18 month groups. This is the non-parametric alternative to the repeated-measures ANOVA. There was no significant change in MMT score over time for either group in either cohort. Figure 4.5 illustrates the mean change in MMT score with time for each group.

Figure 4.5: Change in MMT score with time for the ARMD and normal 18 month groups.



There was, however, a significant increase in AREDS photograph grade for the 18V group ($X^2 = 8.40, p = 0.015$) and a just significant increase for the 18P group ($X^2 = 6.00, p = 0.05$), although these changes were not clinically significant (see table 4.16).

Table 4.16: Summary of the change in AREDS photograph grade from baseline to second follow-up for the 18 months ARMD groups.

Group	Photograph AREDS grade (mean \pm SD)		
	Baseline	First follow-up	Second follow-up
18P	2.1 \pm 1.1	2.4 \pm 1.0	2.6 \pm 1.1
18V	2.8 \pm 0.9	2.9 \pm 0.9	3.2 \pm 0.9

In order to assess the effect of time on the outcome measures over nine months, paired samples t-tests were carried out for DVA, NVA, CS, and EMS score. The Wilcoxon Signed Rank test was carried out for MMT scores. The results for the normal cohort are shown in tables 4.17 and 4.18, and for the ARMD cohort are shown in tables 4.19 and 4.20.

Table 4.17: Summary of the change in each outcome measure over nine months in the normal P group (n = 25).

	Baseline (mean \pm SD)	Nine months (mean \pm SD)	Test statistic	p
DVA	-0.08 \pm 0.10	-0.10 \pm 0.10	t = 2.09	0.047
NVA	-0.04 \pm 0.09	-0.08 \pm 0.08	t = 2.96	0.007
CS	1.55 \pm 0.23	1.63 \pm 0.12	t = -1.87	0.07
MMT	97.78 \pm 3.57	97.06 \pm 3.92	z = -2.15	0.031
EMS	11.24 \pm 4.51	12.16 \pm 5.69	t = -0.713	0.48

Table 4.18: Summary of the change in each outcome measure over nine months in the normal V group (n = 21).

	Baseline (mean \pm SD)	Nine months (mean \pm SD)	Test statistic	p
DVA	-0.09 \pm 0.11	-0.09 \pm 0.09	t = 0.46	0.65
NVA	-0.03 \pm 0.09	-0.04 \pm 0.08	t = -0.69	0.50
CS	1.60 \pm 0.09	1.61 \pm 0.09	t = -0.61	0.55
MMT	96.26 \pm 6.84	95.88 \pm 9.26	z = -0.54	0.59
EMS	12.00 \pm 10.32	10.48 \pm 3.96	t = 0.75	0.46

Table 4.19: Summary of the change in each outcome measure over nine months in the ARMD P group (n = 10).

	Baseline (mean ± SD)	Nine months (mean ± SD)	Test statistic	p
DVA	0.09 ± 0.13	0.07 ± 0.16	t = 0.85	0.42
NVA	0.14 ± 0.15	0.02 ± 0.14	t = 3.02	0.01
CS	1.42 ± 0.19	1.50 ± 0.17	t = -2.52	0.03
MMT	88.4 ± 15.24	88.33 ± 13.58	z = 0	1
EMS	16.60 ± 11.06	13.30 ± 7.32	t = 1.44	0.18

Table 4.20: Summary of the change in each outcome measure over nine months in the ARMD V group (n = 20).

	Baseline (mean ± SD)	Nine months (mean ± SD)	Test statistic	p
DVA	0.18 ± 0.30	0.24 ± 0.32	t = -1.04	0.31
NVA	0.25 ± 0.21	0.24 ± 0.23	t = 0.23	0.82
CS	1.31 ± 0.26	1.31 ± 0.28	t = -0.05	0.96
MMT	79.50 ± 25.89	73.62 ± 24.77	z = -1.72	0.09
EMS	19.55 ± 11.84	25.50 ± 22.18	t = -1.09	0.29

There were significant improvements over nine months in DVA (Eta squared = 0.15), NVA (Eta squared = 0.27), and MMT (Eta squared = 0.16) in the normal P group. There were significant improvements in NVA (Eta squared = 0.28) and CS (Eta squared = 0.21) in the ARMD P group.

4.4 Adverse effects

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

4.5 Summary

No effect of antioxidant supplementation on the outcome measures has been demonstrated over nine or 18 months in either the normal or ARMD cohort.

Chapter 5: Discussion

5.1 Main outcomes

This clinical trial was designed to evaluate the effect of a nutritional supplement containing 6mg lutein, 750 μ g vitamin A, 250 mg vitamin C, 34 mg vitamin E, 10 mg zinc, and 0.5 mg copper on clinical measures of visual function. Participants were divided into two cohorts; those with no ocular abnormality and those with ARMD. The outcome measures were DVA, NVA, CS, MMT, EMS and fundus photography. The study demonstrated that this nutritional supplement had no effect on the outcome measures over nine or 18 months in normal or ARMD affected participants.

These results are not consistent with those of the AREDS ^[58] or the LAST ^[59]. The AREDS investigators reported that the risk of progression to advanced AMD in people presenting with extensive intermediate drusen, at least one large druse, noncentral GA, in one or both eyes, or advanced AMD or vision loss due to AMD in one eye was reduced by 17 % in those taking antioxidants, 21 % in those taking zinc, and 25 % in those taking a combination of antioxidants plus zinc, for an average of 6.3 years. They concluded that people with these characteristics of ARMD should consider taking a supplement containing 500 mg vitamin C, 400 IU vitamin E, 15 mg beta-carotene, 80 mg zinc, and 2 mg copper, unless they are contraindicated from any of the constituents. The AREDS trial formulation contained high levels of vitamins C and E, and zinc. In the Aston study formulation, nutrients were included at levels that were unlikely to increase people's daily intake above advised safe limits. In this way, the risk of toxicity and adverse effects was reduced.

The LAST investigators concluded that supplementing with 10 mg lutein, or 10 mg lutein in combination with other carotenoids and antioxidants/minerals for 12 months significantly improved MPOD, glare recovery and contrast sensitivity function, and improved NVA.

Ninety patients were enrolled in the LAST and 3640 were enrolled in the AREDS from 11 centres. Both of these studies evaluated white populations. It is difficult to compare this study with either the AREDS or the LAST. Far fewer participants were enrolled in the Aston study than in AREDS, and this study population was mixed gender compared with the predominantly male LAST study population.

The data were also analysed to investigate the effect of time on visual function. Although the trial duration was relatively short compared with the AREDS, changes in CS and EMS score were determined using a mixed between-within subjects ANOVA. The trial duration is greater than the LAST for the 18 month groups.

In the normal cohort there was a significant change over 18 months in CS score (from 1.53 ± 0.28 to 1.67 ± 0.10 log units in the P group, and from 1.63 ± 0.04 to 1.66 ± 0.04 log units in the V group) and EMS score (from 9.93 ± 3.53 to 10.67 ± 4.13 s for the P group, and from 8.71 ± 2.16 to 11.29 ± 4.05 s for the V group). This represents an overall improvement in CS scores, but deterioration in EMS scores, although these changes are not clinically significant. Taking the antioxidant formulation had no significant effect on CS or EMS score.

In the ARMD cohort, there was a significant change in EMS score over 18 months (from 17.88 ± 12.09 to 12.00 ± 5.53 s in the P group, and from 19.17 ± 11.06 to 13.33 ± 7.80 s in the V group). It is difficult to account for this overall improvement in EMS scores. A learning effect is unlikely due to the low number of, and the nine month interval between, data collection points. Taking the antioxidant formulation had no effect on EMS score. Friedman tests were carried out to assess the effect of time on AREDS grading. There were statistically significant increases in grade for both the antioxidant and placebo 18 month groups. These changes were not clinically significant, i.e., they were less than one grade. The placebo group changed from grade 2.1 ± 1.1 at baseline to grade 2.6 ± 1.1 at 18 months. The antioxidant group changed from grade 2.8 ± 0.9 at baseline to grade 3.2 ± 0.9 at 18 months.

5.2 Limitations

The main limitation of the ARMD arm of this trial is the low number of participants. The initial aim was to recruit 250 ARMD-affected participants. This aim was considered reasonable, especially considering links with local optometrists, ophthalmologists, and a low vision centre. Recruitment was much more difficult than anticipated, despite local and national publicity, as described in section 2.2.

Power calculations were initially based on using DVA, NVA, and CS as outcome measures. Reliability studies were carried out to allow the EMS and MMT to be included in the power analysis. Retrospectively, the sample sizes required to 80 % chance of detecting a clinically significant effect at the 5 % significance level are shown in table 5.1.

Table 5.1: Sample sizes required for the normal and ARMD cohorts.

	Normal		ARMD	
	Effect size	Sample size	Effect size	Sample size
DVA (logMAR)	0.10	13	0.10	94
NVA (logMAR)	0.10	10	0.10	70
CS (log units)	0.30	2	0.30	9
MMT (MMT points)	14	1	14	45
EMS (seconds)	14	1	14	11
AREDS grade	N/A	N/A	1	20

Effect sizes of 0.1 logMAR were selected for the VA outcomes as a change of five letters has been reported to indicate a significant change ^[253]. Confidence limits may, however be broader for patient groups in whom VA are less consistent, such as the ARMD cohort. For this reason, the sample size calculations were repeated for the ARMD cohort using 0.20 logMAR as the effect size. This yielded required sample sizes of 23 for DVA and 18 for NVA, which were still greater than the number of ARMD participants enrolled. These power calculations will be of use to investigators carrying out research in the area of ARMD.

The intention to recruit 250 ARMD-affected participants related positively to the sample sizes suggested. Power analysis is used to determine the probability that a given study will reject the null hypothesis when it is, in fact, false. In other words, power analysis determines the chance of detecting a true-positive result. Traditionally, a study is considered to be adequately powered if it has at least an 80 % chance of detecting a clinically significant effect when one exists.

Underpowered clinical trials may not adequately test the underlying hypothesis, and so have been described as 'scientifically useless' ^[281]. It has been argued that they are unethical in their exposure of participants to the risks of research ^[281, 282]. It is suggested that the power of the trial to detect an effect should be conveyed to the participants at the beginning of the trial, before they give their informed consent. In this case, power calculations had been carried out at the start of the trial, and the sample sizes suggested were considered to be attainable. The recruitment drive was extensive. In hindsight, the potential for recruitment via the referral of suitable candidates from local optometrists and ophthalmologists was over-estimated. The reluctance of ARMD patients to travel to the University due to mobility problems and cost was underestimated. One incentive for participation may have been cash payment, although this is unlikely to have made any significant improvement to recruitment of ARMD patients. Reimbursement of travel costs may have been beneficial.

The normal arm of the trial was adequately powered. For this cohort, we can conclude that there was no difference in the effect on outcome measures between the placebo and antioxidant interventions.

The choice of intervention formulation may also be considered a limitation. It could be argued that it would be advantageous to compare a single active ingredient against a control. The mixed antioxidant formulation does not permit investigation of the effect of specific nutrients on visual function. The reasons for using a multi-ingredient formulation include the fact that ARMD is of multifactorial aetiology, and so may be affected by more than one nutrient, and also that nutrients are thought to work synergistically together. Relevant examples of this synergism include the mutually dependent recycling of vitamin C, vitamin E ^[283], and facilitation of vitamin A transport from the liver by zinc ^[284].

Another limitation is the fact that the baseline characteristics of the study population may not accurately reflect those of the general population. It could be that those people with ARMD who volunteer for research projects such as this, are generally better informed and have a greater interest in prevention strategies. For example, the ARMD-affected participants may have been less likely to smoke and more likely to take nutritional supplements or maintain a healthy, balanced diet than the general ARMD-affected population. It is interesting that within the ARMD cohort, 3.3 % of the nine month group and 0 % of the 18 month group were current smokers. However, 53.3 % of the nine month and 57.1 % of the 18 month group had previously smoked. It could be that nutritional supplementation is of benefit to those ARMD-affected people who are less healthy than those in this cohort.

Serum antioxidant checks were considered during the design of the trial, and the lack of such testing may be considered a limitation. However, the main reason for not testing the serum is because it only provides a short-term indication of blood antioxidant levels and so would not provide any additionally useful information about compliance. The decay half-life of lutein is reported to be between 10 and 14 days ^[285]. Studies have shown that lutein supplementation increases serum lutein levels ^[223, 224, 286], and MPOD ^[223, 224, 231, 287], however, a poor correlation has been reported between serum lutein levels and MPOD ^[287]. The invasive nature of the procedure may have hindered recruitment further.

5.3 Confounding variables

The randomised controlled trial design was chosen to investigate the research question due to its ability to demonstrate causality. Random assignment of the intervention limits the influence of confounding variables, and double-masking eliminates the possibility that observed effects are due to bias or other treatments.

Baseline data collection included the completion of a health questionnaire, food diary, and food frequency questionnaire. These enabled differences in baseline characteristics to be investigated. There were no differences between placebo and antioxidant groups in baseline age, smoking history, iris colour, years spent living abroad, dietary vitamin E, retinol equivalents, lutein, and zinc, DVA, NVA, CS, MMT, and EMS in either cohort. There was no difference between groups in either cohort for baseline dietary supplementation.

There was no significant difference in gender between groups in either cohort, except for the normal 18 month comparison, where the placebo group contained a majority of females and the antioxidant group contained an equal gender split. There is no reason to believe that the results would have been different if the placebo group had contained a more equal number of males and females.

Within the normal cohort, the nine month placebo group consumed significantly more dietary vitamin C than the antioxidant group. It could be argued that this confounds the results and that a difference between the groups may have been determined if the baseline dietary intake of those nutrients contained in the study formulation had been the same for both groups.

End of trial dietary analysis was carried out on at least 75 % of the participants in each group. There was no change with time in dietary lutein, vitamins C and E, and retinal equivalents in any of the groups. A mean increase in dietary zinc intake was shown in the normal nine month placebo group, and the ARMD nine month antioxidant group. Again, it could be argued that this change in dietary nutrient intake confounded the results of the trial.

5.4 Improvements

It could be argued that longer trial duration was required to determine an intervention effect. However, the LAST reported a positive effect of antioxidant supplementation over 12 months, and so investigation of short term effects of nutritional supplementation on visual function is justified.

Measurement of MPOD would have enhanced the project by directly assessing the effect of lutein supplementation on its deposition in the retina. Studies indicate that MP increases remain stable for significant time periods, many months or years, after supplementation is discontinued [223, 224]. Assessment of the central visual field may have been better served by microperimetry than the MMT, although this instrument was not available for use at the start of the trial. The reliability investigation into the EMS suggests that this was not the best choice of test for assessing PSRT. A more reliable bleaching method would have been macular inspection with a direct ophthalmoscope for 30 s.

5.5 Conclusions

The results of this study suggest that there is no beneficial effect of supplementation with 6mg lutein, 750 μ g vitamin A, 250 mg vitamin C, 34 mg vitamin E, 10 mg zinc, and 0.5 mg copper on visual function in people with healthy eyes. They also suggest that there is no beneficial effect of nine months of daily supplementation with this formulation on CS in ARMD-affected eyes. The trial was not powered to assess the effects of this formulation on DVA, NVA, EMS, and MMT in ARMD-affected eyes.

CS is a particularly relevant outcome measure of for those with ocular disease as it provides an indication of real world visual function [288]. The finding that nine months of antioxidant supplementation, in this case, has no significant effect on CS adds to the literature, and contrasts with the AREDS [104] and the LAST [59]. It could be argued that the positive effect demonstrated by the LAST occurred because 10 mg of lutein was used, compared with the 6 mg used in this study. The recommended daily intake of lutein was 6 mg when the trial

formulation was being designed ^[289]. Future work might involve investigating the effect of lutein supplementation on macular pigment levels, looking at differences between lutein tablet supplementation compared with dietary modification, and investigating the optimum daily intake of lutein.

This project has added to the debate about the use of nutritional supplementation prior to the onset of ARMD. A positive effect of supplementation in the normal cohort may have suggested that there is a role for nutritional supplementation in the prevention or delaying of onset of ARMD. It is impossible to support this hypothesis with the results obtained.

When asked for nutritional advice with respect to ARMD, practitioners should be guided by the results of RCTs. Patients should be advised to consult their GP before commencing a supplementation regime if they are taking prescribed medication. As optometrists, our aim should be to advise patients of the risk factors for ARMD, and to promote the importance of maintaining a healthy, well-balanced diet.

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Appendix

Peer reviewed publications

Title	Page
Bartlett H, Eperjesi F, Ali A, & Fowler CW (2003) <i>Risk Factors Associated with Age-Related Macular Disease</i> Optometry in Practice 5:15-32	94
Bartlett H & Eperjesi F (2004) <i>An Ideal Ocular Nutritional Supplement?</i> Ophthalmic and Physiological Optics 24:339-349	111
Bartlett H & Eperjesi F <i>Possible contraindications and adverse reactions associated with the use of ocular nutritional supplements</i> Ophthalmic and Physiological Optics (In review).	122
Bartlett H & Eperjesi F (2004) <i>A review of the role of carotenoids in ocular disease</i> AgroFOOD Industry HiTech November/December: 19-21	138
Bartlett H & Eperjesi F (2003) <i>Age-related macular degeneration and nutritional supplementation: a review of randomised controlled trials</i> Ophthalmic and Physiological Optics 23:383-399	141
Bartlett H & Eperjesi F (2003) <i>A randomised controlled trial investigating the effect of nutritional supplementation on visual function in normal, and age-related macular disease affected eyes: design and methodology [ISRCTN78467674]</i> Nutrition Journal 2:12	158
Bartlett H & Eperjesi F (2004) <i>Dietary analysis and patterns of nutritional supplement use in normal and age-related macular disease affected subjects: a prospective cross-sectional study</i> Nutrition Journal 3:16	165
Bartlett H, Davies LN & Eperjesi F (2004) <i>Reliability, normative data, and the effect of age-related macular disease on Eger Macular Stressometer photostress recovery time</i> Ophthalmic and Physiological Optics 24:594-599	172

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Appendix 2

Patient information leaflets

Dear Sir/Madam

I am writing with regard to a new research project taking place at Aston University. We are investigating the effects of nutrition on the progression of age-related macular degeneration (ARMD). We need people with and without this condition to act as volunteers.

If you are interested in taking part you should complete and return all the forms in this pack. We will look at these and if you are suitable to take part we will contact you to arrange a 'recruitment appointment'.

At this first appointment several measurements will be taken including how well you see straight ahead, how well you see to the side, and a photograph of the structures inside the eye. In order for us to obtain a good quality photograph we may need to dilate the pupil (make it bigger) of one of your eyes. This will involve an eye drop. These tests are routine and you may already have experienced them during visits to your optometrist or consultant.

If these tests suggest that you are suitable to take part in the project you will be enrolled and issued with nine months worth of tablets. These will either contain the new active ingredient that we are

testing or a harmless ingredient. Neither you, or the person issuing the tablets will know which you have been given.

In nine months we will contact you again to make a review appointment. Most of the tests carried out at the first appointment will be repeated but you will not have the pupil dilated. You will be issued with a further nine months worth of tablets which will be the same as the initial supply.

You will be contacted again in nine months time for the final review where the measurements will be repeated again. We will take another photograph of the eye at this appointment so your pupil may be dilated.

In summary, to take part in the project you will have to be prepared to attend the university on three occasions to have several standard eye measurements made. On the first and last visit we may dilate the pupil of one eye with special drops. You will also need to agree to take one tablet per day for eighteen months. If you do not feel happy with these requirements then it is best not to apply for enrolment. Unfortunately, we will not be able to reimburse your travel costs.

If you have any queries please contact Hannah Bartlett on 07952 685884. Alternatively, e-mail bartlehe@aston.ac.uk

Otherwise, please complete the following and return them in the self-addressed envelope:

1. Health questionnaire
2. Food frequency questionnaire
3. Food diary
4. Consent form

Consent Form

Please read through the following statements and sign below if you agree:

1. I am willing to make three visits to the Neurosciences Institute at Aston University during the eighteen month study period.
2. I understand that I will not be reimbursed for my travel costs.
3. I am prepared to take one nutritional tablet daily throughout the study period.
4. I understand that I will not know whether the tablet contains the active ingredient being tested or not.

5. I am prepared to give accurate information about my health, medication, diet and eye condition throughout the study period.

6. I understand that if I feel my condition deteriorates or changes I must contact my GP, optometrist or consultant immediately.

7. I understand that the study appointments DO NOT replace scheduled appointments with my GP, optometrist or consultant.

8. I am aware that results from the study may be used in published literature but that my personal details will not be disclosed.

Name _____ Date _____

Signature _____

HUMAN SCIENCE ETHICAL COMMITTEE

CONSENT FORM FOR VOLUNTEERS

PROJECT TITLE

The effect of a lutein based nutritional supplement on non-exudative age-related macular degeneration (ARMD): a double-masked randomised controlled trial.

RESEARCH WORKERS, SCHOOL AND SUBJECT AREA RESPONSIBLE

Frank Eperjesi (academic member of staff)

Hannah Bartlett (research postgraduate)

School of Life and Health Sciences

Subject area Optometry

EXPLANATION OF ANY POSSIBLE HAZARDS AND THE PROCEDURES TO BE USED

There are no significant risks associated with this project.

Each participant will undergo an assessment involving a series of tests to determine levels of visual function. Some of these involve standard measurements such as assessing levels of vision and contrast detection using everyday vision charts. Other tests involve novel techniques such as measurement of side vision using a computer system and measurement of how quickly vision recovers following exposure to a camera flash. Volunteers will also be asked to complete a food frequency questionnaire and the information will be analysed using a new computer programme. These measurements will be made at the start of the study, and repeated during the study and at the end of the study.

All participants will be required to take one tablet daily throughout the study period (18 months). No participant will be informed whether they have been supplied with the study tablet (vitamins) or a harmless substitute.

CONFIDENTIALITY OF INFORMATION

The confidentiality of personal information and the anonymity of all volunteers involved in this investigation will be preserved in the following way:

All volunteers will remain anonymous. They will be assessed individually, in private and only the investigators will be present. Their names will not be recorded in the data set and the investigators will not verbally divulge the identity of any volunteer to anyone else. The results will be stored electronically and will be accessible only to the investigators.

VOLUNTEER'S STATEMENT

I have read and understand the above explanation. I have had the opportunity to discuss it with the investigators and to ask any questions. I agree to take part in the above project and I have been informed that I am free to withdraw at any time.

Signed:

Dated:

JGW/HSEC

26.5.00

Health questionnaire

Please answer the following questions in block capitals

1. Name _____

2. Male/Female _____

3. Date of Birth _____

4. Please list any general health problems you have (e.g. blood pressure or diabetes)

5. Do you take any tablets or medicines? If so, please list the names below

6. Do you take any nutritional supplements (e.g. vitamins)? If so, please list the name or brand below

7. Do you smoke? _____

8. If so, how many cigarettes per day _____

9. If you don't smoke at the moment but you have done previously, please give details below _____

10. Please give information about any previous visits to an eye clinic or eye specialist

11. If you know the name of your eye condition, please give details below

12. How does your eye condition affect your vision?

13. Have you always lived in the UK? _____

14. If you have lived abroad for more than a year please give details of where, when and for how long

Contact address:

Telephone number:

Thank you for your time.

Food frequency questionnaire

This questionnaire is designed to find out how often you eat particular types of food.

Please answer as accurately as possible. Do not leave any blank questions.

For each item of food please indicate how many times a week you eat it by simply circling the appropriate number.

Thank you for your co-operation and time.

<u>Meat</u>	<u>Times per week</u>
1. Chicken	0 1 2 3 4 5 6 7
2. Beef (including mince)	0 1 2 3 4 5 6 7
3. Lamb	0 1 2 3 4 5 6 7
4. Pork	0 1 2 3 4 5 6 7
5. Bacon	0 1 2 3 4 5 6 7
	<u>Times per week</u>
6. Ham	0 1 2 3 4 5 6 7
7. Turkey	0 1 2 3 4 5 6 7
8. Sausages	0 1 2 3 4 5 6 7

- | | |
|--|-----------------|
| 9. Beef burgers | 0 1 2 3 4 5 6 7 |
| 10. Liver | 0 1 2 3 4 5 6 7 |
| 11. Kidney | 0 1 2 3 4 5 6 7 |
| 12. Meat pies/pasties | 0 1 2 3 4 5 6 7 |
| 13. Ready meals | 0 1 2 3 4 5 6 7 |
| 14. Meat free/vegetarian
meals (soya/tvp) | 0 1 2 3 4 5 6 7 |

Breakfast cereals

Times per week

- | | |
|------------------|-----------------|
| 1. Cornflakes | 0 1 2 3 4 5 6 7 |
| 2. Rice krispies | 0 1 2 3 4 5 6 7 |
| 3. Weetabix | 0 1 2 3 4 5 6 7 |

Times per week

- | | |
|--|-----------------|
| 4. Muesli | 0 1 2 3 4 5 6 7 |
| 5. Porridge | 0 1 2 3 4 5 6 7 |
| 6. All bran | 0 1 2 3 4 5 6 7 |
| 7. Shreddies | 0 1 2 3 4 5 6 7 |
| 8. Fruit 'n' fibre | 0 1 2 3 4 5 6 7 |
| 9. Any other brands, please
specify _____ | 0 1 2 3 4 5 6 7 |

Vegetables

Times per week

- | | |
|---|-----------------|
| 1. Leafy greens
(e.g. Broccoli, spinach) | 0 1 2 3 4 5 6 7 |
| 2. Salad vegetables
(e.g. Tomatoes, lettuce) | 0 1 2 3 4 5 6 7 |
| 3. Potatoes (boiled,
mashed, baked or fried) | 0 1 2 3 4 5 6 7 |
| 4. Peas | 0 1 2 3 4 5 6 7 |

Times per week

- | | |
|--|-----------------|
| 5. Carrots | 0 1 2 3 4 5 6 7 |
| 6. Cabbage | 0 1 2 3 4 5 6 7 |
| 7. Peppers
(green, red or yellow) | 0 1 2 3 4 5 6 7 |
| 8. Baked beans | 0 1 2 3 4 5 6 7 |
| 9. Broad beans | 0 1 2 3 4 5 6 7 |
| 10. Chick peas | 0 1 2 3 4 5 6 7 |
| 11. Lentils
(green/brown) | 0 1 2 3 4 5 6 7 |
| 12. Kidney beans | 0 1 2 3 4 5 6 7 |
| 13. Soya beans | 0 1 2 3 4 5 6 7 |
| 14. Any other, please specify
<hr/> | 0 1 2 3 4 5 6 7 |

Fruits

Times per week

1. Apples

0 1 2 3 4 5 6 7

Times per week

2. Oranges

0 1 2 3 4 5 6 7

3. Bananas

0 1 2 3 4 5 6 7

4. Pears

0 1 2 3 4 5 6 7

5. Grapefruit

0 1 2 3 4 5 6 7

6. Peaches

0 1 2 3 4 5 6 7

7. Strawberries

0 1 2 3 4 5 6 7

8. Apricots

0 1 2 3 4 5 6 7

9. Mangoes

0 1 2 3 4 5 6 7

10. Dried fruit (apricots,
raisins, currants, sultanas)

0 1 2 3 4 5 6 7

11. Any other, please specify

0 1 2 3 4 5 6 7

Others

Times per week

1. Cheese

0 1 2 3 4 5 6 7

2. Cream

0 1 2 3 4 5 6 7

	<u>Times per week</u>
3. Yoghurt	0 1 2 3 4 5 6 7
4. Eggs	0 1 2 3 4 5 6 7
5. Chocolate	0 1 2 3 4 5 6 7
6. Crisps	0 1 2 3 4 5 6 7
7. Fish	0 1 2 3 4 5 6 7
8. White bread	0 1 2 3 4 5 6 7
9. Wholemeal bread	0 1 2 3 4 5 6 7
10. Brown bread	0 1 2 3 4 5 6 7
11. Naan/pitta bread	0 1 2 3 4 5 6 7
12. Pizza	0 1 2 3 4 5 6 7
13. Pasta/rice/spaghetti	0 1 2 3 4 5 6 7
14. Chapattis (white flour)	0 1 2 3 4 5 6 7
15. Chapattis (wholemeal)	0 1 2 3 4 5 6 7
16. Spicy foods (curries/chilli)	0 1 2 3 4 5 6 7

End

Once you have completed this food frequency questionnaire, the food diary, the health questionnaire and the consent form, please return all four in the self-addressed envelope provided.

Thank you.

Instructions on how to fill in your food diary

Firstly choose two week days and one weekend day to fill in your diary. For example you could choose Monday and Wednesday as your chosen week days and then Saturday as your weekend day.

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.

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.

Day 1 (First week day)	
Breakfast:	Supper:
Lunch:	Snacks:

Day 3 (Weekend day)	
Breakfast:	Supper:
Lunch:	Snacks:

Day 2 (Second week day)	
Breakfast:	Supper:
Lunch:	Snacks: