Macular carotenoid supplementation and visual function in early age-related macular degeneration

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Abstract

The carotenoids (lutein [L], zeaxanthin [Z] and *meso*-zeaxanthin [MZ]) are found at the macula, where they are collectively known as macular pigment (MP). The macula is a specialized part of the retina responsible for central vision. MP acts as a blue light filter, and its constituent carotenoids have antioxidant and anti-inflammatory properties. MP is believed to protect against progression of age-related macular degeneration (AMD), which is the leading cause of blindness in the developed world by protecting against oxidative stress. This PhD thesis answers three main research questions as follows: 1. what is the prevalence of AMD in the Republic of Ireland (ROI)? (research question 1); 2. what is the impact of supplementation using three different macular carotenoid formulations on MP and visual function in patients with non-advanced AMD over a three-year period? (research question 2); 3. Does the addition of MZ to the standard of care confer advantages or disadvantages to patients with non-advanced AMD in terms of visual outcomes and in terms of MP augmentation? (research question 3, main study). This thesis provides prevalence estimates of AMD in the ROI for the first time, and adds to the evidence with respect to the impact of macular carotenoid supplementation on visual function among patients with this condition.

Declaration

No element of the work described in this Thesis or the Thesis itself, except where otherwise acknowledged, has been previously submitted for a degree at this or any other institution. The work described in this Thesis has been performed entirely by the author.

Signature _____ Date _____

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List of Abbreviations (in alphabetical order)

AF – Fundus autofluorescence
AGES – Age, Gene/Environment Susceptibility
AMD – age-related macular degeneration
AREDS – Age-Related Eye Disease Study
ARIC – Atherosclerosis Risk in Communities
ARM – Age-Related Maculopathy
BCVA – best corrected visual acuity
BMI – Body mass index
BOSS – Beaver Dam Off-Spring Study
C – cortical
C3d – Complement factor 3d
C5a – Complement factor 5a
CAPI – Computer-assisted personal interviewing
CARMA – Carotenoids in Age-Related Maculopathy
CFF – Critical flicker frequency
cHFP – customized heterochromatic flicker photometry
CI – Confidence Interval
CLEAR – Combination of Lutein Effects in the Aging Retina
CONSORT – Consolidated Standards of Reporting Trials
COX-2 – cyclooxygenase 2
cpd – cycles per degree
CREST – Central Retinal Enrichment Supplementation Trial
CS – contrast sensitivity
DHA – Docosahexaenoic acid
DSMC – Data and Safety Monitoring Committee
ELISA – Enzyme-linked immunosorbent assay
EPA – Eicosapentaenoic acid
ERC – European Research Council
ETDRS – Early Treatment Diabetic Retinopathy Study
FACT – Functional Acuity Contrast Test
FSD – foveal shape discrimination
GA – geographic atrophy
GD – glare disability
HFP – Heterochromatic flicker photometry
HPLC – High performance liquid chromatography
IC – International Classification and Grading System for Age-related Macular Degeneration
IL-1β – interleukin 1β
IL-6 – interleukin 6
iNOS – inducible nitric oxide
ITT – Intention-to-treat
KVF – kinetic visual fields
L – lutein
LED – light emitting diode
LOCF – Last Observation Carried Forward
LOCS – Lens Opacities Classification System
LogMAR – Logarithm of the minimum angle of resolution
LogRAD – Logarithm of the reading acuity determination

MEHRC – Moorfields Eye Hospital Reading Centre
MESA – Multi-ethnic Study of Atherosclerosis
MOST – Meso-Zeaxanthin Ocular Supplementation Trial
MP – macular pigment
MPOD – macular pigment optical density
MPRG – Macular Pigment Research Group
mRNA – messenger Ribonucleic acid
MZ – *meso*-zeaxanthin
NC – nuclear colour
NEI-VFQ – National Eye Institute Visual-Function Questionnaire
NHANES – National Health and Nutrition Examination Survey
NO – nitric oxide
NO – nuclear opalescence
NV-AMD – neovascular age-related macular degeneration
OCT – optical coherence tomography
PAMDI – Prevalence of Age-Related Macular Degeneration in Italy
POM – Primary Outcome Measure
PRT – photostress recovery time
PSC – posterior subcapsular
RCT – randomised control trial
ROI – Republic of Ireland
ROS – reactive oxygen species
RPE – retinal pigment epithelium
SCQ – Self-completion questionnaires
SD – Standard Deviation
SEE – Spanish Eyes Epidemiology
SHARE – Survey of Health, Ageing and Retirement in Europe
SPSS – Statistical Package for Social Scientists
TILDA – The Irish Longitudinal Study on Ageing
TNF-α – tumor necrosis factor α
UK – United Kingdom
USA – United States of America
VA – visual acuity
VAR – visual acuity rating
VFQ – visual function questionnaire
VIP – Visual Impairment Project
WARMGS – Wisconsin Age-Related Maculopathy grading system
Z – zeaxanthin

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Chapter 1. Background

1.1 Introduction and Overview

Age-related macular degeneration (AMD) is characterised by a spectrum of degenerative changes at the macula, which include drusen and/or hyper-/hypo-pigmentary changes (known as early AMD or non-advanced AMD [Figure 1]), atrophic changes (geographic atrophy, GA, a form of advanced AMD [Figure 2]) and choroidal neovascularisation (neovascular AMD, another form of advanced AMD [Figure 3]).[1] Patients with untreated or untreatable advanced AMD invariably suffer from impairment of central vision, with consequential loss of social independence as a result of a concomitant inability to read, recognise faces, watch television or drive.[2]

Macular pigment (MP) is a yellow pigment found in the macular region of the human retina (see Figure 4), and is composed of the carotenoids, lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (MZ).[3] MP filters short-wavelength blue light (and therefore limits photooxidative damage passively) and its constituent carotenoids act as antioxidants by neutralizing free radicals.[4, 5] In addition, L and MZ have anti-inflammatory properties.[6-9] The carotenoids (L and Z) have been identified in various parts of the visual pathway and brain (e.g. frontal lobe, occipital cortex, cerebellum and pons) [10-12] and are believed to play important roles in brain health and cognition with putative benefits for visual function.

This thesis has four chapters. Chapter 1 gives the introduction and background of the research area pertaining to this thesis. Chapters 2, 3 and 4 are each divided into 5 sections, with an introduction (rationale and objectives), methods, results, discussion, and conclusion presented within each chapter.

This PhD thesis presents and discusses the results of three studies and answers three main research questions. The first study (Chapter 2), using baseline data from the Irish Longitudinal Study on Ageing (TILDA) answers the research question: *what is the prevalence of AMD in the Republic of Ireland (ROI)?* The second study (Chapter 3), known as the MZ Ocular Supplementation Trial (MOST) is an exploratory study which answers the research question: *what is the impact of supplementation using three different macular carotenoid formulations on MP and visual function in non-advanced AMD over a three-year period?* The third study (main study; Chapter 4), known as the Central Retinal Enrichment Supplementation Trial (CREST), answers the research question: *Does the inclusion of MZ to the standard of care confer advantages or disadvantages to patients with non-advanced AMD?* Finally, Chapter 5 presents the conclusions and contributions of this thesis to the research field as well as recommendations for future studies.

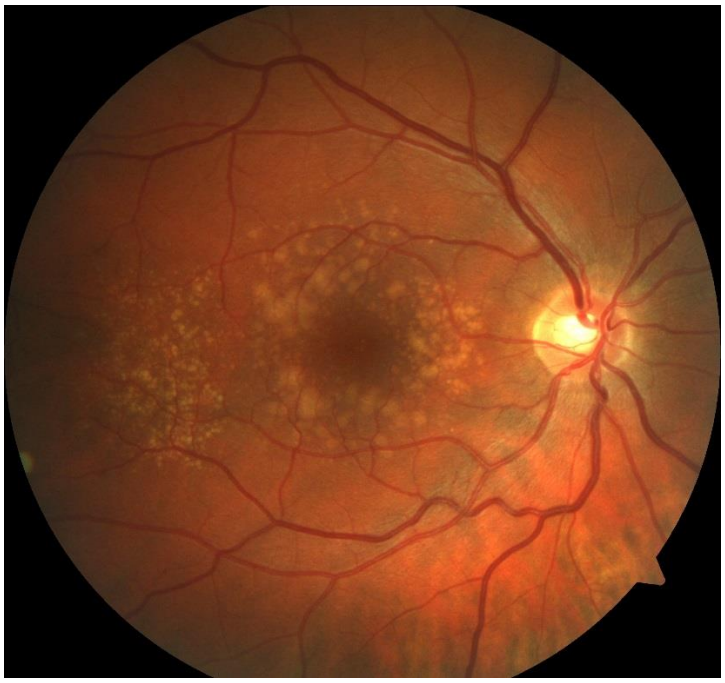


Figure 1: Early age-related macular degeneration. Image courtesy of Kwadwo Akuffo, Nutrition Research Centre Ireland

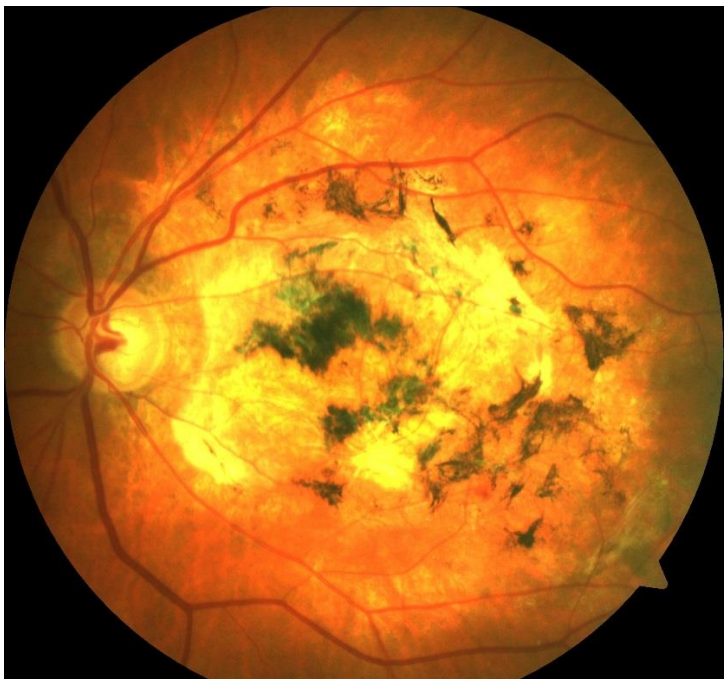


Figure 2: Atrophic age-related macular degeneration. Image courtesy of Kwadwo Akuffo, Nutrition Research Centre Ireland

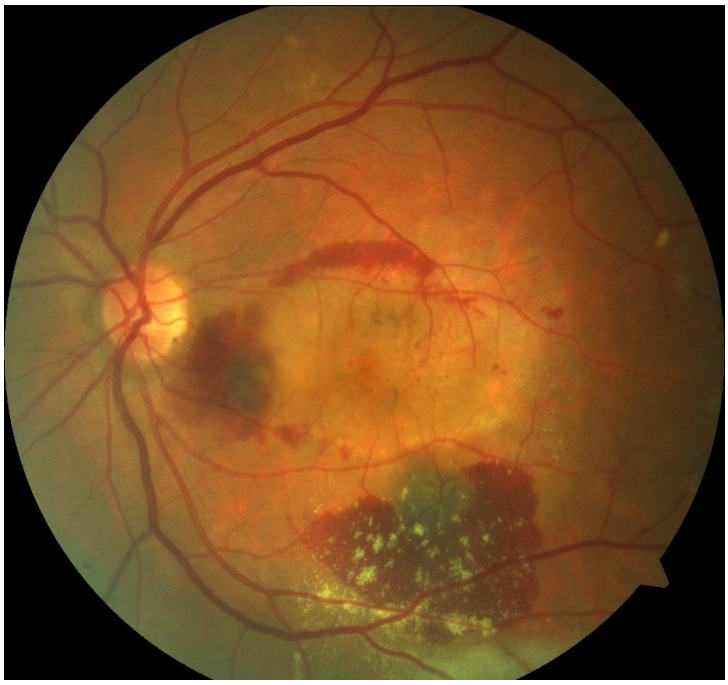


Figure 3: Neovascular age-related macular degeneration. Image courtesy of Kwadwo Akuffo, Nutrition Research Centre Ireland

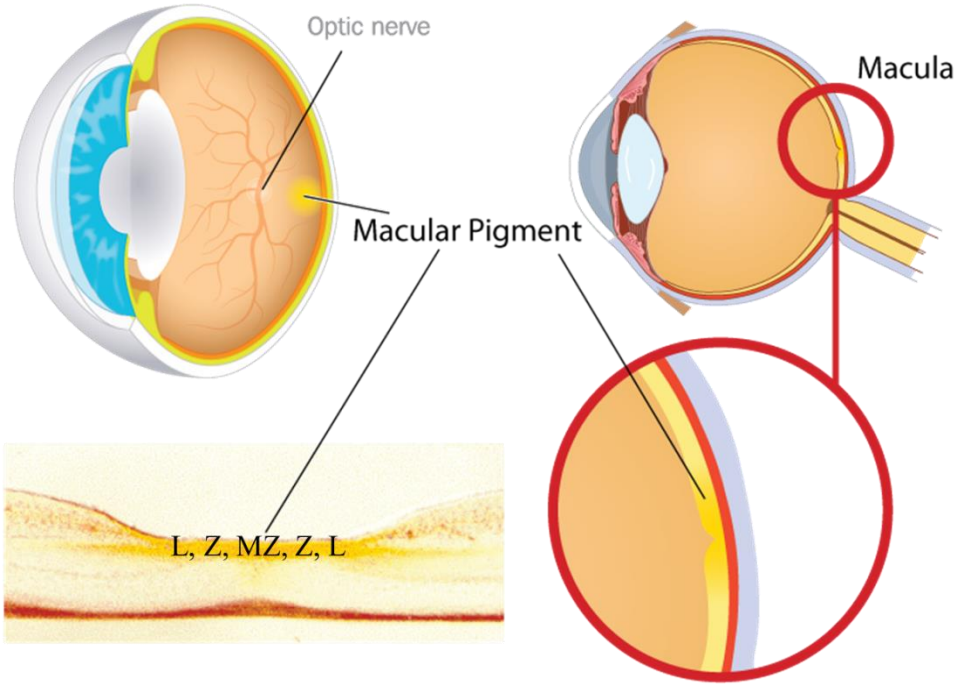


Figure 4: Location of macular pigment. Image courtesy of Professor Max Snodderly, Austin, USA, and Professor John Nolan, Waterford, Ireland

1.2 Classification and Grading Systems for Age-Related Macular Degeneration

Different classification, nomenclature and grading systems for AMD (see below) have been proposed and utilized in epidemiological and clinical studies. These classifications and grading protocols allow for standardized qualitative and/or quantitative assessment of AMD.

1.2.1 Wisconsin Age-Related Maculopathy Grading System

The Wisconsin Age-related Maculopathy Grading System (WARMGS)[13] is a semi-quantitative system for evaluating and grading age-related maculopathy (ARM) on retinal photographs. Grading is conducted using a grid which defines subfields, and standard circles which are used to estimate the size and area covered by various lesions. The grid consists of three concentric circles, centred on the macula with four radial lines superimposed. Characteristics assessed in this classification system include: drusen (size, type, area and confluence), retinal pigment epithelium (RPE) degeneration, increased pigment, subretinal scar and GA. Drusen confluence describes two or more drusen (at least 125μm) that have merged. Reticular drusen describes drusen that forms ill-defined networks of broad interlacing ribbons. GA is defined as a sharply defined area (at least 175μm) of RPE depigmentation with choroidal vessels visible.

1.2.2 International Classification and Grading System for Age-Related Maculopathy and Age-Related Macular Degeneration

In 1995, the Age-Related Maculopathy (ARM) Epidemiological Study Group described a system to identify and grade AMD in epidemiological studies with the intention of providing a consistent nomenclature.[1] In this system, early ARM was defined as the presence of drusen and RPE pigmentary abnormalities (hyperpigmentation and/or hypopigmentation) whereas late ARM, also known as age-related macular degeneration (AMD) in this system, was defined as GA (dry AMD) or neovascular AMD (wet AMD). GA was defined as the presence of a roughly round or oval area (at least 175μm) of hypopigmentation or depigmentation with clearly visible choroidal vessels. Neovascular AMD was defined as the presence of any of the following characteristics: serous and haemorrhagic RPE detachment, retinal haemorrhage, scar/glial/fibrous tissue, and hard exudates (not associated with other retinal vascular disease). A modified version of this classification system has been used in Chapter 2.

1.2.3 The Age-Related Eye Disease Study Severity Scale

The Age-Related Eye Disease Study (AREDS) severity scale was developed using data from the AREDS study.[14] It has multiple severity steps and provides an eye-specific grade on a scale from 1 to 11.[15] This AMD severity scale allows various cut points anywhere along the scale, and is a useful tool for assessing progression to advanced AMD, especially in research. Grades 1 to 9 combine a six-step drusen area scale with a five-step pigmentary abnormality scale. The five-year risk of progression to advanced AMD is less than 1% in grade 1, and this increases to about 50% in grade 9. Grades 9, 10 and 11 represent non-central GA, central GA

and neovascular AMD, respectively. GA was defined as an area of partial or complete depigmentation of the RPE with at least two of the following characteristics: roughly round or oval shape, sharp margins, and visibility of underlying large choroidal vessels. Neovascular AMD was defined as the presence of at least one of the following characteristics: serous sensory retinal detachment, RPE detachment, subretinal haemorrhage, or subretinal fibrosis or previous photocoagulation treatment. The definition for "early AMD" on this scale is unclear. This severity scale has been used in the clinical trials reported in Chapters 3 and 4.

1.2.4 Age-Related Eye Disease Study Simplified Severity Scale

The AREDS simplified classification system[16] was designed to help clinicians identify patients at risk of progression to advanced AMD. It was developed by modifying the original AREDS 11-step severity scale.[15] It has five risk categories on a scale from 0 to 4, which represent the estimated five-year percentage risk of progression to advanced AMD (i.e. Grade 0: 0.5%; Grade 1: 3%; Grade 2: 12%; Grade 3: 25%; and Grade 4: 50%). The main clinical signs of AMD recognised in this classification system are drusen size (intermediate drusen ≥63μm and/or large drusen ≥125μm) and the presence or absence of pigmentary abnormalities (hyperpigmentation, hypopigmentation and non-central GA). In this classification system, grades are assigned using the following criteria:

a) Patients with no advanced AMD in either eye: 1. one is assigned for each eye with large drusen; 2. one is assigned for each eye with pigmentary abnormalities;

3. one is assigned if both eyes have intermediate drusen and have no large drusen in each eye.

b) Patients with advanced AMD in one eye: 1. two is assigned for the eye with neovascular AMD; 2. one is assigned if eye at risk has large drusen; one is assigned if eye at risk has pigmentary abnormalities.

1.2.5 Clinical Classification System of Age-Related Macular Degeneration

The Clinical Classification System of AMD[17] was designed using a modified Delphi technique by a working group (26 AMD experts, 1 neuro-ophthalmologist, 2 committee chairpersons and 1 methodologist) in an attempt to provide a consistent nomenclature for clinicians. "Age-related macular degeneration" was designated as the term for the disease. This system has five stages: 1. No AMD: no visible drusen or pigmentary abnormalities; 2. Normal aging changes: drupelets (small drusen [≤63μm]); 3. Early AMD: medium drusen (>63μm and ≤125μm) with the absence of pigmentary abnormalities; 4. Intermediate AMD: large drusen (>125μm) and/or pigmentary abnormalities; 5. Late AMD: the presence of neovascular AMD and/ or any GA. Pigmentary abnormalities are defined as definite hyperpigmentation or hypopigmentation associated with medium or large drusen but not related to known disease.

1.3 Prevalence and Incidence of Age-Related Macular Degeneration

Several population-based studies have reported the prevalence of AMD (see Table 1 and 2) and incidence of AMD (see Table 3) using different definitions, classification and grading systems (as described above in Section 1.2), as well as retinal photography protocols. In general, the prevalence of AMD increases with increasing age. In addition, cumulative incidence data from epidemiological studies (e.g. Beaver Dam Eye Study and Blue Mountains Eye Study in Table 3) show that the number of new cases of AMD increases over time.

Given the growing and aging world population, the number of people suffering from AMD continues to rise. Using pooled data from 39 studies and applying a Hierarchical Bayesian approach, Wong *et al* estimated the prevalence of any AMD (globally) to be 8.7% in those aged 45 to 85 years, affecting Europeans more than persons of African or Asian origin.[18] It is predicted that the number of people afflicted with AMD worldwide will be 196 million by 2020, and this figure is expected to rise to, increasing to 288 million by 2040.[18] Beyond the personal suffering of those afflicted with advanced AMD, which includes loss of central vision, and associated adverse clinical events such as increased risk of falls,[19] depression, loneliness, suicide, etc.,[20] the growing prevalence of AMD represents a huge socioeconomic burden to society and to healthcare providers.[21]

Table 1: Study characteristics of selected epidemiological studies in white populations reporting prevalence of age-related macular degeneration

Study name	Country	Age (years)	Dilation	Photography	Grading
Baltimore Eye Survey; 1985-1988[22]	USA	≥40	yes	Two simultaneous stereoscopic photographs (one centred on optic disc and one on the macula)	IC
Beaver Dam Eye Study; 1988-1990[23]	USA	≥43	yes	Stereoscopic 30° photographs centred on macula, the optic disc and temporal to but including the fovea	WARMGS
Rotterdam Study; 1990 -1993[24]	Netherlands	≥55	yes	Two 35° colour photographs centred on the macula	WARMGS
Blue Mountains Eye Study; 1992-1993[25]	Australia	≥49	yes	Stereoscopic 30° photographs centred on macula, the optic disc, and temporal to but including the fovea	WARMGS
VIP Study; 1992-1996[26]	Australia	≥40	yes	Colour stereo photographs centred on both the optic disc and fovea	WARMGS/IC
ARIC study; 1993-1995[27]	USA	≥48	no	45° retinal photograph centred on the optic disc and macula	WARMGS
Reykjavik Eye Study; 1996[28]	Iceland	≥50	yes	Two simultaneous colour stereo fundus photographs 30°; one centred on fovea and the other on the optic disc	IC
MESA; 2000-2002[29]	USA	≥45	no	Two photographic fields; first centred on optic disc and the second centred on the fovea	WARMGS
Greenland Inuit Eye Study; 2000-2001[30]	Greenland	≥60	yes	Three fields photographed for each eye; 30° and 45° centred on macula and 30° centred on optic disc	IC
AGES Reykjavik Study; 2002-2006[31]	Iceland	≥66	yes	Two photographic fields (one centred on the optic disc and the other centred on the fovea)	WARMGS

Study name	Country	Age (years)	Dilation	Photography	Grading
PAMDI Study; 2005-2006[32]	Italy	≥61	yes	30° colour fundus photographs	IC
EUREYE Study[33]	Norway, Estonia, Northern Ireland, France, Italy, Greece, Spain	≥65	yes	Two 35° non-simultaneous stereoscopic colour fundus images; centred on the fovea	IC
Oslo Macular Study; 2002[34]	Norway	≥51	yes	Digital stereo fundus photographs centred on the macula	IC
NHANES; 2005-2008[35]	USA	≥40	no	Two 45° nonmydriatic digital retinal images - One image centred on macula, and the other centred on the optic disc	WARMGS
BOSS; 2005-2008[36]	USA	≥21	yes	Two photographic fields (one centred on the optic disc and the other centred on the fovea)	WARMGS
Tromsø Eye Study; 2007-2008[37]	Norway	≥65	yes	5-field 45° colour retinal photographs and one 30° photograph centred on the fovea	IC
SEE Study[38]	Spain	≥65	yes	Colour fundus photographs centred on the macula	IC
TILDA Study; 2009-2011[39]	ROI	≥50	no	One 45° monoscopic colour photograph, centred on the macula	IC

SEE, Spanish Eyes Epidemiology; BOSS, Beaver Dam Off-Spring Study; NHANES, National Health and Nutrition Examination Survey; VIP, Visual Impairment Project; PAMDI, Prevalence of Age-Related Macular Degeneration in Italy; MESA; Multi-ethnic Study of Atherosclerosis; ARIC, Atherosclerosis Risk in Communities; AGES, Age, Gene/Environment Susceptibility; IC, International Classification and Grading System for Age-related Macular Degeneration; WARMGS, Wisconsin Age-Related Maculopathy grading system; TILDA, The Irish Longitudinal Study on Ageing.

Table 2: Prevalence of age-related macular degeneration (AMD) in white population-based studies

Study name	Age (years)	Early AMD (%)	Late AMD (%)	GA (%)	NV-AMD (%)
Baltimore Eye Survey[22]	**All**				
*Only whites	40-49		0.00	0.00	0.00
	50-59		0.52	0.17	0.35
	60-69		0.73	0.73	0.00
	70-79		2.94	1.76	1.63
	80+		7.00	4.00	5.62
Beaver Dam Eye Study[23]	**All**	**15.6**	**1.6**		
	43-54	8.4	0.1		
	55-64	13.8	0.6		
	65-74	18.0	1.4		
	75+	29.7	7.1		
Rotterdam Study[24]	**All**		**1.7**	**0.6**	**1.1**
	55-64		0.2	0.1	0.1
	65-74		0.8	0.4	0.4
	75-84		3.7	1.3	2.4
	85+		11	3.7	7.4
Blue Mountains Eye Study[25]	**All**	**7.2**	**1.9**		
	49-54	1.3	0.0		
	55-64	2.6	0.2		
	65-74	8.5	0.7		
	75-84	15.5	5.4		
	85+	28.0	18.5		
VIP Study[26]	**All**	**15.1**	**0.68**	**0.27**	**0.39**
ARIC study[27]	**All**	**5.4**	**0.2**		
Data on only whites*					
Reykjavik Eye Study[28]	**All**	**17.9**	**3.5**	**3.2**	**0.7**
	50-59	8.9	0.3	0.3	0.0
	60-69	16.4	1.2	1.2	0.0
	70-79	27.5	5.8	5.3	0.5
	>80	37.1	30.8	25.0	9.8
MESA[29]	**All**	**4.8**	**0.6**		
*only whites	45-54	1.8	0.0		
	55-64	2.8	0.1		
	65-74	5.5	0.3		
	75-84	13.3	2.9		
Greenland Inuit Eye Study[30]	**All**	**52.3**	**9.5**	**2.3**	**5.9**
	60-69	50.0	3.9	0.7	3.1
	70-79	58.8	14.6	3.4	9.9
	≥80	44.7	43.2	12.5	15.0

Study name	Age (years)	Early AMD (%)	Late AMD (%)	GA (%)	NV-AMD (%)
AGES Reykjavik Study[31]	**All**	**21.3**		**2.4**	**3.3**
	66-69	10.9		0.2	0.6
	70-74	13.0		0.5	1.0
	75-79	23.9		1.9	2.4
	80-84	29.5		5.2	6.1
	≥85	36.0		7.6	11.4
Oslo Macular Study[34]	**All**	**43.1**	**2.8**		
	51-60		0.0		
	61-70		2.5		
	71-80		5.6		
	81-90		8.5		
PAMDI Study[32]	**All**	**58.6**	**4.1**	**1.6**	**2.1**
	61-64		1.3		
	65-69		2.9		
	70-74		3		
	75-79		2.7		
	80+		10.4		
EUREYE Study[33]	**All**		**3.32**	**1.2**	**2.3**
NHANES[35]	**All**	5.7	0.8		
	40-59	2.8			
	≥60	11.1	2.2	1.4	0.9
BOSS Study[36]	**All**	**3.4**			
*No signs of late AMD in cohort	21-34	2.4			
	35-44	2.1			
	45-54	2.6			
	55-64	5.0			
	65-84	9.8			
Tromso Eye Study[37]	**All**		**3.5**	**1.0**	**2.5**
	65-69		0.8	0.3	0.5
	70-74		2.2	0.4	1.8
	75-79		5.8	1.4	4.3
	80-87		10.9	3.7	7.2
SEE Study[38]	**All**	**10.3**	**3.4**	**1.5**	**1.9**
	65-74		1.3	0.3	1.0
	≥75		5.7	3.0	2.7
TILDA Study[39]	**All**	**6.6**	**0.6**	**0.3**	**0.3**
	50-64	4.9	0.1	0.1	0.1
	65-74	7.3	0.5	0.2	0.2
	≥75	11.0	2.2	1.3	1.0

SEE, Spanish Eyes Epidemiology; BOSS, Beaver Dam Off-Spring Study; NHANES, National Health and Nutrition Examination Survey; VIP, Visual Impairment Project; PAMDI, Prevalence of Age-Related Macular Degeneration in Italy; MESA; Multi-ethnic Study of Atherosclerosis; ARIC, Atherosclerosis Risk in Communities; AGES, Age, Gene/Environment Susceptibility; IC, International Classification and Grading System for Age-related Macular Degeneration; WARMGS, Wisconsin Age-Related Maculopathy grading system; USA, United States of America; ROI, Republic of Ireland; AMD, age-related macular degeneration; GA, geographic atrophy; NV-AMD, neovascular AMD; TILDA, The Irish Longitudinal Study on Ageing.

Table 3: Incidence of age-related macular degeneration in two population-based studies with 15-year follow up

			Early AMD (%)			Late AMD (%)		
Study name	**Country**	**Age**	**5-y**	**10-y**	**15-y**	**5-y**	**10-y**	**15-y**
Beaver Dam Eye Study[40-42]	USA	**All**	**8.2**	**12.1**	**14.3**	**0.9**	**2.1**	**3.1**
		43-54	3.9	4.1	6.9	0.0	0.1	0.4
		55-64	4.7	10.7	12.7	0.3	1.0	2.6
		65-74	16.1	23.6	25.3	1.3	4.4	5.8
		≥75	22.8	36.7	24.4	5.4	9.5	7.6
Blue Mountains Eye Study[43-45]	Australia	**All**	**8.7**	**14.1**	**22.7**	**1.1**	**3.7**	**6.8**
		<60	3.2	4.2	8.7	0.0	0.2	1.1
		60-69	7.4	14.7	26.9	0.6	3.0	6.8
		70-79	18.3	28.7	51.4	2.4	9.1	20.2
		≥80	14.8	32.5	29.3	5.4	24.3	21.3

USA, United States of America; AMD, age-related macular degeneration; Age in years; y, year

1.4 Aetiopathogenesis of Age-Related Macular Degeneration

1.4.1 Oxidative Stress

Oxidative stress refers to tissue damage caused by reactive oxygen species (ROS) such as singlet oxygen, free radicals and hydrogen peroxide. The free radical theory of aging postulates that aging and age-related diseases are as a result of cumulative damage from ROS. It is believed that oxidative stress plays an important role in the pathogenesis of AMD.[46] ROS are produced from oxygen metabolism in the retina. The retina is an ideal substrate for the generation of ROS because of its high oxygen consumption, the high levels of cumulative irradiation, the presence of polyunsaturated fatty acids in photoreceptor outer segment membranes and abundant photosensitizers (e.g. visual pigments, lipofuscin). ROS are generated from both cellular systems (e.g. mitochondria, enzymatic-reactions, phagocytosis, photosensitizers, and inflammatory processes) as well as external lifestyle and environmental (e.g. cigarette smoking, light irradiation). The cumulative effect of the generation of these ROS results in tissue damage (oxidative stress). Oxidative stress is modulated by inherent antioxidant and repair systems which balance the oxidative and reductive processes within the retina. However, when the oxidative load is greater than counteracting effect of the antioxidant and repair systems (which may be due to increasing age and/or reduced antioxidant capacity), oxidative damage occurs.

1.4.2 Inflammation

A growing body of evidence suggests that the inflammation contributes to the pathogenesis of AMD.[47, 48] Local inflammation and the complement system may contribute to the formation of drusen, degeneration of the photoreceptors, and the disruption of Bruch's membrane.[49, 50] Drusen contains many pro-inflammatory components including the activated complement cascade.[51-53] In a recent study by Nishiguchi *et al*, which analysed blood samples from 945 primates, drusen was positively associated with age (OR: 1.10 per year, 95% CI: 1.07–1.12) and white blood cell count (OR: 1.01 per 1 × 103/μl, 95% CI: 1.00–1.01).[54] Macrophages have been identified close to or within AMD lesions in the retina.[55-57] These inflammatory components promote inflammation in the RPE/Bruch's membrane and choroid, and subsequently contribute to AMD progression.[52] Polymorphisms in genes encoding for CFH, CFB/C2, C3 and C5 have a strong association with AMD.[58-61] Radu *et al* demonstrated that cultured human RPE cells with AMD-predisposing CFH haplotype (HH402/VV62) are more susceptible than AMD-protective CFH haplotype (YY402/II62) to complement-mediated attack following exposure to bisretinoid-containing Abca4(-/-) photoreceptor outer segments.[62] Markers of systemic inflammation have also been shown to be associated with AMD.[63-68] For instance, in a case-control study, C-reactive protein was significantly higher in patients with advanced AMD when compared to those without AMD.[63] Shankar *et al* reported that an elevated white blood cell count was independently associated with the incidence of early AMD.[64] Lechner *et al* found that plasma levels of C3a, C4a and C5a were significantly higher in patients with neovascular AMD compared to controls.[66] Taken together, these studies

provide support for the role of inflammation and immune-mediated processes in the pathogenesis of AMD.

1.4.3 Choroidal Mechanisms

Abnormal choroidal blood perfusion may contribute to the pathogenesis of AMD.[69, 70] The hemodynamic model posits that in AMD, there is an increase in resistance to choroidal blood flow which is caused by decreased compliance of the sclera and choroidal vessels.[71] Indeed, studies have shown a decrease in choroidal blood flow in patients with AMD.[72-77] In a case-control study, choroidal filling of the perifoveal regions were delayed and were heterogeneous between regions, in patients with non-neovascular AMD compared to age-matched controls.[72] Choroidal blood flow is more impaired in eyes in neovascular AMD than in fellow eyes with non-neovascular AMD.[73] Grunwald *et al* found that foveolar choroidal perfusion parameters (blood velocity, blood volume, and blood flow) decreased with increasing risk of neovascular AMD.[75] Some studies have also demonstrated choroidal thinning in patients with AMD[78-80] while others have not.[81, 82]

1.5 Risk Factors of Age-Related Macular Degeneration

Epidemiological and clinical studies have identified many risk factors for AMD.[83, 84] Established risk factors are those that have been shown to be consistently associated with AMD, and these include a genetic predisposition for AMD,[85-87] increasing age[23, 25, 87] and cigarette smoking.[87-90] Of note, it has also been shown that established risk factors for AMD are associated with a relative lack of MP decades before disease onset.[91] Risk factors which are not consistently associated with AMD are referred to as putative risk factors.

Obesity is putative risk factor for AMD and is a potential modifiable risk factor for AMD. In a case-control study by the AREDS investigators, persons in neovascular AMD were more likely to have an increased body mass index, when compared to controls with fewer than 15 small drusen.[92] In a clinic-based prospective cohort study which included participants with early or intermediate AMD, Clemons *et al* reported that greater body mass index is associated with incident central geographic atrophy (OR: obese vs. non-obese, 1.93; 95% CI, 1.25-2.65).[93] In the Atherosclerosis Risk in Communities Study, a decrease in waist-hip ratio (which is a measure of abdominal obesity) of ≥3% was associated with 29% lower reduced risk of any AMD (OR: 0.71; 95% CI, 0.52-0.97).[94] In the Melbourne Collaborative Cohort an increase of 0.1 in waist-hip ratio among men was associated with a 13% increase in the odds of early AMD (OR: 1.13, 95% CI: 1.01, 1.26; P = 0.03) and a 75% increase in the odds of late AMD (OR: 1.75, 95% CI: 1.11, 2.76; P = 0.02).[95] However, no statistically significant associations were observed between any AMD (early or late AMD) and body mass index.[95] Among women in that same study, statistically significant inverse associations with early AMD were observed for all measures of adiposity (waist-hip ratio, body mass

index, fat mass), but no associations were observed for late AMD.[95] Furthermore, in a recent systematic review which included 31,151 participants from seven prospective cohort studies, a 32% increased risk of progression to advanced AMD was observed among obese participants (RR: 1.32, 95% CI: 1.11–1.53), whereas no significant association between obesity and early AMD was demonstrated (RR: 0.91, 95% CI: 0.74–1.08).[96]

Race is another risk factor for AMD. In the Baltimore Eye Study, white participants were more likely than black participants to have pigmentary abnormalities, large drusen and advanced AMD.[22] In the Salisbury Eye Evaluation (SEE) Project, white participants were more likely than black participants to have medium or large drusen, pigment abnormalities, and advanced AMD.[97] Furthermore, in a clinic-based prospective cohort study which included participants with early or intermediate AMD, Clemons et al reported that race was significantly associated with incident neovascular AMD (OR: white vs. black, 6.77; 95% confidence interval [CI], 1.24-36.9).[93]

Cardiovascular disease is a putative risk factor for AMD. In the Beaver Dam Eye Study, higher systolic blood pressure and 10-year incidence of RPE depigmentation and neovascular AMD.[98] In addition, higher pulse pressure was associated with the 10-year incidence of hypo- and hyper- pigmentation, and neovascular AMD.[98] In the Blue Mountains Eye Study, history of stroke or any cardiovascular disease is associated with incident early AMD and incident indistinct soft or reticular drusen.[99] However, pulse pressure, systolic or diastolic blood pressure, or presence of hypertension were not associated with incident AMD.[99] In a case-control study by the AREDS investigators, persons with

neovascular AMD were significantly more likely to have hypertension when compared to controls with fewer than 15 small drusen.[92] These studies provide some evidence of the links between cardiovascular disease risk factors and AMD.

Alcohol intake is another putative risk factor for AMD. Whiles some studies have reported an association between alcohol consumption and increased risk of AMD,[100-104] other studies have found no association with AMD or reduced risk of AMD.[105-109] In the Beaver Dam Eye Study, consumption of beer in the previous year was associated with an increased odds of hyperpigmentation (OR: 1.13; 95% CI: 1.02, 1.25) and neovascular AMD (OR: 1.41; 95% CI: 1.05, 1.88).[104] Alcohol consumption was significantly associated with an increased risk of incident early AMD (OR: 1.57; 95% CI: 1.18 to 2.11) in the Study of Osteoporotic Fractures (SOF).[100] In the Melbourne Collaborative Cohort Study, a daily alcohol consumption of more than 20g per day was associated with about 20% increased odds of early AMD (OR: 1.21, 95% CI: 1.06, 1.38) when compared with those who reported no consumption of alcohol at baseline.[101] In a systematic review and meta-analysis comprising five cohort studies, Chong et al reported that heavy alcohol consumption was associated with an increased risk of early AMD (pooled OR: 1.47; 95% CI: 1.10 to 1.95), but not risk of late AMD.[103]

Diet is another putative risk factor for AMD and is a potential modifiable risk factor for AMD. There is an inverse relationship between dietary intake of MP's constituent carotenoids (L and Z) and risk of AMD.[110-113] In the Eye Disease Case Control Study, after adjusting for other risk factors, participants in the highest quintile of carotenoid intake had a 43% reduced risk of advanced AMD when compared with participants in the lowest quintile of carotenoid intake (OR

0.57; 95% CI, 0.35 to 0.92).[110] In a systematic review and meta-analysis comprising of six longitudinal cohort studies, dietary intake of L and Z was found to be significantly associated with a reduced risk of late AMD (RR: 0·74; 95 % CI 0·57, 0·97) as well a reduced risk of neovascular AMD (RR: 0·68; 95 % CI 0·51, 0·92).[111] However, in this meta-analysis, dietary intake of L and Z was not associated with a reduced risk of early AMD.[111] The AREDS2 study examined the role of supplementation with two of MP's constituent macular carotenoids (L and Z, in combination with co-antioxidants) in patients with intermediate AMD.[114] The primary outcome measure (progression to advanced AMD) in AREDS2 did not demonstrate a beneficial effect of supplemental L and Z.[115] However, secondary analysis, where data were dichotomized to those supplemented with L and Z versus those not being supplemented with these macular carotenoids, did demonstrate a beneficial effect in terms of progression to the advanced AMD, especially in those with a low dietary intake of these carotenoids.[115, 116] Furthermore, in a recent report which utilized data from the Nurses' Health Study and the Health Professionals Follow-up Study, higher dietary intake of L and Z is associated with a 40% lower risk of advanced AMD following two decades of prospective follow up.[117]

1.6 Macular Pigment

1.6.1 Anatomical Location, Distribution and Absorbance Spectrum

MP is found at the macula (the specialized part of the retina that mediates central vision) between the receptor axon layers and inner plexiform layer (Henle's fibre layer) in the retina (see Figure 4).[118, 119] Interestingly, the pigment is captured and distributed at the macula in such a way that it normally peaks centrally at the foveola and declines with increasing retinal eccentricity.[120]

MP absorbs light between 400 to 540nm with its peak absorption maximum at 460nm and therefore MP acts as a blue light filter (see Figure 5).[118] Higher MP denotes high blue light attenuation ability. MP's anatomical location (i.e. Henle's fibre layer[118, 119]) is ideal for filtering short wavelength blue light before it reaches the photoreceptors and it is believed to enhance visual function by these optical properties. The blue light filtering properties also confer protection by limiting photooxidative damage passively.

MZ and Z are the predominant carotenoids in the foveal region, whereas L predominates in the parafoveal region.[121] The concentration of MZ peaks centrally, with an MZ:Z ratio of 0.82 in the central retina (within 3mm of the fovea) and 0.25 in the peripheral retina (11-21mm from the fovea).[122] This shows the unique spatial distribution of the macular carotenoids at the macula.

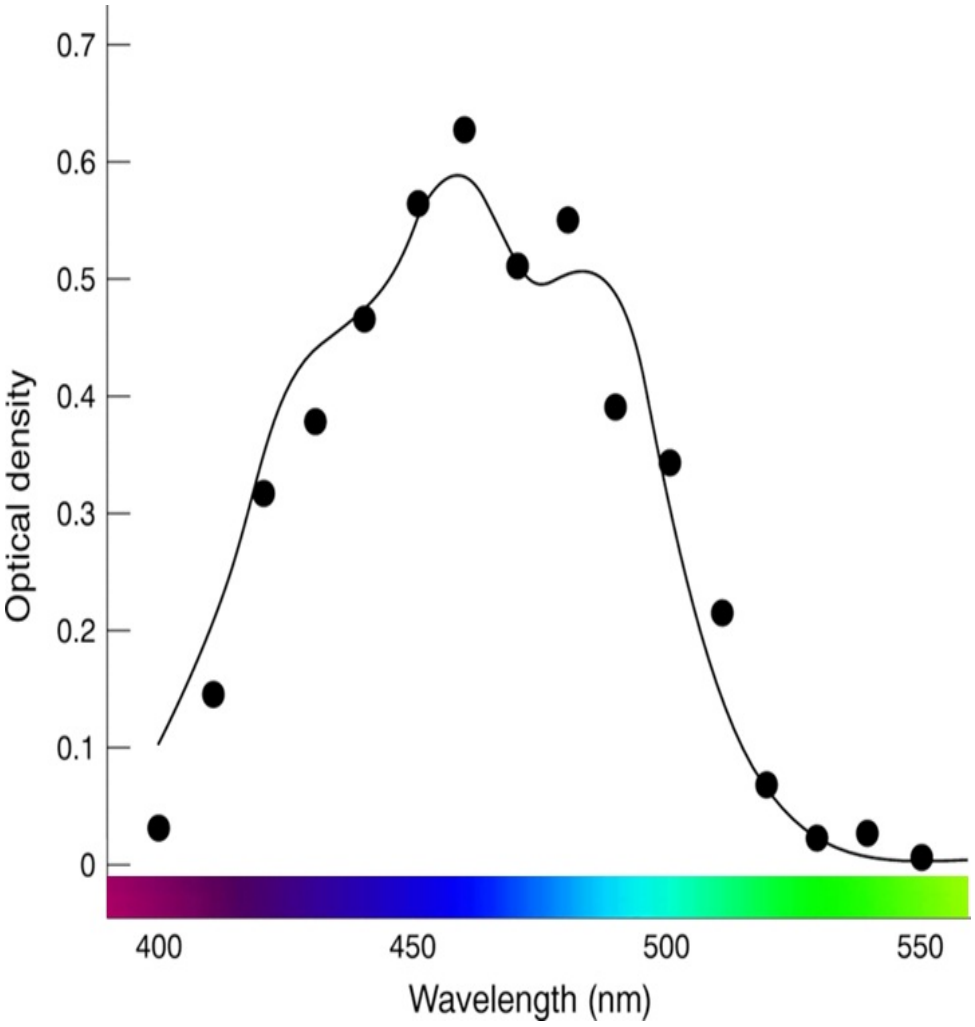


Figure 5: The absorbance spectrum of macular pigment. Image courtesy of Professor John Nolan

1.6.2 Source of Macular Pigment

MP's constituent carotenoids can be obtained through the diet or by taking nutritional supplements containing the macular carotenoids. The average dietary intake of combined L and Z in the western diet is 1.3-3 mg per day.[123, 124] L and Z are found in fruits (e.g. kiwi, red grapes, oranges) and vegetables (e.g. spinach, lettuce, kale, pea).[125] Z is the major carotenoid found in orange peppers, corn, and corn products.[125, 126] Egg yolk is also a good source of dietary L and Z.[125, 126] Possible dietary sources of MZ include shrimp, certain marine fish, and turtles.[127] Nolan *et al* has also recently reported the presence of MZ in salmon skin, sardine skin, trout skin and flesh.[128] However, Rasmussen *et al* reported the presence of MZ in the eggs of hens fed with MZ, but not in any species of fish analysed in that study.[129] In addition, the generation of MZ from L at the macula has been suggested as the origin of MZ in the eye by two studies conducted in animal models.[130, 131] The first study (by Johnson *et al*) was performed in carotenoid-deficient Rhesus monkeys (*Macaca mulalla*), in which one group was fed with supplemental L whiles another group was fed with supplemental Z.[130] Analyses of macular samples from these monkeys revealed that MZ was present only in the 4mm annular (central punch) of monkeys fed with L, but not in those fed with Z.[130] In another study, two groups of Japanese quails (*Coturnix japonica*) were supplemented with either deuterated L or Z.[131] Following supplementation, MZ (in the quail retina) was more deuterated in the supplemental L group than in the supplemental Z group (42% in L group versus <10% in Z group).[131] These studies support the notion that MZ is derived from L at the macula. However, the specific biochemical mechanism by which this retinal isomerization of L to MZ occurs is still unknown. In an attempt to shed light on this mechanism, Gorusupudi *et al*

studied the carotenoid composition of fertilized White Leghorn chicken eggs during embryonic development, and concluded that the RPE/choroid is the most likely site for the MZ isomerase enzyme.[132] Furthermore, Gorusupudi *et al* hypothesized that the Interphotoreceptor Retinoid-Binding Protein (IRBP) is involved in the transport of MZ.[132]

1.6.3 Macular Pigment Measurement

There are a variety of techniques available for measuring MP (and its constituent carotenoids) and the measurement techniques can be broadly classified as *ex vivo* (i.e. outside cell/tissue) and *in vivo* techniques (i.e. inside cell/tissue). *Ex vivo* techniques include high performance liquid chromatography (HPLC) and microdensitometry. However, these *ex vivo* techniques can only be performed in post-mortem eyes. *In vivo* techniques include physical (objective) techniques (e.g. fundus autofluorescence (AF), fundus reflectometry and Raman spectroscopy) and psychophysical (subjective) techniques (e.g. heterochromatic flicker photometry [HFP], customized heterochromatic flicker photometry [cHFP], colour matching and motion photometry), and these methods are desirable because they can be performed non-invasively in the living subject. The AF technique can also be used *ex vivo*. However, there remains debate, as to which technique, if any, should be deemed as the "gold standard" for measuring MP. A review of the literature shows that HFP and cHFP are the most commonly used, but it is important to note that each technique has its own advantages and limitations.[133]

1.6.4 Functions of Macular Pigment

MP filters short-wavelength blue light (optical filter function) and its constituent carotenoids have antioxidant and anti-inflammatory properties. It is believed that MP may confer protection against AMD and plays a role in visual function via these properties.

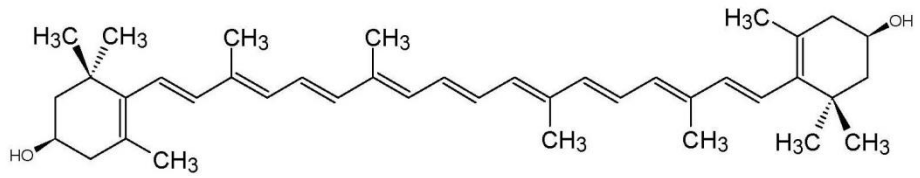
1.6.4.1 Optical filter

The absorption spectrum of the macular carotenoids peaks at 460 nm and, thus, MP is a filter of blue light, and may limit photooxidative damage to retinal cells.[134] Of note, short-wavelength (blue) light is the most harmful to the retina.[135] Importantly, both the absorptive characteristics of MP and its anatomical location (in the anterior portion to the photoreceptors) enable the pigment to attenuate blue light incident on the photoreceptors, with associated protective benefits.[120] The orientation of L (which lies both parallel and perpendicular to the cell membrane) facilitates greater blue light filtering potential than Z (which only lies parallel to the cell membrane), because L absorbs blue light incident from all directions.[136] However, L, Z and MZ have slightly different absorption spectra and, thus, the combination of these pigments at the macula results in the pre-receptorial absorption of a wider range of short-wavelength light than if any were present in isolation.

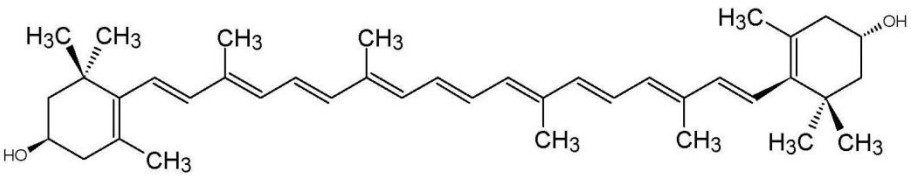
1.6.4.2 Antioxidant

MZ, Z, and L are structural isomers of one another (see Figure 6), and their antioxidant properties rests on the high number of double bonds (and, therefore,

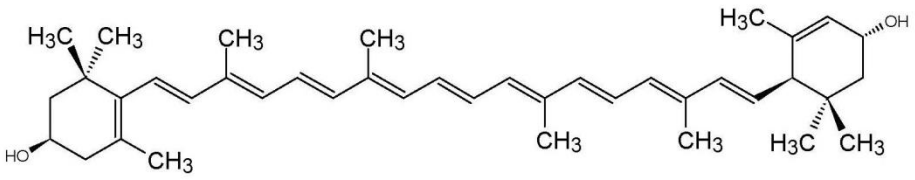
readily available electrons) present in their molecular structure.[137] These carotenoids quench reactive oxygen species (e.g. singlet oxygen, free radicals, and triplet state photosensitisers), thus limiting membrane phosopholipid peroxidation.[136] Khachik *et al* demonstrated the presence of direct oxidation products of L and Z in the retina (e.g. 9-cis-lutein, 9'-cislutein, 13-cis-lutein, 13'-cis-lutein, 9-cis-zeaxanthin, and 13-cis-zeaxanthin) and therefore provided evidence that these carotenoids could function as antioxidants in the human retina.[4] Notably, a study by Li *et al* has shown that a combination of all three macular carotenoids (MZ, Z, and L, in a 1:1:1 ratio) can quench more singlet oxygen than can be achieved by any of these carotenoids individually.[138] This suggests a synergistic effect of these carotenoids when present in combination.



Zeaxanthin



Meso-zeaxanthin



Lutein

Figure 6: Chemical structure of the macular carotenoids, lutein, zeaxanthin and *meso*-zeaxanthin.

1.6.4.3 Anti-inflammatory

The macular carotenoids have potential anti-inflammatory activity with putative benefits for patients with AMD. Tian *et al* examined the effect of L on plasma levels of inflammatory markers associated with the complement pathway (i.e. Factor D, C5a and C3d) in 70 patients with early AMD. Plasma samples were collected as part of the Combination of Lutein Effects in the Aging Retina (CLEAR) study,[139] and were analysed at baseline, 4, 8 and 12 months by enzyme-linked immunosorbent assay (ELISA). L significantly reduced circulating levels of the complement factors - Factor D, C5a and C3d - when compared to the placebo group.[7] In another study, levels of the membrane attack complex (sC5b-9) were significantly reduced in patients with early AMD who were given supplemental L compared to placebo.[6] An *in vitro* study using cultured human adipocytes showed that L inhibits the synthesis of complement Factor D both at the level of Factor D messenger Ribonucleic acid (mRNA) expression and Factor D protein release,[140] providing a possible mode of action for L in the complement pathway. Furthermore, Firdous *et al* demonstrated the anti-inflammatory activity of MZ using inflammatory models in mice.[9] In that study, paw edema induced using either carrageenan, dextran or formalin was significantly reduced by MZ. In addition, MZ inhibited the production of C-reactive protein, pro-inflammatory cytokines (e.g. tumor necrosis factor α [TNF-α], interleukin 1β [IL-1β], and interleukin 6 [IL-6]), nitric oxide (NO), and the expression of TNF-α, inducible nitric oxide (iNOS), and cyclooxygenase 2 (COX-2) genes in lipopolysaccharide-stimulated macrophages.[9] These studies provide support for the role of the macular carotenoids in reducing the effect of inflammatory mediators in AMD, although further work is needed to understand the exact mechanism of action.

1.7 Macular Pigment and Age-Related Macular Degeneration

MP (and its constituent carotenoids) may protect against AMD by reducing oxidative stress (see Section 1.4.1) via its antioxidant properties in the pathogenesis of this condition.[141] The blue light filtering properties of MP also limit photooxidative damage passively and thereby confer protection to the macula.

In 2001, the AREDS study presented the evidence and rationale for antioxidant supplementation in the management of AMD.[14] AREDS found a 26% risk reduction for progression to advanced AMD following supplementation with vitamins C, E, beta-carotene, copper and zinc.[14] AREDS was criticised because it had not included the macular carotenoids in the formulation, and this shortcoming prompted the subsequent AREDS2 study, which examined the impact of supplementation with L, Z and/or omega 3 fatty acids on progression to advanced AMD, and was powered to detect an additional 25% risk reduction beyond that attained when using the original AREDS formula.[114] It is important to emphasise that almost all subjects in AREDS2 were also given a variation of the original AREDS formula, and, as such, AREDS2 was not truly placebo-controlled.[114] Thus, AREDS2 assessed the additive effect of L and Z on progression to advanced AMD. The primary outcome measure in AREDS2 failed to reveal a beneficial effect of supplemental L and Z. Secondary analysis, however, where data were dichotomized to those supplemented with L and Z versus those not being supplemented with these macular carotenoids, did reveal a beneficial effect in terms of progression to the advanced form of the disease, especially in those with a low dietary intake of these carotenoids.[115, 116] Musch, in an editorial review following publication of AREDS 2, seeks further clarity with respect to the

AREDS2 findings, including whether the AREDS2 formulation comprised the optimal doses of L and Z, and whether L and Z are the ideal carotenoids for retarding progression to advanced AMD.[142] MZ is one of MP's constituent carotenoids, and it is possible that the inclusion of MZ to the AREDS2 formulation may offer potential benefits to patients with AMD. Of interest, MZ is believed to be particularly important for the following reasons. Firstly, MZ is the pre-dominant macular carotenoid at the foveal epicentre,[8] and is therefore ideally located to exert optimal antioxidant activity and short-wavelength light filtration at the central macula, the specialized part of the retina responsible for colour vision and high spatial resolution.[143] Secondly, it has been shown (*in vitro*) that the antioxidant properties of the macular carotenoids (L, Z, and MZ) are enhanced when all three carotenoids are present.[138] We present data (in Chapters 3 and 4) from clinical trials examining the potential benefits of MZ for visual function in patients with non-advanced AMD. These clinical trials are not sufficiently powered to examine progression to advanced AMD (which will require a follow-up period of at least five years), but will contribute to our understanding of the role of the macular carotenoids on the natural course of AMD.

1.8 Macular Pigment and Visual Function in Age-Related Macular Degeneration

AMD is a multifactorial disease which causes degenerative changes at the macula, resulting in central vision impairment. Central vision impairment affects normal daily activities (e.g. reading, driving, watching television, and recognising faces) and consequently leads to an overall loss of social independence and reduced quality of life among sufferers of this condition.[2] MP's anatomic location (central

macula), short-wavelength light filtering (optical) properties, antioxidant and anti-inflammatory (biochemical) properties make this pigment important for visual function.

Several hypotheses (e.g. acuity hypothesis,[144] visibility hypothesis,[145] glare hypothesis[146]) have been put forward to explain how MP may influence visual function. For instance, the acuity hypothesis posits that MP can improve visual acuity (VA) by reducing the effects of chromatic aberration.[144] MP attenuates the penumbra/blur circle formed as a result of this phenomenon.[147] On the other hand, the visibility hypothesis posits that MP can enhance detail of a target by the differential absorption of blue haze.[145] Blue haze is caused by scattered short-wavelength dominant air light (blue light) that produces a veiling luminance when we view objects at a distance.[145] MP accentuates the luminance of an object relative to its background by attenuating this scattered (veiling) short-wavelength visible blue light and, by consequence, extends the visual range. The glare hypothesis posits that MP augmentation could improve GD and PRT via its optical (blue light) filtration properties.[146] MP attenuates shortwavelength light from the glare source before it reaches the photoreceptors, thereby reducing its impact on photopigment bleaching, and consequently, reducing the recovery time (i.e. the time it takes for vision to be restored).[146]

Studies have been performed to examine the role of MP for visual function across diverse populations, including patients with AMD (*the focus of this thesis*). Our visual environment is made up of objects and targets of varying size, contrast, shape, form, illuminance, location/position etc. Therefore, our ability to see (visual function) is influenced by a myriad of factors including the state of the eye's optical system, visual system (which includes the neural apparatus and

brain), the presence of ocular pathology as well as environmental conditions. Given the multiple factors which could influence visual function, the research design most appropriate to investigate the effect of MP augmentation on visual function is one with a randomized controlled trial design.[148] Clinical trials examining the impact of supplementation with MP's constituent carotenoids on a range of visual function parameters in patients with AMD are presented in Table 4. It is evident in Table 4 that differences exist between these supplementation studies in terms of study design, outcome measures, length of follow-up and composition of supplemental macular carotenoids (either with or without co-antioxidants). Some studies have demonstrated improvements in various measures of visual function (including VA, CS, PRT), whereas others report no supplementation-related benefits (see Table 4). None of the studies, to date, have investigated the impact of supplementation on reading speed and retinal straylight. Overall, most interventional studies have shown that supplementation with the macular carotenoids (in varying doses and/ or in combination with co-antioxidants) impacts positively on visual function (see Table 4). However, only one study examined the impact of supplementation with a formulation containing MZ on visual function in patients with AMD (see Table 4). In that study (known as the MOST study), formulations containing MZ appear to offer visual benefit in terms of contrast sensitivity (CS) in patients with non-advanced AMD.[149] Chapter 3 presents data from this exploratory study in detail.[149] Furthermore, the CREST study[150] (described in Chapter 4) presents data on a double-blind randomized clinical trial examining whether the inclusion of MZ confers any visual benefits in patients with non-advanced AMD. The CREST study compares two macular carotenoid supplements: one containing MZ, Z and L along with co-antioxidants,

versus one containing L and Z along with co-antioxidants. Of note, the macular carotenoid concentrations of the supplement formulations utilized in the clinical trials reported in Chapters 3 and 4 were informed by the research studies reported in Table 4, and it is noteworthy that the macular carotenoid concentrations are significantly greater than average dietary consumption levels. A recent meta-analysis by Liu *et al* comprising 1176 patients with (any) AMD from eight placebo-controlled RCTs concluded a visual benefit following supplementation with the macular carotenoids and demonstrated a dose dependent response.[151] However, this meta-analysis was limited by the small number of studies included in the analyses.

Table 4: Clinical trials investigating the impact of supplemental macular carotenoids on visual function in patients with age-related macular degeneration

Authors, year	Design	Sample size	Country	Intervention	Placebo	Duration	Visual function	Outcomes related to supplementation
Richer *et al*, 2004[152]	double-blind RCT	90	USA	10mg L or 10mg L+ antioxidants	Present	12 months	VA, CS, Amsler grid, PRT, VFQ-14	Improved PRT, near VA, CS and Amsler grid score
Bartlett *et al*, 2007[153]	double-blind RCT	30	UK	6mg L, 750µg retinol, 250mg vit. C, 34mg vit. E, 10mg zinc, 0.5mg copper	Present	9 months	CS	No benefit
Weigert *et al*, 2011[154]	double-blind RCT	126	Austria	20mg L from baseline to 3 months and/10mg L from 4 to 6 months	Present	6 months	BCVA	No benefit
Richer *et al*, 2011[155]	double-blind RCT	60	USA	8mg Z or 8mg Z + 9mg L	Present[α]	12 months	VA, FSD, KVF, PRT, CS, Chroma colour	Improved high contrast VA and FSD*
Piermarocchi *et al*, 2011[156]	prospective open-label RCT	145	Italy	10mg L, 1mg Z , 4mg astaxanthin, 180mg vit. C, 30mg vit. E, 22.5mg zinc, 1mg copper	Present[β]	24 months	BCVA, CS, NEI VFQ-25	Improved BCVA, CS and NEI VFQ-25 score
Ma *et al*, 2012[157]	double-blind RCT	108	China	10mg L or 20mg L or 10mg L +10mg Z	Present	48 weeks	BCVA, CS, PRT, Amsler grid	Improved CS
Beatty *et al*, 2013[158]	double-blind RCT	433	Northern Ireland and ROI	12mg L, 0.6mg Z, 15mg vit. E, 150mg vit. C, 20mg zinc, 0.4mg copper	Present	36 months	BCVA, CS	Improved BCVA
Murray *et al*, 2013[139]	double-blind RCT	72	UK and Netherlands	10mg L	Present	12 months	BCVA	No benefit
Berrow *et al*, 2013[159]	single-blind RCT	14	UK	12mg L, 0.6mg Z, 150mg vit. C, 15mg vit. E, 20mg zinc, 400µg copper, 240mg EPA, 840mg DHA	Present[β]	40 weeks	BCVA, CS	No benefit

Authors, year	Design	Sample size	Country	Intervention	Placebo	Duration	Visual function	Outcomes related to supplementation
Dawczynski *et al*, 2013[160]	double-blind RCT	172	Germany	10mg L, 1mg Z + 100mg DHA, 30mg EPA and 60mg vit. C, 20mg vit. E, 10mg zinc, 0.25mg copper or 20mg L, 2mg Z, 200mg DHA, 60mg EPA and 120mg vit. C, 40mg vit. E, 20mg zinc, 0.5mg copper	Present	12 months	BCVA	Improved BCVA
Garcia-Layana *et al*, 2013[161]	double-blind RCT	44	Spain	12mg L, 0.6mg Z and 280mg DHA	Present	12 months	BCVA, CS	No benefit
AREDS2 *et al*, 2013[115]	double-blind RCT	4203	USA	10mg L, 2mg Z; 350mg DHA, 650mg EPA + co-antioxidants	Present$^{\delta}$	5 years	BCVA	No benefit
Huang *et al*, 2015[162]	double-blind RCT	112	China	10mg L or 20mg L or 10mg L +10mg Z	Present	24 months	BCVA, CS, PRT, NEI VFQ-25,	Improved CS and PRT
Akuffo *et al*, 2015[149]	single-blind RCT	67	ROI	20mg L, 0.86mg Z or 10mg MZ, 10mg L, 2mg Z or 17mg MZ, 3mg L, 2mg Z	Absent	36 months	BCVA, CS	Improved CS

α, "Faux placebo": 9mg L; β; no dietary supplementation; δ, original AREDS[14] formula; *, Placebo group showed improvements in low contrast visual acuity, contrast sensitivity and photostress recovery time; RCT, randomized clinical trial; AMD, age-related macular degeneration; VA, visual acuity; BCVA, best-corrected visual acuity; PRT, photostress recovery time; VFQ, visual function questionnaire; CS, contrast sensitivity; FSD, foveal shape discrimination; KVF, kinetic visual fields; NEI-VFQ, National Eye Institute Visual Function Questionnaire; vit., vitamin; L, lutein; Z, zeaxanthin; MZ, *meso*-zeaxanthin; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; ROI, Republic of Ireland; UK, United Kingdom; USA, United States of America.

Chapter 2. Prevalence of Age-Related Macular Degeneration in the Republic of Ireland

2.1 Introduction

TILDA[163] is a prospective cohort study aimed at providing representative and comprehensive data relating to older people and the ageing population in the ROI, by collecting data on the social, economic, and health status of participants aged 50 years and over. TILDA collected vision data, including retinal photographs for grading of AMD, as part of the health assessment at baseline (wave 1).

Although the prevalence of AMD has been reported in population-based studies for many different countries (see Chapter 1, Section 1.3; Tables 1 and 2),[24, 164] the TILDA sample provides an unprecedented opportunity to investigate the prevalence of AMD from a population-based random sample selected from the ROI. Of note, this is the first study of its kind in the ROI.

2.2 Methods

2.2.1 Study Population

The design and methodology of TILDA has been described in detail elsewhere.[163] The TILDA sampling frame was based on a comprehensive record of all residential addresses in the ROI compiled by the Irish Postal Service (An Post) and Ordnance Survey Ireland (RANSAM system, developed by the Economic and Social Research Institute of Ireland), and the sampling method was designed to achieve a population-representative sample of (community-resident) individuals aged 50 years or older. The sampling frame was made up of 3155 clusters (500 to 1180 residential addresses in each cluster). 640 clusters were randomly selected

using proportionate stratification by socioeconomic status (percentage in professional/managerial occupations), age structure (percentage of population aged 50 years or older) and geography. Forty residential addresses were randomly selected from each of the 640 clusters, resulting in a list of 25,600 addresses. A letter of invitation was sent to each of the sampled addresses, furnishing residents with information about the study and informing residents of the proposed visit by a member of the field staff. All sampled addresses (see Figure 7 below) were then visited by a member of the field staff and residents that were deemed eligible were then invited to participate. All persons aged 50 years and over (primary respondents) and their spouses or partners of any age (secondary respondents) were eligible for inclusion in TILDA. Of note, secondary respondents are not included in this analysis.

In all, 8504 participants were sampled, with 8175 participants aged 50 years or older. Enrolled participants completed the computer-assisted personal interviewing (CAPI) questionnaire, self-completion questionnaires (SCQ) and were offered either a health centre assessment or a home-based assessment.[163, 165] Of note, 5035 (62%) participants underwent a health centre assessment, which included retinal photographs for AMD grading. Characteristics of participants who opted for home-based versus health centre assessment have been previously analysed and reported.[165] In brief, participants who opted for home-based assessment had lower levels of global cognitive function, walked more slowly, had weaker handgrip strength, rated their health as poor, had lower levels of education, were more likely to be older, self-report a disability and be a current smoker, than those who opted for health centre assessment.[165] No sex differences were observed between participants who had a home-based versus health centre

assessment.[165] Figure 1 illustrates the TILDA baseline (wave 1) participants included in the current study. Data for this report was collected as part of the first wave of TILDA, which was initiated in October 2009, and completed in July 2011. Additional waves are also scheduled, with interviews taking place on a two yearly basis (e.g. waves 2, 4) and the comprehensive health assessment every four years from baseline (e.g. waves 3, 5).

Ethical approval for the TILDA study was granted by the Faculty of Health Sciences Ethics Committee of Trinity College Dublin, Ireland (see Appendix A). Written informed consent was granted by all participants prior to study enrolment. All experimental procedures adhered to the tenets of the Declaration of Helsinki.

Figure 7: Map of randomly selected residential addresses in the Republic of Ireland. Image courtesy of the Irish Longitudinal Study of Ageing (TILDA)

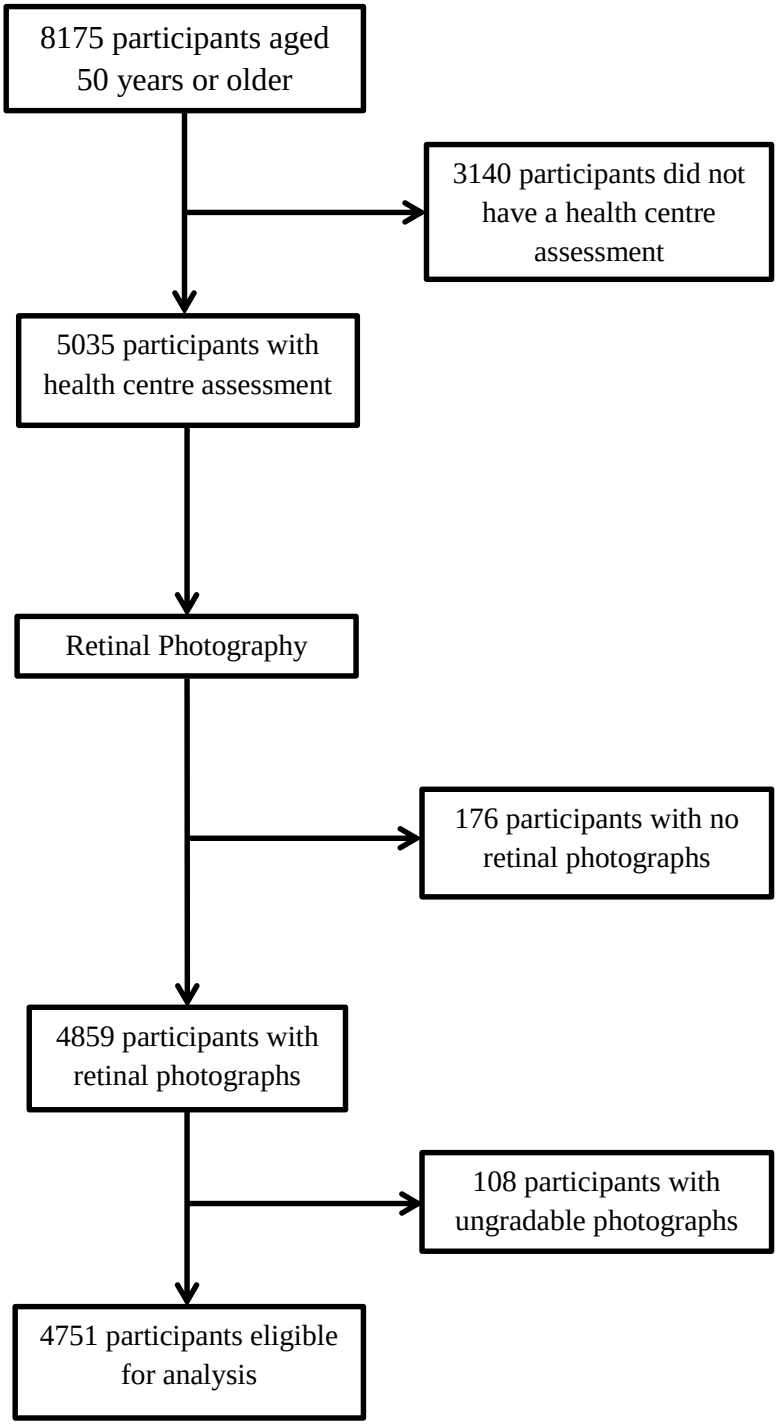


Figure 8: Flow chart showing TILDA Baseline (wave 1) participants included in study analysis. 8175 participants aged 50 years or older completed the TILDA baseline (wave 1) interview. Health assessments were conducted in clinical centres in Dublin and Cork, Republic of Ireland. Participants who refused or were unable to attend clinical centres were given the option of a home-based clinical assessment. Home-based clinical assessment did not include retinal photography. Retinal photographs were taken at the clinical centres using the NIDEK AFC-210 camera. Subjects with no photographs were either due to the following reasons: unable, unwilling, or technical failure. Photographs were judged as ungradable based on photographic quality.

2.2.2 Retinal Photography

Retinal photography was carried out using the NIDEK AFC-210 non-mydriatic auto-fundus camera, through a non-dilated pupil, by TILDA research nurses. TILDA nurses were trained and certified by experts from the Ocular Epidemiology Reading Centre at the University of Wisconsin, Madision, USA. One 45º monoscopic colour photograph, centred on the macula (EDTRS standard field 2), was obtained for each eye. The photographs were anonymised, using a unique identifier and transferred to the Moorfields Eye Hospital Reading Centre (MEHRC), London, United Kingdom and the Macular Pigment Research Group (MPRG), Nutrition Research Centre Ireland, Waterford, Ireland.

2.2.3 Retinal Grading

Retinal photographs were graded at the MPRG by a masked grader (K.O.A) who was trained and certified at the MEH Reading Centre (see Appendix B). A grading station, consisting of two Dell Professional LED monitors (24") with screen resolution (1920 ×1080) and a Dell OptiPlex 7010 MT, was set up at the MPRG, Vision Research Centre, for grading retinal photographs taken as part of the TILDA study. The grading screens were calibrated in ambient lighting with the Spyder 4^{TM} PRO (DataColor, USA). Grading was carried out under the supervision of the MEHRC, using a modified version of the International Classification and Grading System for AMD.[1] A detailed description of the International Classification and Grading System for ARM and AMD is given in Chapter 1 Section 1.2.2.

The retinal photographs were first assessed for photographic quality and judged as excellent; good; fair; poor; wrong field definition but some features are gradable; or ungradable. The presence or absence of AMD features was determined using the following criteria: absent (<50% certainty the lesion is present); questionable (50-90% certain the lesion is present); present (>90% certain the lesion is present); cannot grade (obscuring lesion or photo quality). The following AMD features were evaluated: the presence of more than 10 hard drusen (< 63µm); soft drusen (>125 µm); atrophic AMD; and signs of neovascular AMD (choroidal neovascularization, RPE detachment, disciform scar). Early AMD was defined as the presence of more than 10 hard drusen (< 63µm) and/or the presence of soft drusen (>125 µm). Late AMD was defined as the presence of atrophic AMD and/or neovascular AMD. Mixed AMD was defined as the presence of atrophic AMD in one eye and neovascular AMD in the other eye.

AMD features graded as questionable were adjudicated by the MEHRC. To ensure that valid and reliable data with respect to AMD grading was secured, the following quality assurance measures were taken: First, 10% of images were re-graded by the MEH Reading Centre for concordance. Second, intragrader reliability was assessed by the re-grading of a 3% randomly selected sample of retinal photographs graded by the principal grader (K.O.A) with a minimum interval of 14 days between visualization of the images in question.

2.2.4 Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY; weighted kappa statistics, not available in SPSS, were

obtained using the statistical programming language R.[166] For purposes of statistical analysis, the worst eye, in terms of AMD severity, was assigned to each participant.

Of 5035 TILDA participants who presented at health centres for clinical examination, 4859 had retinal photographs for at least one eye (right eye in 4808 and left eye in 4798). Intragrader reliability was assessed in 300 eyes using the kappa statistic. Demographic characteristics of participants with gradable photographs were compared to those with ungradable photographs using independent samples t-test or chi-squared test of independence. After excluding subjects with ungradable fundus photographs, 4751 participants remained for estimating AMD prevalence.

Selection of households for inclusion in this study was random, but we identified two major sources of subsequent bias. In addition to the usual non-response bias, common to most social surveys, it was evident that non-attendance at health centres was more common e.g. among older subjects, and this introduced additional bias. In order to identify and adjust for bias, study participants were initially classified by three variables - age (three categories, 50-64, 65-74 and ≥ 75), gender (male, female) and education (three categories, primary/none, secondary and tertiary/higher), resulting in a total of eighteen (3 x 2 x 3) sample subgroups. Comparison of numbers in these subgroups, with what would be expected from the corresponding data for the population of the ROI (available from the Central Statistics Office, Dublin),[167] revealed significant discrepancies. For instance, females, third-level educated and younger subjects were over-represented in the sample. However, before developing sample weights to adjust for these discrepancies, we first used logistic regression to investigate the

relationship between AMD prevalence and these three variables jointly. As only the age variable was significantly related to AMD in the regression analysis, sample weights, adjusting for disproportionate representation, were calculated using just this (age) variable. These weights were then applied in all calculations of overall AMD prevalence.

The relationship between the prevalence of AMD, and established or putative risk factors for this condition, other than age, was investigated by logistic regression. Each such investigation controlled for age, and included an age*risk factor interaction term. The rationale for including the age*risk factor interaction term in routinely models was simply to see if the interaction effect was significant. Age is a crucial variable for AMD, and it is of interest to see if it has the same effect at different levels of another factor, or has different effects e.g. it was worth investigating if the age effect (on AMD prevalence) was the same for males and females, or had greater effect for males than for females. In reporting results, however, we elected to stratify by age, and report prevalence with respect to potential risk factors within each age group. The 5% level of statistical significance was applied throughout all risk factor analyses, without adjustment for multiple testing.

2.3 Results

Demographic characteristics of the TILDA participants studied as part of this investigation are reported in Table 5. Participants with ungradable photographs were significantly older and had poorer visual acuity when compared to participants with gradable photographs (mean ± standard deviation [SD]; age:

gradable, 61.61 ± 8.10; ungradable, 68.09 ± 9.23; $p<0.0005$) and (mean ± SD; VA [LogMAR]: gradable, 0.06 ± 0.18; ungradable, 0.12 ± 0.19; $p= 0.001$].

Intragrader reliability showed moderate agreement for all categories.[168] Kappa and weighted kappa scores varied from 0.51 to 0.61 and 0.60 to 0.61 respectively. Exact agreement for AMD features varied from 91% to 96%.

Table 5: Demographic and other characteristics of TILDA Baseline (Wave 1) participants included in study analyses

Characteristic	
Age	61.61 ± 8.10
BMI	28.42 ± 4.51
VA	0.06 ± 0.18
Gender	
Male	2169 (45.7)
Female	2582 (54.3)
Total	4751 (100)
Education	
Primary/none	1013 (21.3)
Secondary	1986 (41.8)
Tertiary/higher	1750 (36.8)
Total	4749 (100)
Location	
Dublin	1383 (29.1)
Other urban	1259 (26.5)
Rural	2104 (44.3)
Total	4746 (100)
Smoking	
Never	2189 (46.1)
Past	1856 (39.1)
Current	706 (14.9)
Total	4751 (100)
Family History	
Don't know	453 (9.5)
No	4043 (85.1)
Yes	255 (5.4)
Total	4751 (100)
Cardiovascular disease	
No	2987 (62.7)
Yes	1771 (37.3)
Total	4751 (100)
Stroke	
No	4690 (98.7)
Yes	61 (1.3)
Total	4751 (100)

Interval data presented as mean ± standard deviation (SD). Categorical data presented as percentages (%). Age, age in years; BMI, body mass index (kg/m^2); VA, visual acuity; Visual acuity recorded in logarithm of the minimum angle of resolution (LogMAR); Visual acuity was measured in both eyes using an Early Treatment Diabetic Retinopathy Study (ETDRS) LogMAR chart at a test distance of four meters; Only eye with best visual acuity is reported; Family History, subjects who reported a family history of age-related macular degeneration; Family history was defined as having a first degree relative, i.e. parent or sibling with AMD; Smoking, smoking status of subjects classified as never (no reported history of smoking), past (past smokers) and current

(current smokers); Education, level of education; Primary/none: subjects who did not have education and those with only primary education; Secondary: subjects who completed a junior certificate or leaving certificate or equivalent; Tertiary: subjects who completed a diploma, first degree or higher degree; Location, location of residence in the Republic of Ireland; Dublin: residence in Dublin city or county; residence in other urban, another town or city in the Republic of Ireland; Rural, residence in rural area in the Republic of Ireland; Cardiovascular disease refers to participants who reported no self-reported doctor's diagnosis of any of the following: angina, heart attack, heart failure, stroke, transient ischaemic attack (TIA), heart murmur; Stroke refers to participants who reported a doctor's diagnosis of stroke.

2.3.1 Prevalence of AMD

Increasing age was the only variable exhibiting a statistically significant association with AMD (defined as any AMD yes/no) in a logistic regression model including the variables age, gender and education. The development of sample weights based on this age variable is presented in Table 6. The age group ≥75 constitutes over 18% of the over 50s in the Irish population, but only 8.5% of the sample reported herein. Therefore, ignoring this under-representation in the sample of the oldest age group would lead to an underestimate of prevalence of AMD. The weights (final column of Table 6) adjust for this: every subject aged ≥75 in the sample is treated (in estimating overall prevalence) as representing 544 subjects in the population, whereas sample subjects in the other two age groups are treated as representative of about 225 subjects in the population.

Table 6: Sample weights for analysis

Age group	Population (%)	Sample (%)	Weight
50-64	700,800 (58.4)	3093 (65.1)	226.6
65-74	280,900 (23.4)	1256 (26.4)	223.6
≥75	218,700 (18.2)	402 (8.5)	544.0

Weights developed from age variable. Population data was based on the Republic of Ireland population census 2011

.

Table 7 shows the prevalence of each category of AMD, as well as the estimated prevalence of AMD (all forms) for those aged 50 years or older in the ROI. These estimates are based on the weights presented in Table 6. Adjusting for age, the prevalence of AMD (any form) was 7.2% (95% CI: 6.5% to 7.9%); the prevalence of early AMD was 6.6% (95% CI: 5.9% to 7.3%); the prevalence of late AMD was 0.6% (95% CI: 0.4% to 0.8%); the prevalence of atrophic AMD was 0.3% (95% CI: 0.1% to 0.5%); and the prevalence of neovascular AMD was 0.3% (95% CI: 0.1% to 0.5%).

Analysis of AMD by other demographic subgroups, stratifying by age, is shown in Table 8. The p-values displayed in Table 8 were obtained from the chi-squared test for contingency tables. Some differences in prevalence of AMD are evident in Table 8 with respect to gender, education and geographic location ("Dublin" versus "other urban" versus "rural"). However, a statistically significant difference was observed only for early AMD with respect to geographic location (with prevalence values of 10.8%, 18.4% and 6.3% of participants categorized as "Dublin", "other urban" and "rural", respectively). Some other, statistically non-significant, findings in Table 8 may be attributable to the small sample sizes of the respective subgroups; e.g. prevalence of (any and early) AMD is clearly greater for females than for males in the ≥75 age group.

The prevalence of drusen within demographic subgroups, stratifying by age, is reported in Table 9.

Table 7: Prevalence of age-related macular degeneration by age category

Age groups (years)	Any AMD n (%)	Early AMD n (%)	Late AMD n (%)	Atrophic AMD n (%)	Neovascular AMD n (%)	Mixed AMD* n (%)
50-64	156 (5.0)	152 (4.9)	4 (0.1)	1 (0.0)	3 (0.1)	0 (0.0)
65-74	98 (7.8)	92 (7.3)	6 (0.5)	3 (0.2)	2 (0.2)	1 (0.1)
≥ 75	53 (13.2)	44 (11.0)	9 (2.2)	5 (1.3)	4 (1.0)	0 (0.0)
Overall unweighted	307 (6.5)	288 (6.1)	19 (0.4)	9 (0.2)	9 (0.2)	1 (0.0)
Overall weighted	86095 (7.2)	78950 (6.6)	7144 (0.6)	3618 (0.3)	3303 (0.3)	224 (0.0)
95% CI (Overall weighted)	6.5-7.9	5.9-7.3	0.4-0.8	0.1-0.5	0.1-0.5	-

Abbreviations: AMD, age-related macular degeneration; n, number; CI, confidence interval; %, percentage; *Mixed AMD – subject has neovascular AMD in one eye and atrophic AMD in the other eye.

Table 8: Prevalence of age-related macular degeneration by demographic subgroups, stratified by age group

	Any AMD			Early AMD			Late AMD			Atrophic AMD			Neovascular AMD		
Characteristic, n (%)	**Age groups**			**Age groups**			**Age groups**			**Age groups**			**Age groups**		
	50-64	**65-74**	**≥ 75**	**50-64**	**65-74**	**≥ 75**	**50-64**	**65-74**	**≥ 75**	**50-64**	**65-74**	**≥ 75**	**50-64**	**65-74**	**≥ 75**
Gender															
Male	71(5.2)	47 (7.8)	21(11.1)	70 (5.1)	44 (7.3)	16 (8.4)	1 (0.1)	3 (0.5)	5 (2.6)	0 (0.0)	2 (0.3)	3 (1.6)	1 (0.1)	1 (0.2)	2 (1.1)
Female	85(4.9)	51(7.9)	32(15.3)	82 (4.8)	48 (7.4)	28 (13.4)	3 (0.2)	3 (0.5)	4 (1.9)	1 (0.1)	1 (0.2)	2 (1.0)	2 (0.1)	1 (0.2)	2 (1.0)
	p=0.771	p=0.947	p=0.211	p=0.672	p=0.927	p=0.113	p=0.435	p=0.933	p=0.630	p=0.372	p=0.524	p=0.577	p=0.700	p=0.961	p=0.924
Education															
Primary/None	28 (5.7)	34 (8.5)	15 (12.0)	27 (5.5)	33 (8.3)	9 (7.2)	1 (0.2)	1 (0.3)	6 (4.8)	0 (0.0)	0 (0.0)	3 (2.4)	1 (0.2)	1 (0.3)	3 (2.4)
Secondary	72 (5.2)	30 (6.9)	21 (13.8)	69 (4.9)	27 (6.2)	19 (12.5)	3 (0.2)	3 (0.7)	2 (1.3)	1 (0.1)	1 (0.2)	1 (0.7)	2 (0.1)	1 (0.2)	1 (0.7)
Tertiary	56 (4.6)	34 (8.1)	17 (14.2)	56 (4.6)	32 (7.6)	16 (13.3)	0 (0.0)	2 (0.5)	1 (0.8)	0 (0.0)	2 (0.5)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
	p=0.628	p=0.658	p=0.863	p=0.744	p=0.501	p=0.242	p=0.277	p=0.656	p=0.069	p=0.545	p=0.377	p=0.382	p=0.356	p=0.603	p=0.147
Location															
Dublin	33(4.0)	27(7.0)	21(13.3)	33 (4.0)	24 (6.2)	17 (10.8)	0 (0.0)	3 (0.8)	4 (2.5)	0 (0.0)	1 (0.3)	2 (1.3)	0 (0.0)	2 (0.5)	2 (1.3)
Other urban	48(4.9)	27(7.8)	18(18.4)	46 (5.6)	26 (7.5)	18 (18.4)	2 (0.2)	1 (0.3)	0 (0.0)	1 (0.1)	1 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Rural	75(5.2)	43(8.3)	14(9.8)	73 (5.1)	41 (7.9)	9 (6.3)	2 (0.1)	2 (0.4)	5 (3.5)	0 (0.0)	1 (0.2)	3 (2.1)	2 (0.1)	0 (0.0)	2 (1.4)
	p=0.185	p=0.776	p=0.156	p=0.264	p=0.618	**p=0.013**	p=0.379	p=0.584	p=0.191	p=0.248	p=0.955	p=0.355	p=0.569	p=0.106	p=0.515

Abbreviations: AMD, age-related macular degeneration; n, number; %, percentage; p, statistical significance; level of significance set at $p<0.05$; Statistical significance tested with chi-squared test for contingency tables; Education, level of education; Primary/none: subjects who did not have education and those with only primary education; Secondary: subjects who completed a junior certificate or leaving certificate or equivalent; Tertiary: subjects who completed a diploma, first degree or higher degree; Location, location of residence in the Republic of Ireland; Dublin: residence in Dublin city or county; residence in other urban, another town or city in the Republic of Ireland; Rural, residence in rural area in the Republic of Ireland

Table 9: Prevalence of drusen by demographic subgroups, stratified by age group

	Hard drusen(<63µm) *			**Soft drusen(>125µm)**		
Characteristic, n (%)	**Age groups**			**Age groups**		
	50-64	**65-74**	**≥ 75**	**50-64**	**65-74**	**≥ 75**
Gender						
Male	36 (2.6)	15 (2.5)	2 (1.1)	34 (2.5)	29 (4.8)	14 (7.4)
Female	49 (2.9)	20 (3.1)	9 (4.3)	33 (1.9)	28 (4.3)	19 (9.1)
	p=0.702	p=0.515	**p=0.047**	p=0.289	p=0.688	p=0.533
Education						
Primary/None	18 (3.7)	13 (3.3)	4 (3.2)	9 (1.8)	20 (5.0)	5 (4.0)
Secondary	40 (2.9)	11 (2.5)	4 (2.6)	29 (2.1)	16 (3.7)	15 (9.9)
Tertiary	27 (2.2)	11 (2.6)	3 (2.5)	29 (2.4)	21 (5.0)	13 (10.8)
	p=0.240	p=0.789	p=0.938	p=0.735	p=0.559	p=0.104
Location						
Dublin	23 (2.8)	10 (2.6)	3 (1.9)	10 (1.2)	14 (3.6)	14 (8.9)
Other urban	21 (2.6)	11 (3.2)	5 (5.1)	25 (3.1)	15 (4.3)	13 (13.3)
Rural	41 (2.9)	13 (2.5)	3 (2.1)	32 (2.2)	28 (5.4)	6 (4.2)
	p=0.929	p=0.816	p=0.262	**p=0.033**	p=0.445	**p=0.040**

n, number; %, percentage; *, more than 10 hard drusen (<63µm); p, statistical significance; level of significance set at p<0.05; Statistical significance tested with chi-squared test for contingency tables; Education, level of education; Primary/none: subjects who did not have education and those with only primary education; Secondary: subjects who completed a junior certificate or leaving certificate or equivalent; Tertiary: subjects who completed a diploma, first degree or higher degree; Location, location of residence in the Republic of Ireland; Dublin: residence in Dublin city or county; residence in other urban, another town or city in the Republic of Ireland; Rural, residence in rural area in the Republic of Ireland.

2.3.2 Risk Factors for (any) AMD

Each risk factor for AMD (as listed in Table 5) was investigated separately, via logistic regression models containing that risk factor; each such model also included age, and the interaction of age with that risk factor. The dependent variable in these analyses was any AMD (yes/no); logistic analyses for smaller categories of AMD were deemed statistically infeasible. Subjects with ungradable photographs, and subjects unsure of family history for AMD, were omitted from all regression analyses.

Age was highly statistically significant in all logistic regression analyses ($p<0.005$ in all analyses). Family history was also statistically significant (odds ratio = 0.28, 95% CI for odds ratio = [0.11, 0.69], $p=0.006$), but the age*family history interaction was not ($p=0.17$). None of the other risk factors analysed (gender, education, geographic location, body mass index (BMI), stroke, cardiovascular disease, smoking), nor their respective interactions with the age risk factor, were statistically significant ($p>0.05$ for all). For example, we obtained $p=0.10$ for BMI and $p=0.16$ for the interaction term; $p=0.44$ for cardiovascular disease and $p=0.76$ for the interaction; $p=0.32$ for stroke and $p=0.38$ for the interaction.

We considered that the other risk factors merited further exploration, beyond the basic regression findings, and that the best way to do this was to stratify by age, and analyse each risk factor separately within each age group. Table 8 (first three age columns) contains this information for any AMD, and for each of the three demographic risk factors (gender, education, location). The p-values displayed in Table 8 were obtained from the chi-squared test for contingency tables; all p-values exceed 0.05 (with the sole exception of

geographic location differences in early AMD in the ≥75 age-group [possibly a type 1 error]) and so support the earlier findings from the logistic regression analyses.

Positive family history was defined as having a first degree relative, i.e. parent or sibling, with AMD. The relationship of family history to (any) AMD, stratifying by age, is presented in Table 10. The prevalence of AMD was significantly higher in those who reported a positive family history in the age group 65-74 (14.5% with AMD, p=0.017) and in the age group ≥75 (33.3% with AMD, p=0.002). These significant findings support the earlier findings from the logistic regression analysis. Persons with an unsure family history were only removed from the analyses investigating the relationship between prevalence of (any) AMD and self-reported family history of AMD. For example, in the contingency table analyses reported in Table 10, persons with an unsure family history were not removed from the analyses investigating smoking as a risk factor.

The remaining risk factor (smoking) was not significantly associated with (any) AMD, after controlling for age (p=0.59 for smoking, p=0.44 for the interaction, in the logistic regression). Nevertheless, we have included some details of the smoking-AMD relationship in Table 10. While not statistically significant, it is worth noting that in all three age groups, in Table 10, prevalence of (any) AMD was higher for current smokers than for either of the other smoking groups. It is also worth reporting that, in the case of neovascular AMD, six of nine study subjects (67%) with this condition are past or current smokers, whereas just 54% of the TILDA sample are past or current smokers.

Table 10: Risk factors for prevalence of age-related macular degeneration, stratified by age group

Characteristic, n (%)	Any AMD Age groups		
	50-64	**65-74**	**≥ 75**
Smoking			
Never	77 (5.4)	39 (6.7)	24 (13.0)
Past	50 (4.4)	44 (8.5)	25 (13.1)
Current	29 (5.5)	15 (10.1)	4 (16.7)
	p=0.420	p=0.290	p=0.881
Family History			
No	134 (5.0)	75 (7.2)	40 (11.7)
Yes	9 (6.1)	12 (14.5)	8 (33.3)
	p=0.564	**p=0.017**	**p=0.002**

n, number; %, percentage; p, statistical significance; level of significance set at $p<0.05$; Statistical significance tested with chi-squared test for contingency tables; AMD, age-related macular degeneration; Family History, subjects who reported a family history of age-related macular degeneration; Family history was defined as having a first degree relative, i.e. parent or sibling with AMD; Smoking, smoking status of subjects classified as never (no reported history of smoking), past (past smokers) and current (current smokers).

2.3.3 Other Results

While logistic regression was not considered feasible for risk factor analysis for the rarer forms of AMD, Table 8 has some interesting contingency table results for these. A statistically significant difference was observed for early AMD with respect to geographic location (with prevalence values of 10.8%, 18.4% and 6.3% of participants categorized as “Dublin”, “other urban” and “rural”, respectively). Of note, no statistically significant differences in gender were observed in the current study. Some other, statistically non-significant, findings in Table 8 may be attributable to the small sample sizes of the respective subgroups.

The prevalence of drusen within demographic subgroups, stratifying by age, is reported in Table 9. There are three statistically significant results

highlighted in Table 9, but in general, definitive conclusions based on Table 9 results (as in Tables 8 and 10) are problematic because of the small numbers of subjects in certain subgroups.

2.4 Discussion

This study was undertaken to investigate the prevalence of AMD in the ROI using the TILDA wave 1 (baseline) sample. Subjects were randomly selected from the ROI population and therefore representative of the community dwelling population aged 50 years or older. The prevalence of AMD (any form) in the ROI is estimated at 7.2%, after adjusting for different non-response rates (and different attendance rates at the health centres) in different age groups.

The prevalence estimates (and all other results presented) were obtained from subjects with gradable photographs only. Including the 108 ungradable subjects, and assuming these have the same prevalence rates within age groups as the gradable subjects, leads to some changes in AMD sample numbers within each age group, but also to changes in weights. The net effect is an overall age-adjusted estimate of 7.17% for any AMD i.e. practically identical to the estimate from the gradable subjects only.

Different population-based studies reporting prevalence estimates of AMD have adopted various photography/grading protocols and definitions for AMD (see Chapter 1, Section 1.2 and 1.3; Tables 1 and 2). Table 2 provides AMD prevalence estimates from other studies which are compared to estimates from our TILDA study. Some large differences in reported AMD prevalence are evident in Table 2, and could be either attributable to differences between the populations

studied, or differences in study design (e.g. grading techniques, photography protocols, sampling and recruitment strategies, age range of sample, etc.). A recent meta-analysis of the prevalence of AMD in populations of European ancestry found substantial variability in prevalence rates between studies, with differences in late AMD primarily due to differences in age profile and study design.[169] For the purpose of emphasising the important role of such variables on published findings, it is noteworthy that the prevalence of early AMD was as high as 52.3% and 58.6% in the Greenland Inuit Eye Study[170] and PAMDI study,[171] respectively. However, in our study, we estimate the prevalence of early AMD in the ROI to be 6.6%, consistent with some reports of ethnically comparable populations (e.g. NHANES 2005-2008 US population:[164] 5.7%). Of note, one of the characteristics graded in this study was more than 10 hard drusen (<63µm). Large areas of small drusen have been found to be associated with incidence of AMD as well as risk of progression and development of AMD.[40, 172, 173] However, in the Clinical Classification System of AMD[17] (described in detail in Chapter 1, Section 1.2.5), drusen (<63µm) is considered as druplets and are classified as changes associated with normal aging. Therefore, applying the Clinical Classification System of AMD to our study will reduce the prevalence of early AMD to 3.3% (unadjusted). Thus, different classifications systems give different prevalence of early AMD and subsequently, overall prevalence of AMD.

The prevalence of late AMD in the current study was 0.6%, consistent with some previous reports (e.g. NHANES 2005-2008 US population:[164] 0.8% ; Visual Impairment Project:[26] 0.68%) but less than that reported by others (e.g. Beaver Dam Eye Study:[23] 1.6%; Rotterdam Study:[24] 1.7%). However, the prevalence of neovascular AMD and atrophic AMD is known to vary between

studies. In our study, we report prevalence for each of the two forms of late AMD (atrophic and neovascular) to be equal (at 0.3% each), whereas some previous studies have reported the atrophic form to be more common than the neovascular form (e.g. Reykjavik eye study:[174] atrophic 3.2%, neovascular 0.7%). In contrast, however, the neovascular form of AMD has been reported to be more prevalent than the atrophic form of the condition in many other studies (e.g. Beaver Dam Eye Study:[23] atrophic 0.6%, neovascular 1.2%; Blue Mountains Eye Study:[25] atrophic 0.7%, neovascular 1.3%).

In general, we found that differences in prevalence of AMD between demographic subgroups were not statistically significant, after controlling for age (Tables 8 and 9). However, especially for the rarer forms of AMD, these findings are based on small cell frequencies, and should be treated circumspectly.

For both males and females in this study, the impact of age on prevalence appears much stronger for the more severe forms of the disease. For example, in Table 8, the prevalence of late AMD in the ≥75 age group (at 2.6%) is 5.2 times the prevalence observed for the 60-74 age group for males, and 3.8 times the observed prevalence for females. In contrast, for early AMD, the corresponding risk ratios are 1.2 and 1.8 for males and females, respectively. Similarly, in Table 9, prevalence of soft drusen in the ≥75 group is 1.5 times and 2.1 times the prevalence observed in the 60-74 group for males and females respectively, whereas the corresponding risk ratios for hard drusen are just 0.4 and 1.4 for males and females, respectively.

While primarily concerned with the prevalence of AMD, we also investigated possible associations with this condition, especially for variables that

have been previously identified as risk factors for AMD. In this regard, we report that the prevalence of AMD increases with increasing age, consistent with all other studies.[23, 25] Also, family history for AMD was strongly associated with prevalence of this condition, consistent with other studies.[85, 86] In fact, in the 65-74 and ≥75 age groups, the prevalence of AMD is strikingly greater for subjects who reported a family history of this condition. Self-reported data with respect to family history for AMD is problematic for the following reasons: reporting of AMD amongst siblings is subject to influence by the number of siblings; reporting of AMD amongst parents is subject to influence by the longevity of those parents; and reporting of AMD amongst participants who were adopted will be irrelevant with respect to a genetic predisposition for AMD; finally, reporting of early AMD is likely to be under-represented because it is typically asymptomatic. Nevertheless, and with full appreciation of these limitations, and given that we excluded subjects who replied that they did not know whether or not a first degree relative suffered from AMD, we believe that our findings that self-reported family history of AMD is a risk-factor for the condition is important.

However, with respect to other potential risk factors for which no statistically significant associations with AMD were observed in the current study, it should be appreciated that the small numbers of participants identified as having late AMD may have contributed to the non-identification of some potentially significant associations with AMD.

The strengths of our study include: 1) the use of a population-representative cohort of subjects aged 50 years and older in the ROI; 2) the study population is racially homogeneous, over 99% being white, precluding comparison to other non-white populations; 3) retinal photographs were graded in

a masked fashion using standard protocols by the same person and therefore reducing inter-grader variability. The large sample size (nearly 5000) could also be considered a strength, but the need to stratify by age group meant that, for some analyses, subgroup sizes were small.

The limitations of this study include the use of monoscopic retinal photographs through undilated pupils, rendering it difficult to obtain quality photographs in the presence of significant media opacities. The TILDA investigators elected to use monoscopic retinal photographs in the study because other health assessment measures (e.g. gait) were to be conducted immediately following retinal photography, and the results of such tests would have been influenced and confounded by pharmacological pupillary dilation. Also, subjects with ungradable images were more likely to be older and have poor vision, although (upon investigation) this did not appear to have much effect on our prevalence estimates. In addition, new imaging techniques (e.g. optical coherence tomography) which are more sensitive to detect AMD lesions were not included in this study and this may represent a limitation.

The possible consequences of non-pharmacological pupillary dilation and non-stereoscopic images on different aspects of AMD grading warrant discussion. If mydriatic photography was feasible and therefore performed, it is likely that the number of participants with ungradable photographs due to photographic quality may have been lower than reported in the current study. Dark areas due to photographic quality (as a result of non-mydriatic photography) could obscure grading of macular areas for different phenotypes. Although the retinal grading will be affected by the degree of photographic quality, non-mydriatic photography is unlikely to affect the results on large drusen and signs of late AMD. However,

it is possible that small drusen may have been missed. Furthermore, it is possible that subtle drusenoid pigment epithelial detachment and subtle fluid on CNV could have been missed in the absence of stereoscopic images. Of these advanced AMD phenotypes, drusenoid pigment epithelial detachment may have influenced our grading results if there was no other sign of neovascular AMD.

The response rate in the TILDA study (62% of eligible households participated) is in line with other national household surveys of older people e.g. in the Survey of Health, Ageing and Retirement in Europe (SHARE), the average response rate across all countries was 55%.[175] Moreover, a non-response rate of this magnitude had been anticipated (from pilot surveys prior to the main survey), and built into the sample size calculations for the TILDA study. However, non-attendance at health centres reduced the effective participation rate further, so that just 5035 of 8175 participants (61.6%), who were successfully enrolled in the broader TILDA study, actually took part in this AMD study; this represents just 38% of the individuals originally selected. This has to be acknowledged as a weakness of our study, although we were able to adjust our prevalence calculations, to take account of the distorted sample age structure which arose from this non-participation. Of note, while some studies listed in Table 2 (see Chapter 1, Section 1.3) reported much higher response rates than our AMD study (e.g. 83.1% for the Beaver Dam Study), most of these were not nationally representative population-based studies, and are not directly comparable. For obvious reasons (e.g. travel to and from study centres), response is better in non-nationally representative, population-based studies, but the findings are more local and therefore less generalizable.

2.5 Conclusion

This study reports the prevalence of AMD in the ROI for the first time, and will inform healthcare providers and planners involved in the delivery of care to those suffering with this condition. This work was published in the British Journal of Ophthalmology (BJO; see Appendix T), under the title, "***Prevalence of age-related macular degeneration in the Republic of Ireland***."[39]

Chapter 3. Supplementation with three different carotenoid formulations on visual function in Non-Advanced Age-Related Macular Degeneration: 36 months' Follow-up

3.1 Introduction

The MOST study compared the effect of supplementation with some or all of MP's constituent carotenoids on visual function, and evaluated the impact of such supplementation on vision and disease progression. Observations that MZ, the dominant carotenoid in the epicentre of the MP's spatial profile, may offer advantages in terms of MP augmentation across its spatial profile[176] and in terms of enhancement of visual function[177] prompted this investigation. In the current study, we present data on a three-year follow up of subjects in the MOST study. Of note, this is the first study to monitor MP, visual function, and AMD status in response to supplementation with all three macular carotenoids in patients with non-advanced AMD, over a 36-month period.

3.2 Methods

The design and methodology of the MOST study has been previously reported.[178] In brief, MOST is a single-blind, randomised controlled clinical trial (ISRCTN60816411). Clinical assessments were carried out at the Institute of Eye Surgery (www.ioes.ie), Waterford, Ireland. Prior to study enrolment, an eligibility screening visit was conducted by an ophthalmologist with a special interest in retinal disease (S.B.). Eligibility criteria included: non-advanced AMD (one to eight on AREDS 11-step severity scale[15] [see Chapter 1, Section 1.2.3] in at least one eye [the study eye], confirmed by the Ocular Epidemiology Reading Center at

the University of Wisconsin, Madison, USA); BCVA ≥ 6/12 in the study eye; no other ocular pathology. The study eye was chosen by adhering to the eligibility criteria with particular emphasis on the presence of early AMD, BCVA of 6/12 (20/40), and no other retinal pathology beyond AMD. The study eye could be either the right or left eye. If both eyes had early AMD, the eye with the best BCVA was chosen as the study eye. However, if both eyes had the same BCVA, the right eye was selected.

Subjects were randomly assigned to one of three parallel groups: Group 1: 20mg L, 0.86mg Z (Ultra Lutein™ supplied by Nature's Plus, Europe); Group 2: 10mg MZ, 10mg L, 2mg Z [Macushield™ (UK) /Macuhealth LMZ3™ (North America)]; Group 3: 17 mg MZ, 3 mg L, 2mg Z (supplied by Industrial Organica, Monterrey, Mexico [not commercially available]). The above treatment groups (formulations) were selected to be comparable total concentrations of macular carotenoids (i.e. 22 mg). Of note, however, discrepancies between label claim and measured values of the supplements used in this trial have been previously reported, and in particular, the finding that the Group 1 supplement contained small amounts of MZ (0.30mg).[179, 180] This has implications for the findings presented below.

The supplements were prepared in a soft gel capsule. Subjects were instructed to take one capsule daily with a meal. All study supplements were indistinguishable in terms of external appearance, and were packaged in identical containers. Study visits were conducted at baseline, 12 months, 24 months and 36 months.

3.2.1 Ethics

Ethics approval was granted by the Waterford Regional Hospital Ethics Committee (see Appendix C). Written and informed consent was obtained from each subject prior to study enrolment. The tenets of the Declaration of Helsinki were adhered to in all study procedures.

3.2.2 Outcome Measures

The primary study outcome measure was change in MP as measured by customized heterochromatic flicker photometry (cHFP) at 36 months. Secondary outcome measures included BCVA, letter contrast sensitivity (CS), serum concentrations of macular carotenoids and grade of AMD.

3.2.3 Study Procedures

3.2.3.1 Macular Pigment Measurement by Customized Heterochromatic Flicker Photometry

MP was measured using the Macular Densitometer^{TM} (Macular Metrics, Corp., Providence, Rhode Island, USA) at 0.25^{o}, 0.5^{o}, 1.0^{o} and 1.75^{o} retinal eccentricity, with a reference point at 7^{o}.[181] This protocol has been described in detail elsewhere and has been validated in AMD subjects by comparing the *in vivo* spectral absorption curves from this device to the *ex vivo* spectral absorption curves of the macular carotenoids.[182] Two wavelengths of light, one at 458nm (blue light; wavelength that is well absorbed by MP) and the other at 550nm (green light; wavelength that is not absorbed by MP) are arranged in a stimulus-surround configuration where the stimulus consists of a target presented in counterphase flicker (alternating blue to green). The blue and green alternating

lights are inverse-yoked so that when the blue light is adjusted to be more intense, the green light is commensurately decreased and vice versa. The radiance of the blue and green lights are adjusted by turning a dial until the flicker of the disk stops (null flicker) or it is at a point of minimal flicker. Thus, null flicker occurs when there is iso-luminance of the blue and green lights.

Prior to MP measurements, the testing procedure was explained and the subject's critical flicker frequency (CFF) was estimated using a prediction table based on age. Setting the flicker rate according to an expected optimal for a narrow null zone helps to minimize the variance in radiance values obtained during MP measurements at a given retinal eccentricity. If the subject could not reach the null flicker, the CFF was increased by 1Hz in a stepwise fashion until null flicker was perceived. Also, if the subject exhibited a wide variation in null flicker reading, the CFF was decreased in steps of 1Hz until an acceptable null range was achieved. During the test, subjects were instructed to turn the radiance knob clockwise or counter clockwise until the flickering stops or it is at a point of minimal flicker. The starting radiance is alternated, so that the knob is not always turned in the same direction. Throughout the testing, subjects were reminded to blink, and instructions were repeated where necessary. Six measurements at each of the targets (0.25º, 0.5º, 1º, 1.75º and a reference point at 7º) were taken for each subject. The MP measurement at a specified retinal eccentricity was computed from the radiance values obtained where the subject reported null flicker and these radiance values were deemed reliable and acceptable only when the standard deviation of the MP value was below 0.1 optical density units (ODU). The log ratio of the difference in radiance values between the measurement at a particular

retinal eccentricity (0.25º, 0.5º, 1º, 1.75º) and the measurement at 7º yields the MP optical density at the specified test locus.

3.2.3.2 Serum L, Z, and MZ Analysis

Serum L, Z and MZ were quantified by high performance liquid chromatography (HPLC) using methodology previously described.[179, 183, 184] This carotenoid analysis was performed by a biochemical analyst (R.M).

3.2.3.3 Best-Corrected Visual Acuity

BCVA measures the spatial resolving power of the visual system at 100% contrast and involves three aspects – resolution, recognition and identification. BCVA was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (LogMAR) chart (Test Chart 2000 PRO™; Thomson Software Solutions, Hatfield, UK) viewed at 4 metres. The Sloan ETDRS letterset was chosen for this test. At the first incompletely read line, the letters of the line are randomized three times using the testing software's randomization function. At each letter randomization, the number of letters correctly identified was recorded and an average of three scores was taken. Each line consists of five letters and therefore one point was awarded for each correctly identified letter. After this, the next (smaller) line is presented and subjects are encouraged to read it. If any letters are identified correctly on this line, one point per letter was added to the previous score. A subject achieving a Snellen value of 6/6 or LogMAR 0.00 receives a visual acuity rating (VAR) score of 100. Each extra correctly identified letter has a score of 1 (and similarly, if a letter is missed,

-1 is taken from the VAR score). Therefore, the BCVA score was calculated as follows: BCVA = VAR value of Snellen Fraction – (number of letters missed) + (number of extra letters).

3.2.3.4 Letter Contrast Sensitivity

Contrast sensitivity (CS) can be described as our ability to discriminate an object from its background and is determined by measuring the contrast threshold between visible and invisible at given spatial frequency.[185] The spatial frequency represents the size of the target whereas the threshold is the point where an object is just detectable (can just be seen). Letter CS was assessed using the LogMAR EDTRS (Test Chart 2000 PRO™; Thomson Software Solutions, Hatfield, UK) chart at five different spatial frequencies (1.2 [cycles per degree] cpd, 2.4cpd, 6.0cpd, 9.6cpd, 15.15cpd) viewed at 4 metres. The Sloan optotypes were chosen and subjects were asked to read the letters aloud while fixating on the chart at a distance of 4m. The letter set is randomized during the test at each change of contrast. The percentage contrast of letter optotypes is decreased in 0.15 log CS steps until the lowest contrast value for which subjects see at least three letters is reached. The test is then repeated for the other spatial frequencies. Each letter has a nominal log CS value of 0.03. Missed letters at any contrast level are noted. The resultant log CS value for the subject at a particular spatial frequency is calculated by adding any extra letter(s) and/or subtracting missed letters from best log CS value corresponding to the lowest percentage contrast.

3.2.3.5 Retinal Photography and AMD Grading

Following prior pharmacological pupillary dilation (0.5% proxymetacaine hydrochloride [Chauvin Pharmaceuticals Ltd, London UK], 2.5% phenylephrine hydrochloride [Chauvin Pharmaceuticals Ltd, London UK], and 1% tropicamide [Chauvin Pharmaceuticals Ltd, London UK]), 45º stereoscopic color fundus photographs were taken in three retinal photographic fields (optic disc, macula, temporal to macula) using a Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany). Photographs were transferred to the Ocular Epidemiology Reading Center at the University of Wisconsin, Madison, USA via an encrypted system. Photographs were graded in a masked fashion using a modified version of the WARMGS[13, 186] (see Section 1.2.1) and adhered to the AREDS 11-step severity scale (see Section 1.2.3).[15]

3.2.4 Statistical Analysis

One eye (the study eye) of each subject comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0. Armonk, NY. In order to compare the effects of the three supplements (on each outcome measure, over time), we used Repeated Measures Analysis of Variance, and contingency table analysis, as appropriate. Cognisant that this exploratory study would likely have insufficient power for such analyses, however, we did some additional analyses. In fact, and beyond the previously reported 12-month data,[178] we decided upon two strands of analysis: (a) between supplement group analysis over time: Despite the small sample sizes, supplement groups were compared with each other, for changes in each outcome variable over the three years of the study. For interval outcome variables (MP, serum carotenoids,

BCVA, CS), the method of analysis was Repeated Measures Analysis of Variance, with time as a within-subjects factor and supplement as a between-subjects factor; we used the Greenhouse-Geisser correction for lack of sphericity. Post-hoc analysis, with Bonferroni adjustment for multiple testing, was used where appropriate. For categorical outcome variables (AMD grade), we used contingency table analysis to compare supplements; (b) within-supplement group changes in each outcome variable, over the three years of the study. We used paired t-tests analysis here.

Tests of significance, for all t-test analyses, were two-tailed, and the 5% level of significance was used throughout. With the exception of post-hoc analyses for the Repeated Measures Analysis of Variance, we did not correct for multiple tests.

3.3 Results

67 subjects were enrolled at baseline, with 47 subjects completing the final study visit at 36 months. Only those subjects that completed each study visit were included in analysis. Therefore, if a subject attended his/her 12 or 24-month visit, but did not complete the 36-month visit, he/she was not included in the analysis. Where a subject did complete a study visit, but where a variable was not measured or recorded, that subject was also excluded from all analyses relating to that variable. Exclusions occurred only in the MP and CS analysis because data was not available at all study visits (MP analysis: 5 subjects; CS analysis: 6 subjects). We have also included the sample size in all tables for clarity.

Baseline characteristics (e.g. age, gender, smoking status, education) of participants in intervention groups have been previously described, and the intervention groups were statistically comparable in terms of these variables.[178]

3.3.1 Macular Pigment and its Constituent Carotenoids in Serum

3.3.1.1 Macular Pigment

Primary Analysis: baseline vs 36 months

Comparing MP at 36 months with MP at baseline, a statistically significant increase in MP was observed at three years in all groups, at each measured eccentricity ($p<0.05$), with the exception of a non-significant increase in MP at 1.75° in Group 1 ($p=0.160$).

Secondary Analysis

(a) Comparing supplement groups

In the repeated measures analysis of change in MP (at 0.25°, 0.5°, 1.0°, 1.75°), the within-subjects Time*Supplement interaction effect was not significant ($p=0.759$, 0.726, 0.703, 0.110, respectively, using the Greenhouse-Geisser adjustment for lack of sphericity). Thus, the effect (on MP levels) over time, at any eccentricity, does not differ significantly between supplement groups. The boxplots in Figure 9 graphically illustrate these findings.

(b) Within-supplement group analyses of MP are given in Table 11.

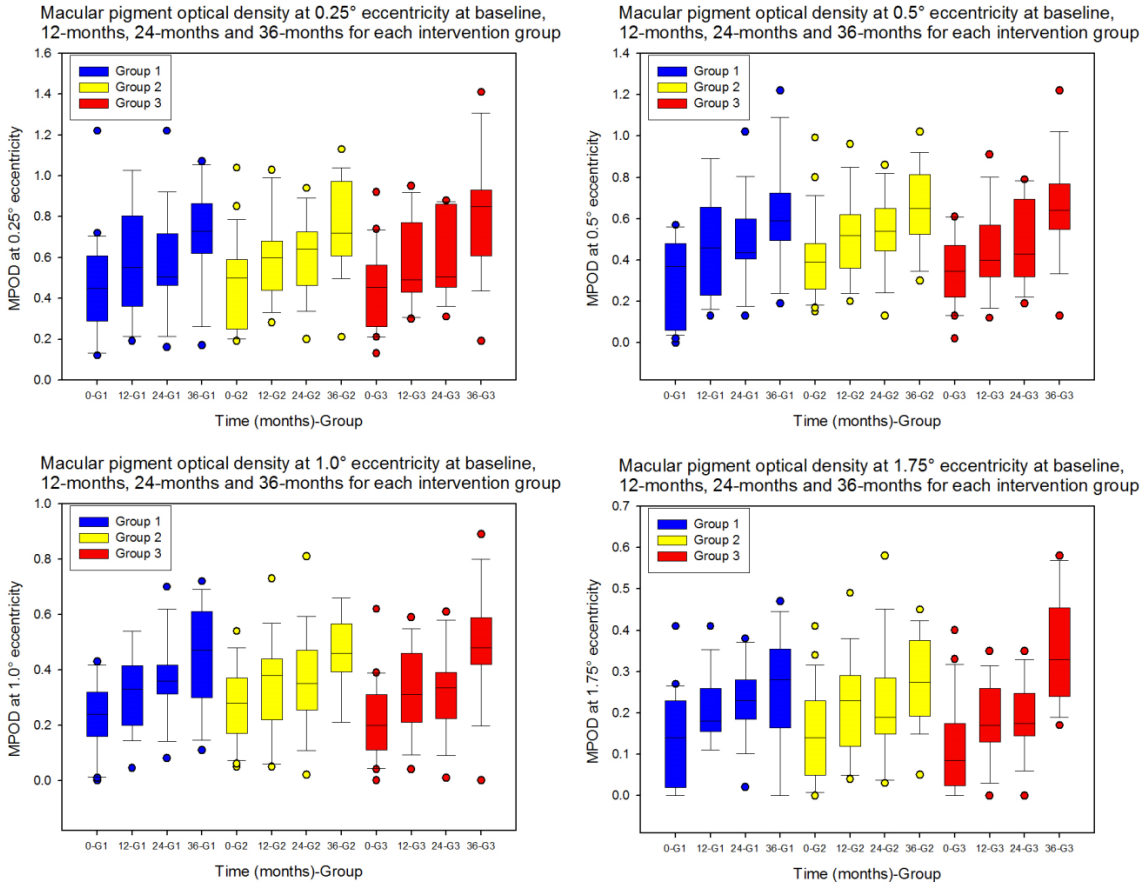


Figure 9: Macular pigment response at different retinal eccentricities over the course of the Meso-zeaxanthin Ocular Supplementation (MOST) study. Box plots representing macular pigment optical density at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group; Group 1: 20mg Lutein and 0.86mg Zeaxanthin; Group 2: 10mg Meso-zeaxanthin, 10mg Lutein and 2mg Zeaxanthin; Group 3: 17 mg Meso-zeaxanthin, 3 mg Lutein and 2mg Zeaxanthin; MPOD: macular pigment optical density; Macular pigment measured at 0.25, 0.5, 1.0 and 1.75 degrees eccentricity using customized heterochromatic flicker photometry; 0-G1: Baseline Group 1; 12-G1: 12 months Group 1; 24-G1: 24 months Group 1; 36-G1: 36 months Group 1; 0-G2: Baseline Group 2; 12-G2: 12 months Group 2; 24-G2: 24 months Group 2; 36-G2: 36 months Group 2; 0-G3: Baseline Group 3; 12-G3: 12 months Group 3; 24-G3: 24 months Group 3; 36-G3: 36 months Group 3.

Table 11: Within–supplement group analysis of macular pigment by intervention groups

Int.	N	Baseline mean ± SD	12 months mean ± SD	% Δ	Sig.	Int.	N	12 months mean ± SD	24 months mean ± SD	% Δ	Sig.	Int.	N	24 months mean ± SD	36 months mean ± SD	% Δ	Sig.
MP at 0.25						**MP at 0.25**						**MP at 0.25**					
G 1	**13**	0.51 ± 0.29	0.61 ± 0.30	20%	**0.039**	**G 1**	**13**	0.61 ± 0.30	0.61 ± 0.25	0%	0.896	**G 1**	**13**	0.61 ± 0.25	0.72 ± 0.24	18%	0.134
G 2	**16**	0.50 ± 0.24	0.63 ± 0.21	26%	**0.001**	**G 2**	**16**	0.63 ± 0.21	0.64 ± 0.17	2%	0.802	**G 2**	**16**	0.64 ± 0.17	0.76 ± 0.23	19%	0.095
G 3	**12**	0.51 ± 0.20	0.62 ± 0.19	22%	**0.021**	**G 3**	**12**	0.62 ± 0.19	0.62 ± 0.19	0%	0.924	**G 3**	**12**	0.62 ± 0.19	0.85 ± 0.25	37%	**0.003**
MP at 0.5						**MP at 0.5**						**MP at 0.5**					
G 1	**13**	0.41 ± 0.28	0.47 ± 0.27	15%	**0.194**	**G 1**	**13**	0.47 ± 0.26	0.53 ± 0.21	13%	0.092	**G 1**	**13**	0.53 ± 0.21	0.62 ± 0.26	16%	0.087
G 2	**16**	0.45 ± 0.21	0.54 ± 0.18	20%	**0.011**	**G 2**	**16**	0.54 ± 0.18	0.55 ± 0.16	2%	0.343	**G 2**	**16**	0.55 ± 0.16	0.64 ± 0.20	16%	**0.034**
G 3	**12**	0.39 ± 0.19	0.50 ± 0.20	22%	**0.016**	**G 3**	**12**	0.50 ± 0.20	0.50 ± 0.20	0%	0.879	**G 3**	**13**	0.50 ± 0.20	0.68 ± 0.20	36%	**0.011**
MP at 1.0						**MP at 1.0**						**MP at 1.0**					
G 1	**13**	0.30 ± 0.19	0.38 ± 0.15	27%	**0.053**	**G 1**	**13**	0.38 ± 0.15	0.40 ± 0.14	5%	0.339	**G 1**	**13**	0.40 ± 0.14	0.45 ± 0.18	13%	0.298
G 2	**16**	0.29 ± 0.13	0.37 ± 0.16	28%	**0.010**	**G 2**	**16**	0.37 ± 0.16	0.38 ± 0.16	3%	0.73	**G 2**	**16**	0.38 ± 0.16	0.46 ± 0.15	21%	0.071
G 3	**12**	0.26 ± 0.17	0.37 ± 0.14	42%	**0.010**	**G 3**	**12**	0.37 ± 0.14	0.35 ± 0.13	-6%	0.473	**G 3**	**12**	0.35 ± 0.13	0.52 ± 0.16	49%	**0.011**
MP at 1.75						**MP at 1.75**						**MP at 1.75**					
G 1	**13**	0.17 ± 0.11	0.22 ± 0.09	29%	0.055	**G 1**	**13**	0.22 ± 0.09	0.24 ± 0.08	9%	0.256	**G 1**	**13**	0.24 ± 0.08	0.23 ± 0.19	-4%	0.87
G 2	**16**	0.15 ± 0.12	0.24 ± 0.11	60%	**0.007**	**G 2**	**16**	0.24 ± 0.11	0.24 ± 0.13	0%	0.793	**G 2**	**16**	0.24 ± 0.13	0.28 ± 0.11	17%	0.383
G 3	**12**	0.12 ± 0.13	0.21 ± 0.09	75%	**0.006**	**G 3**	**12**	0.21 ± 0.09	0.21 ± 0.07	0%	0.899	**G 3**	**12**	0.21 ± 0.07	0.34 ± 0.14	62%	**0.003**

Int.: Intervention; G: Group; N: number; Group 1: 20mg Lutein and 0.86mg Zeaxanthin; Group 2: 10mg *Meso*-zeaxanthin, 10mg Lutein and 2mg Zeaxanthin; Group 3: 17 mg *Meso*-zeaxanthin, 3 mg Lutein and 2mg Zeaxanthin; MP: macular pigment; Macular pigment measured at 0.25, 0.5, 1.0 and 1.75 degrees eccentricity using customized heterochromatic flicker photometry; Statistical significance tested using paired t-test; SD: standard deviation; Sig.: level of significance set at $p < 0.05$; %Δ: percentage change; the calculated percentage change from baseline to 12 months, calculated as the 12 month value minus baseline value divided by baseline value, multiplied by 100 (- = negative change and + = positive change); the calculated percentage change from 12 to 24 months, calculated as the 24 month value minus the 12 month value divided by 12 month value, multiplied by 100 (- = negative change and + = positive change); the calculated percentage change from 24 to 36 months, calculated as the 36 month value minus the 24 month value divided by 24 month value, multiplied by 100 (- = negative change and + = positive change

3.3.1.2 Serum concentrations of lutein

Primary analysis: baseline vs 36 months

Comparing serum L at 36 months with serum L at baseline, serum L increased significantly in all three supplement groups ($p<0.05$).

Secondary analysis

(a) Comparing supplement groups

In the repeated measures analysis of change in serum L, the within-subjects Time*Supplement interaction effect was significant ($p=0.029$, using the Greenhouse-Geisser adjustment for lack of sphericity). Thus, the effect (on serum L levels) over time differs significantly between the supplements used. Post Hoc analysis indicates that increases in serum L over time in Groups 1 and 2 are comparable ($p = 1$, after Bonferroni adjustment for multiple testing), and each of these Groups exhibit significantly greater increases than Group 3 ($p = 0.029$ and $p = 0.004$, respectively, after Bonferroni adjustment for multiple testing). The boxplots in Figure 10(a) graphically illustrate these findings.

(b) Within-supplement group analyses of serum L are given in Table 12.

3.3.1.3 Serum concentrations of meso-zeaxanthin

Primary analysis: baseline vs 36 months

Comparing serum MZ at 36 months with serum MZ at baseline, serum MZ increased significantly in all three supplement groups ($p<0.05$).

Secondary analysis

(a) Comparing supplement groups

In the repeated measures analysis of change in serum MZ, the within-subjects Time*Supplement interaction effect was significant (p=0.011, using the Greenhouse-Geisser adjustment for lack of sphericity). Thus, the effect over time (on serum levels of MZ) differs significantly between the supplement groups. Post Hoc analysis indicates that increases in MZ over time in Groups 2 and 3 are comparable (p = 1, after Bonferroni adjustment for multiple testing), and each of these Groups exhibits significantly greater increases than Group 1 (p =0.001 for both, after Bonferroni adjustment for multiple testing). The boxplots in Figure 10(b) graphically illustrate these findings.

(b) Within-supplement group analyses of serum MZ are given in Table 12.

3.3.1.4 Serum concentrations of zeaxanthin

Primary analysis: baseline vs 36 months

Comparing serum Z at 36 months with serum Z at baseline, serum Z increased significantly in Groups 2 and Group 3 ($p<0.05$); the increase in Group 1 was not statistically significant (p=0.124).

Secondary analysis

(a) Comparing supplement groups

In the repeated measures analysis of change in serum Z, the within-subjects Time*Supplement interaction effect was not significant (p=0.081, using the Greenhouse-Geisser adjustment for lack of sphericity). Thus, the effect over time

does not differ significantly between the supplements. The boxplots in Figure 10(c) graphically illustrate these findings.

(b) Within-supplement group analyses of serum Z are given in Table 12.

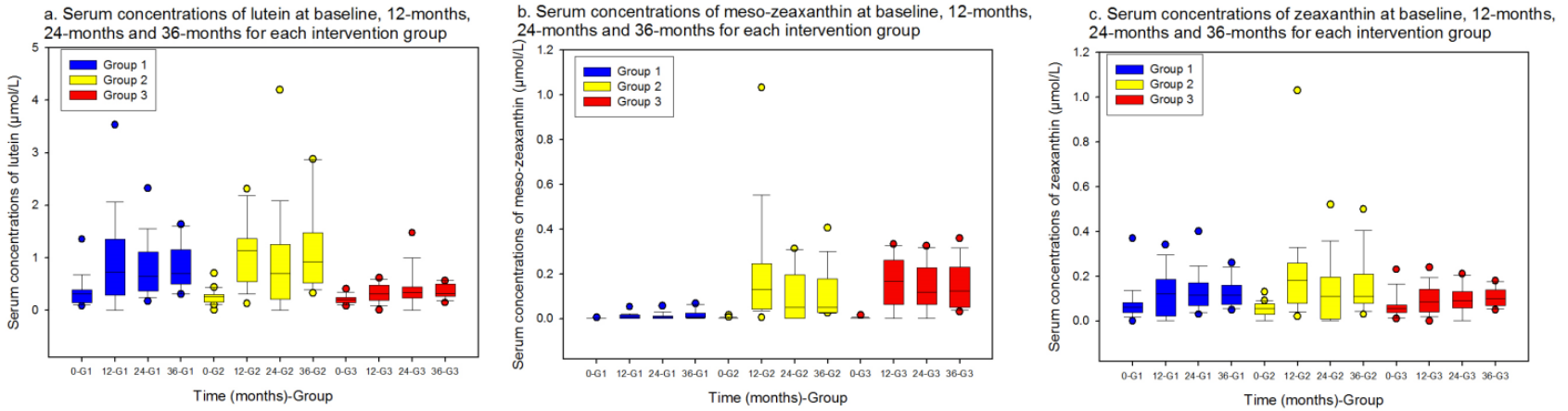


Figure 10: Serum response of lutein, meso-zeaxanthin and zeaxanthin over the course of the Meso-zeaxanthin Ocular Supplementation (MOST) age-related macular degeneration (AMD) study. Box plots representing serum concentrations of lutein (4a), meso-zeaxanthin (4b) and zeaxanthin (4c) at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group; Group 1: 20mg Lutein and 0.86mg Zeaxanthin; Group 2: 10mg Meso-zeaxanthin, 10mg Lutein and 2mg Zeaxanthin; Group 3: 17 mg Meso-zeaxanthin, 3 mg Lutein and 2mg Zeaxanthin; Serum macular carotenoids analysed by high performance liquid chromatography (HPLC); 0-G1: Baseline Group 1; 12-G1: 12 months Group 1; 24-G1: 24 months Group 1; 36-G1: 36 months Group 1; 0-G2: Baseline Group 2; 12-G2: 12 months Group 2; 24-G2: 24 months Group 2; 36-G2: 36 months Group 2; 0-G3: Baseline Group 3; 12-G3: 12 months Group 3; 24-G3: 24 months Group 3; 36-G3: 36 months Group 3.

Table 12: Within–supplement group analysis of serum macular carotenoids by Intervention groups

Int.	N	Baseline mean ± SD	12 months mean ± SD	% Δ	Sig.	Int.	N	12 months mean ± SD	24 months mean ± SD	% Δ	Sig.	Int.	N	24 months mean ± SD	36 months mean ± SD	% Δ	Sig.
Lutein						**Lutein**						**Lutein**					
G 1	**14**	0.39 ± 0.31	0.81 ± 0.58	108%	**0.014**	**G 1**	**14**	0.81 ± 0.58	0.90 ± 0.57	11%	0.616	**G 1**	**14**	0.90 ± 0.57	0.81 ± 0.44	-10%	0.412
G 2	**15**	0.24 ± 0.11	1.11 ± 0.67	363%	**<0.0005**	**G 2**	**15**	1.11 ± 0.67	0.85 ± 1.05	-23%	0.336	**G 2**	**15**	0.85 ± 1.05	1.14 ± 0.83	34%	0.250
G 3	**13**	0.20 ± 0.08	0.31 ± 0.17	55%	**0.021**	**G 3**	**13**	0.31 ± 0.17	0.39 ± 0.36	26%	0.367	**G 3**	**13**	0.39 ± 0.36	0.36 ± 0.13	-8%	0.694
Zeaxanthin						**Zeaxanthin**						**Zeaxanthin**					
G 1	**14**	0.09 ± 0.09	0.12 ± 0.09	33%	0.314	**G 1**	**14**	0.12 ± 0.09	0.15 ± 0.09	25%	0.251	**G 1**	**14**	0.15 ± 0.09	0.13 ± 0.06	-13%	0.202
G 2	**15**	0.04 ± 0.03	0.22 ± 0.24	450%	**0.012**	**G 2**	**15**	0.22 ± 0.24	0.13 ± 0.14	-41%	0.221	**G 2**	**15**	0.13 ± 0.14	0.16 ± 0.12	23%	0.298
G 3	**13**	0.06 ± 0.05	0.09 ± 0.06	50%	**0.031**	**G 3**	**13**	0.09 ± 0.06	0.10 ± 0.07	11%	0.653	**G 3**	**13**	0.10 ± 0.07	0.11 ± 0.04	10%	0.799
Meso-zeaxanthin						**Meso-zeaxanthin**						**Meso-zeaxanthin**					
G 1	**14**	0.00 ± 0.00	0.01 ± 0.01		**0.008**	**G 1**	**14**	0.01 ± 0.01	0.01 ± 0.02	0%	0.393	**G 1**	**14**	0.01 ± 0.02	0.02 ± 0.02	100%	0.371
G 2	**15**	0.00 ± 0.00	0.22 ± 0.27		**0.007**	**G 2**	**15**	0.22 ± 0.27	0.09 ± 0.11	-59%	0.083	**G 2**	**15**	0.09 ± 0.11	0.11 ± 0.11	22%	0.314
G 3	**13**	0.00 ± 0.00	0.16 ± 0.11		**0.000**	**G 3**	**13**	0.16 ± 0.11	0.15 ± 0.11	-6%	0.911	**G 3**	**13**	0.15 ± 0.11	0.14 ± 0.10	-7%	0.743

Int.: Intervention; G: Group; N: number; Group 1: 20mg Lutein and 0.86mg Zeaxanthin; Group 2: 10mg *Meso*-zeaxanthin, 10mg Lutein and 2mg Zeaxanthin; Group 3: 17 mg *Meso*-zeaxanthin, 3 mg Lutein and 2mg Zeaxanthin; SD: standard deviation; Statistical significance tested using paired t-test; Sig.: level of significance set at $p < 0.05$; %Δ: percentage change; the calculated percentage change from baseline to 12 months, calculated as the 12 month value minus baseline value divided by baseline value, multiplied by 100 (- = negative change and + = positive change); the calculated percentage change from 12 to 24 months, calculated as the 24 month value minus the 12 month value divided by 12 month value, multiplied by 100 (- = negative change and + = positive change); the calculated percentage change from 24 to 36 months, calculated as the 36 month value minus the 24 month value divided by 24 month value, multiplied by 100 (- = negative change and + = positive change).

3.3.2 Changes in Visual Function

3.3.2.1 Best-Corrected Visual Acuity

Primary Analysis: baseline vs 36 months

Comparing BCVA at 36 months with BCVA at baseline, there were no significant changes in BCVA ($p>0.05$, for all).

Secondary Analysis

(a) Comparing supplement groups

There were no significant Time*Supplement interaction effects for BCVA indicating that the observed effects over time did not differ between intervention groups.

3.3.2.2 Letter Contrast Sensitivity

Primary analysis: baseline vs 36 months

Comparing letter CS at 36 months with letter CS at baseline, statistically significant improvements in letter CS ($p<0.05$) were seen at all spatial frequencies (with the sole exception of 2.4 cycles per degree [cpd]) in Group 2, at some spatial frequencies (6cpd, 9.6cpd and 15.15cpd) in group 3, and at a single spatial frequency (15.15cpd) in group 1.

Secondary Analysis

(a) Comparing Supplement Groups

There were no significant Time*Supplement interaction effects for letter CS at any spatial frequency indicating that the observed effects over time did not differ between intervention groups.

3.3.3 Changes in Grade of AMD

Because of the limited number of subjects in this study, we collapsed adjacent grades of AMD, as follows: AREDS grades 1-3 (representing eyes at low risk of progression to advanced AMD), and AREDS grades 4-8 (representing eyes at high risk of progression to advanced AMD). In terms of this collapsed and simplified classification, intervention groups were statistically similar in terms of baseline findings (p=0.44, chi-square test). Using this simplified and modified system, no study eye in any intervention group progressed from low risk to high risk of progression to advanced AMD over the course of the study period, and no study eye regressed from high risk to low risk of progression to advanced AMD in any intervention group, and finally, no subject progressed to advanced AMD (AREDS grades 9 to11) over the study period. Given that findings were identical for all three intervention groups, there was no need for statistical investigation of differences between intervention groups in terms of changes in risk for progression to advanced AMD.

We also investigated clinically meaningful change in AMD grade along the AREDS 11-step scale, defined as a change of at least two steps along this scale. Thus, an increase of two steps between baseline and final visit at 36 months was considered clinically meaningful disease progression and a decrease of two steps was considered a clinically meaningful disease regression. On this basis, there was no clinically meaningful change in AMD grade in 43 (93%) study eyes, while 3 (7%) study eyes (1 subject in Group 1 and 2 subjects in Group 3) exhibited a clinically meaningful progression along the AREDS 11-step scale, and these observed changes were not statistically different between intervention groups (p=0.29, Fisher exact test).

3.4 Discussion

The present study reports on the impact of sustained supplementation using different carotenoid formulations on serum concentrations of MP's constituent carotenoids, MP, visual function (BCVA and letter CS) and disease progression in subjects with non-advanced AMD.

The strengths of this study include: 1. It is a randomized clinical trial comparing three different formulations containing some or all of MP's constituent carotenoids, with a follow-up of three years; 2. MP was measured using a validated technique at regular intervals throughout the study period; 3. assessment of visual function was not restricted to BCVA, and included CS; 4. assessment of AMD morphology was performed by an accredited reading centre in a masked fashion.

Serum response to supplementation reflected the carotenoid content of the supplement used. For example, serum L exhibited an increase in all three supplementation groups, but to a greater extent in Groups 1 and 2 where intake of L was at least three times the typical dietary intake of this carotenoid.[187, 188] Similarly, a significant rise in serum Z was noted following supplementation, but that was comparable across supplement groups, reflecting similar concentrations of this carotenoid in each of the three formulations tested. Finally, serum MZ response is noteworthy for several reasons. First, MZ was detected in the serum of patients supplemented with a formulation with no declared MZ content. However, we have shown that MZ is present in commercially available formulations containing L, including Ultra Lutein, the Group 1 supplement used in this study.[179, 180] Finally, it is also noteworthy that serum L and serum Z responses were unaffected by the presence of substantial concentrations of MZ (10 mg or

more) in the formulation used, thereby allaying any concerns that the inclusion of MZ in a supplement may adversely impact upon the circulating bioavailability of the other two macular carotenoids.

MP increased significantly in all groups at each eccentricity (with the exception of Group 1 at 1.75°) at three years. It is surprising to see that MP did not increase at 1.75° in Group 1, given that L is the dominant carotenoid at this locus, and this seemingly counterintuitive observation might be because subjects in Group 1 were bioconverting L to MZ at the macula.[130, 189] Consistent with this hypothesis, only groups which received supplemental MZ exhibited significant augmentation of MP across the spatial profile of this pigment.

In terms of MP increase over the course of the study, it was observed that MP continues to increase further and significantly in the third year of supplementation (but only in groups supplemented with meaningful concentrations of MZ) following a relative plateau in the second year of supplementation. Indeed, MP did not increase significantly between 12 and 24 months in any intervention group, at any eccentricity. Although the exact mechanism of macular carotenoid uptake has not been fully elucidated, it is plausible that there are several mediators (e.g. binding proteins, enzymes) which influence the capture, accumulation and stabilisation of these carotenoids at the macula,[190] but further research is needed to understand these mechanisms.

There was no significant change in BCVA over the course of the present study, other than a transient improvement between 12 and 24 months in Group 3. Murray *et al*[191] reported the impact of supplemental L on MP and BCVA in patients with early AMD in a randomised, double-blind, placebo-controlled,

multicentre, 12-month trial (see Chapter 1, Table 4). At the end of their study, there was no change in BCVA in the L group, whereas BCVA in the placebo group had deteriorated significantly.[191] In the present study, there was a non-significant increase in BCVA in all intervention groups, consistent with the view that BCVA stabilized over the three-year period of the study in this cohort of patients with early AMD. The CARMA trial, a randomized controlled trial of L, Z and co-antioxidants versus placebo, reported no significant change in BCVA at one year, although there was a demonstrable benefit in terms of differential BCVA between intervention and placebo groups at three years.[192, 193] Of note, visual acuity, which is a measure of the spatial resolving power of the visual system and remains the most commonly used measure of vision in clinical practice, is probably not sensitive enough to detect subtle but important changes in visual function experienced when monitoring subjects with non-advanced AMD.[194]

CS can be described as our ability to discriminate an object from its background and is determined by measuring the contrast threshold between visible and invisible at given spatial frequency,[185] and is a better tool than BCVA for assessing visual function in non-advanced AMD.[194] In Group 2 (a supplement with a formulation containing all three of MP's constituent carotenoids), there was a statistically significant improvement in CS at the lowest spatial frequency (2.4cpd), whereas this was not observed for Groups 1 and 3. At the highest spatial frequency (15.15cpd), letter CS improved in Groups 1 and 3 at 36 months, but not in Group 2. At intermediate spatial frequencies (6cpd and 9.6cpd), however, only supplementation with formulations containing appreciable amounts of MZ (Groups 2 and 3) resulted in a significant improvement in letter CS. Although

some, but not all, previous studies have reported improvements in CS following supplementation with macular carotenoids in subjects with non-advanced AMD (see Chapter 1, Table 4), our results suggest that those studies that failed to report an improvement in CS may be explained, at least in part, by a lack of MZ in the supplement formulation used.[193, 195] Finally, an important and novel finding of the current study rests on the observation that further and significant improvements in CS are experienced beyond 24 months of supplementation with MP's constituent carotenoids, suggesting that sustained supplementation is indeed necessary to exert a beneficial effect on visual function.

With respect to AMD, only three study eyes exhibited clinically meaningful disease progression (1 subject from Group 1 and 2 subjects from Group 3), and no study eye progressed to advanced AMD over the three-year study period. This study is not adequately powered or designed to make meaningful comment on AMD progression.

The current study compared the impact of supplementation with different carotenoid formulations on visual function, and our findings suggest that a formulation containing MZ yields benefits in terms of MP augmentation and in terms of CS enhancement. Further, sustained supplementation appears necessary, for at least three years, if MP is to be augmented maximally and CS is to be optimised over that period of time. Of note, modest visual benefits were observed in the current study. Future clinical trials should examine the impact of supplementation with formulations containing MZ and Z at similar doses. The CREST study (described in Chapter 4) will also add to our understanding of the role of the macular carotenoids, including MZ, on vision in patients with AMD.[196]

Limitations of the MOST study include its small numbers and the fact that it is a single blind clinical trial with no placebo arm. With respect to the use of placebo in the current study, we believe that the findings arising from the secondary analysis of the AREDS2 may render the use of placebo in patients with non-advanced AMD ethically questionable.[115, 116] Of note, the term non-advanced AMD in this study includes patients with intermediate AMD (as defined by AREDS). However, the absence of placebo may render it difficult to demonstrate clinical efficacy of the different carotenoid formulations used in this study and our results should be interpreted with full appreciation of this limitation. We employed the single blind design because the current study was the first clinical trial to compare the impact of supplementation with three different carotenoid formulations (including MZ) on visual function in subjects with non-advanced AMD (see Chapter 1, Section 1.8, Table 4) and therefore we wanted to monitor more closely the effects of the three carotenoid formulations in terms of response among these subjects. Statistically, this exploratory study was under-powered for a direct comparison of the three supplements. Differences in effects between supplements were, in general, likely to be small, meaning that impractically large numbers of subjects would have been required to obtain statistically significant results.

3.5 Conclusion

The inclusion of MZ in a supplement formulation seems to confer benefits in terms of MP augmentation and in terms of enhanced CS in subjects with non-advanced AMD. An important and novel finding rests on the observation that sustained supplementation with the macular carotenoids seems necessary to maximally augment MP and to optimise CS over a three-year period in patients with non-advanced AMD. This work was published in Eye, the official journal of The Royal College of Ophthalmologists (see Appendix T), under the title, "***Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration***."[149]

Chapter 4. Macular Carotenoid and Co-Antioxidant Supplementation on Visual Function in Non-Advanced Age-Related Macular Degeneration, CREST Randomized Clinical Trial

4.1 Introduction

AMD is a multifactorial disease characterized by a spectrum of degenerative changes at the macula, ultimately leading to central vision impairment in many cases. Given the growing and aging world population, the number of people suffering from AMD continues to rise. Using pooled data from 39 studies and applying a Hierarchical Bayesian approach, Wong *et al* estimated the prevalence of any AMD (globally) to be 8.7% in those aged 45 to 85years, and predicted that the number of people afflicted with AMD worldwide will be 288 million by 2040.[18] We have shown in the ROI that the current prevalence of (any) AMD among persons 50 years and older is estimated to be 7.2% (see Chapter 2).[39] Beyond the personal suffering of those afflicted with advanced AMD, which includes loss of central vision, and associated adverse clinical events such as increased risk of falls,[19] depression, loneliness, suicide, etc.,[20] the growing prevalence of AMD represents a huge socioeconomic burden to society and to healthcare providers.[21] In order to address this challenge, preventative, retarding and vision-optimizing strategies for non-advanced AMD need to be explored, and prior work in diseased and non-diseased eyes indicate that ocular nutrition represent a biologically plausible rationale to pursue in this endeavor.[197]

MZ, Z, and L represent the three constituent carotenoids that make up MP, a yellow pigment found in the macula. Their anatomic (central and pre-receptorial location), biochemical (antioxidant and anti-inflammatory) and optical (short-

wavelength [blue] light-filtering) properties make these compounds ideal candidates to enhance vision and potentially protect against AMD and its progression.[197] The AREDS2, published in May 2013, examined the role of supplementation with two of MP's constituent macular carotenoids (L and Z, in combination with co-antioxidants) in patients with intermediate AMD.[114] The primary outcome measure (progression to advanced AMD) in AREDS2 failed to reveal a beneficial effect of supplemental L and Z.[115] However, secondary analysis, where data were dichotomized to those supplemented with L and Z versus those not being supplemented with these macular carotenoids, did demonstrate a beneficial effect in terms of progression to the advanced form of the disease, especially in those with a low dietary intake of these carotenoids.[115, 116] It is important to note that AREDS2 was designed and powered to investigate the impact of supplementation with macular carotenoids plus co-antioxidants on AMD morphology and on visual acuity, whereas the current trial (CREST AMD) was designed and powered to investigate a change in psychophysical (visual) function following supplementation with the macular carotenoids plus co-antioxidants. In other words, the aim of the current study is to investigate the impact of antioxidant supplementation on visual function in eyes with non-advanced AMD, and the current study is not designed to make a meaningful comment on disease progression.

In terms of assessing visual function in patients with AMD, a number of studies have examined the impact of supplementation with macular carotenoids (see Chapter 1, Table 4). Indeed, recent studies have reported positive outcomes on visual function (e.g. CS and GD) in patients with AMD and other retinal diseases, following supplementation with the macular carotenoids using a

formulation of MZ: L: Z in a ratio (mg/day) of 10:10:2.[149, 198, 199] However, given the exploratory nature of those studies, a double-blind randomized controlled trial (RCT) with appropriate methodology was warranted. Originally, the CREST AMD trial planned a placebo-controlled design, but following publication of AREDS2, the CREST Data Safety and Monitoring Committee (DSMC) recommended that the design be amended to reflect the new standard of care and that, accordingly, the placebo group should be replaced with an AREDS2 formula with lower dose of zinc (25mg). In the amended protocol, we chose a lower zinc dose (25mg) because the AREDS2 study found no efficacy-lowering effect of reducing zinc from 80mg to 25mg on either VA or AMD progression.

In summary, CREST AMD was designed and conducted to investigate the impact of macular carotenoid supplementation with co-antioxidants in patients with non-advanced AMD over a two-year period (ISRCTN13894787).[150] We also investigated whether the addition of 10mg of MZ to a formulation containing standard AREDS2 doses of L and Z and in combination with co-antioxidants offered advantages in terms of a wide array of measures of visual function and MP response.

4.2 Methods

4.2.1 Design and Registration

The protocol for this study has been published.[150] The trial was registered on the International Standard RCT register (ISRCTN13894787) and was conducted as a single centre study at the MPRG, Nutrition Research Centre Ireland. CREST AMD was initially designed as a double-blind RCT, investigating the impact of

supplementation with 10mg/day MZ, 10mg/day L, 2mg/day Z versus placebo, on vision in patients with non-advanced AMD. However, the study protocol was revised following a recommendation from the CREST DSMC, after publication of the AREDS2 study.[115, 116] Following the AREDS2 study, the National Eye Institute recommended that L and Z replace beta-carotene in the original AREDS formula. Thus, this new formulation was then considered to represent standard of care (standard therapy) for persons with at least intermediate AMD. Accordingly, the CREST protocol was amended to a double-blind, head-to-head, RCT (ISRCTN13894787), in which participants were randomly assigned to two parallel groups, both receiving active supplements: Group 1 (10mg/day MZ, 10mg/day L, 2mg/day Z plus 500mg/day vitamin C, 400 international units (IU)/day of vitamin E, 25mg/day zinc and 2mg/day copper; Macushield Gold™ [Europe]; Macuhealth Plus™ [North America]), and Group 2 (10mg/day L, 2mg/day Z plus 500mg vitamin C, 400 IU/day of vitamin E, 25mg zinc and 2mg copper; AREDS 2 like formula). The Group 2 intervention, therefore, represents the standard of care (AREDS2 like formula) whereas Group 1 also represents the standard of care, but with the addition of 10mg of MZ. All protocol changes (see Appendix E and H) were approved by the DSMC and the Research Ethics Committee of the Waterford Institute of Technology (WIT), Waterford, Ireland, and the Ethics Committee of the European Research Council (ERC). In addition, protocol changes were published on the International Standard RCT registration website (www.isrctn.com/ISRCTN13894787) and in the published methodology[150] for this project. Participants in both groups, were instructed to take the study intervention daily with a meal (see Appendix I). The trial was conducted at the Macular Pigment Research Group, Nutrition Research Centre

Ireland from November 2013 (first visit of first participant) to May 2016 (last visit of last participant).

4.2.2 Study Oversight

An independent Data and Safety Monitoring Committee (DSMC) was appointed to examine and review data collected during this study. The CREST DSMC consisted of a statistician, medical ophthalmologist, a health science researcher and a vision scientist. The DSMC had full access to the randomization code for the trial and the authority to break the code if needed. The DSMC had the authority to recommend any of the following: continuation of the study uninterrupted, alteration of any arm of trial, or termination of any arm of trial. The DSMC were blinded to intervention assignment and gave recommendations when there were any reported potential/perceived adverse events. The DSMC did not break the randomization code at any point during the study (see Appendix S).

4.2.3 Eligibility Criteria

Inclusion criteria included: 1) non-advanced AMD in at least one eye [study eye], based on the grading of a fundus photograph (from one [drusen absent or questionable or small hard drusen present, total drusen area < 125 microns diameter, without retinal pigment abnormalities] to eight [drusen ≥ 0.5 disc area (DA) with RPE depigmentation ≥ 350 microns to < 0.5 DA or any drusen with ≥ 0.5 DA RPE depigmentation]) on the AREDS severity scale[15] (see Chapter 1, Section 1.2.3); 2) BCVA of 6/12 or better in the study eye; 3) no more than five diopters spherical equivalence of refraction in the study eye; 4) no previous

consumption of supplements containing the macular carotenoids (L, Z and/or MZ); 5) no other retinal pathology other than AMD; 6) no diabetes mellitus (by self-report). The study eye could be either right or left eye. If both eyes had non-advanced AMD, the eye with the best BCVA was chosen as the study eye. However, if both eyes had the same BCVA and non-advanced AMD, the right eye was selected.

4.2.4 Ethical Assessment and Approval

All subjects provided a written informed consent prior to study enrolment (see Appendix G). Ethical approval (see Appendix D and E) for the study was granted by the Research Ethics Committee of the Waterford Institute of Technology, Waterford, Ireland, and the Ethics Committee of the European Research Council (ERC). CREST adhered to the tenets of the Declaration of Helsinki, and followed the full code of ethics with respect to subject recruitment, subject testing and data protection.

4.2.5 Research Question

Does supplementation with all three macular carotenoids in a ratio (mg/day) of 10:10:2 (L: MZ: Z) plus 500mg vitamin C, 400 IU of vitamin E, 25mg zinc and 2mg copper for 24-months, enhance visual function in patients with non-advanced AMD when compared to 10:2 (L: Z) plus 500mg vitamin C, 400 IU of vitamin E, 25mg zinc and 2mg copper?

4.2.6 Primary Outcome Measure

The primary outcome measure was change in contrast sensitivity (CS) at 6 cycles per degree (cpd) following 24 months of supplementation. The Test Chart 2000PRO (Thomson Software Solutions, Hatfield, UK) was used to assess this measure

4.2.7 Secondary Outcome Measures

Secondary outcome measures included: CS at the other spatial frequencies, visual acuity, glare disability (GD), photostress recovery, MP, retinal straylight, AMD morphology, reading acuity, reading speed, and subjective visual function (National Eye Institute Visual Function Questionnaire -25).

4.2.8 Randomization and Intervention

Participants were randomly assigned to intervention groups using block randomization (block size: 4; randomization ratio: 1:1 with no stratification). Block randomization was used to assign subjects to intervention groups. The use of blocking is designed to ensure that an equal number of subjects are assigned to intervention groups. The randomization code list was generated by the study statistician (J.S.) who has no contact with study subjects and no access to data until study completion. Random allocation was carried out by a pharmacist (C.K.) at Whitfield Clinic, Waterford, who had no contact with study subjects. The study investigator (K.O.A.) only received a box of supplements with subject ID label. At study completion, after a masked database review, and following direction from the CREST DSMC, the randomization sequence was revealed.

The interventions consisted of a softgel capsule containing 10mg L, 10mg MZ and 2mg Z in a sunflower oil suspension plus two multivitamin capsules each containing 250mg vitamin C, 200 IU of vitamin E, 12.5mg zinc, 1mg copper (provided by Macuvision Europe Limited, Solihull, UK prepared by EuroCaps Limited, Tredegar, South Wales, UK) [Group 1] or a softgel capsule containing 10mg L, 2mg Z in a sunflower oil suspension plus two multivitamin capsules each containing 250mg vitamin C, 200 IU of vitamin E, 12.5 mg zinc, 1mg copper (provided by Macuvision Europe Limited, Solihull, UK prepared by EuroCaps Limited, Tredegar, South Wales, UK) [Group 2]. The macular carotenoid capsules were indistinguishable from each other in external appearance. Subjects were instructed to take one macular carotenoid capsule and two multivitamin capsules daily with a meal.

4.2.9 Compliance

Frequent phone calls and reminder text messages were sent to subjects to ensure compliance with consumption of the study intervention. Subjects were asked to come along with their supplement containers to follow up study visits where the capsules were counted. Capsule counting considered the expected number of capsules to be consumed between study visits as well as the total number of capsules left (returned at study visit). Expected number of capsules to be consumed between study visits was calculated as the product of the daily dose and the number of days since dispensed. The percentage capsule count was calculated as:

$$\%Capsule\ Count\ = \frac{Total\ capsules\ dispensed - Number\ of\ capsules\ left}{Expected\ number\ of\ capsules\ to\ be\ taken} \times 100\%$$

In addition, compliance was assessed at the end of the study (after the randomization code was broken) by analyzing serum carotenoid concentrations using HPLC. Serum analysis was conducted by the CREST Senior Scientist (D.K.).

4.2.10 Adverse Events

Subjects were frequently called by the CREST research team to assess adherence to study intervention and also to ascertain whether they had experienced any unusual signs/symptoms following the intervention. Any potential or perceived adverse events were documented (see Appendix J) and reported to the CREST Data and Safety Monitoring Committee (DSMC).

4.2.11 Sample Size Calculation

Pilot studies were conducted to inform CREST with respect to power and sample size (ISRCTN81595685). From this pilot work, estimates of standard deviation of CS, and the correlation between CS pre- and post-intervention were available and were used in the sample size calculations.

Using a clinically significant effect size of 0.15 logCS units (an improvement of one line on a Letter CS [Thomson Test Chart 2000 PRO]), a two-tailed test and on standard assumptions (5% level of significance, 80% power, equal group sizes), the required minimum sample size was 112 (56 per treatment group). [200] However, assuming a 25% dropout rate, we decided on a total sample a total sample size of 150.

4.2.12 Screening Visits to Assess and Confirm Eligibility

Subjects were recruited through an organized advertising campaign. National and local media were informed of the trial and many mainstream Irish newspapers published the call for volunteers. Radio and online adverts were also carried out. In addition, flyers were developed for distribution to the general public (see Appendix E and F). Subjects were also recruited from hospitals in the ROI. This was facilitated by raising awareness of the trial at each hospital. Educational events for general practitioners, optometrists and ophthalmologists were held to create awareness of the trial and to solicit help with recruitment. Interested and potential volunteers were invited to attend the Vision Research Centre for assessment to confirm eligibility (with particular emphasis placed on presence of non-advanced AMD). During the screening visit, demographic information was collected (see Appendix L). This was followed by measuring BCVA. In addition, anterior and posterior segment examination using the Haag-Streit BM 900 Slit lamp biomicroscope (Haag-Streit AG, Switzerland) was carried out by a consultant ophthalmologist with a special interest in AMD (S.B). Subjects who were deemed suitable following the ophthalmological examination had their stereo fundus photographs taken. These stereo fundus photographs were sent to the MEHRC for a non-detailed grading of AMD in order to confirm that the subject had non-advanced AMD. Only patients who had such confirmation from the MEHRC at eligibility screening visit were invited to participate in the study.

4.2.13 Study Visits

Study visits were conducted at baseline, six months, 12 months, 18 months and 24 months. At each visit, subjects undergo a series of tests and procedures, which are described in detail below. Table 13 summarizes the clinical procedures conducted at each study visit. A typical study visit took approximately 150 minutes.

Table 13: Study procedures in Central Retinal Enrichment Supplementation Trial (CREST) age-related macular degeneration (AMD) study

Study Procedures	Baseline	6 M	12 M	18 M	24 M
Demographic and lifestyle questionnaire	●				
NEI VFQ-25 questionnaire	●				●
Dietary carotenoid screener	●				●
Visual acuity assessment	●	●	●	●	●
Reading acuity	●	●	●	●	●
Reading speed	●	●	●	●	●
Letter contrast sensitivity	●	●	●	●	●
Contrast sensitivity with functional vision analyzer	●	●	●	●	●
Light scatter	●	●	●	●	●
Photostress recovery	●	●	●	●	●
MP measurement by customized heterochromatic flicker photometry	●	●	●	●	●
MP measurement by dual-wave autofluorescence	●	●	●	●	●
Optical coherence tomography	●	●	●	●	●
Fundus photography	●	●	●	●	●
Fundus grading	●				●

Abbreviations: M, months.

4.2.14 Study Procedures

4.2.14.1 Demographic and lifestyle questionnaire

The demographic and lifestyle questionnaire (see Appendix O) obtains the following details: contact details; ethnicity; education; occupation; smoking habits (history and frequency); alcohol intake (average consumption per week, frequency); exercise (number of sessions per week, duration of each session in minutes); light exposure (time spent outdoors, use of protective eyewear such as sunglasses, photochromic lenses); body mass index (BMI); blood pressure; medical and ocular history.

4.2.14.2 NEI VFQ-25 Questionnaire

Subjective visual function was assessed using the validated[201, 202] National Eye Institute Visual Function questionnaire-25 (NEI VFQ-25).[203] NEI VFQ-25 contains 25 questions that measure various aspects of visual health across 12 subscales (general vision, near vision, distance vision, driving, peripheral vision, colour vision, ocular pain, general health, and vision-specific role difficulties, dependency, social function, and mental health) with 13 optional questions that enhance the reliability of subscale scores. In this study, the NEI VFQ-25 was self-administered with three optional questions added to both the near and distance vision subscales. Responses were scored using the NEI VFQ-25 scoring algorithm. The overall vision score is the average of all subscale scores, excluding the general health subscale score. Scores range from zero (worst) to 100 (best).

4.2.14.3 Dietary Carotenoid Screener

The dietary carotenoid screener (xanthophyll screener or L/Z screener; see Appendix N) is a simplified questionnaire which assesses the dietary intake of four carotenoid-rich food substances (eggs, broccoli, corn, dark green leafy vegetables). Subjects indicate their serving size by ticking any of six categories (less than 1 per week; 1 per week; 2-3 per week; 4-6 per week; 1 per day; more than 1 per day) with respect to each of the food substances. The responses are entered into a computer program developed by Professor Elizabeth Johnson, Tufts University, USA which weighs responses based on the frequency of food intake and the bioavailability of L and Z within these food substances, and calculates a dietary score. The dietary scores generated range from zero to 75, and are further divided into three subgroups (Low Intake, Category 1, 0-15: ≤ 2mg/day; Medium Intake, Category 2, 16-30: 3-13mg/day; High Intake, Category 3, 31-75: > 13mg/day). This method has been used previously by our group. [176, 204]

4.2.14.4 Best-corrected visual acuity

BCVA was measured with a computerized LogMAR ETDRS test chart (Test Chart 2000 Xpert; Thomson Software Solutions [see Chapter 3, Section 3.2.3.3 for detailed description]) viewed at 4 metres (m).

4.2.14.5 Letter contrast sensitivity

Letter CS was assessed using the computerized LogMAR ETDRS test chart (Test Chart 2000 Xpert; Thomson Software Solutions [see Chapter 3, Section 3.2.3.4 for detailed description; see Appendix N]) at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, 15.15 cpd).[205]

4.2.14.6 Contrast sensitivity with functional acuity contrast test

The Optec® Functional Vision Analyzer™ [206] (Stereo Optical Co., Inc., Chicago, IL, USA) uses the functional Acuity Contrast Test (FACT)[207, 208] to assess CS at five different spatial frequencies (1.5, 3, 6, 12, 18 cpd; see Appendix N). The device uses an inbuilt light emitting diode (LED) system with a customized glare source. Subjects identify the orientation of nine sinusoidal gratings presented as gabor patches at five different spatial frequencies (1.5, 3, 6, 12, 18 cpd). The sinusoidal gratings, which are inclined vertically at -15º, 0º, or +15º, in order to keep their orientation within the spectrum of the visual channel, are presented in 0.15 log CS decrements. These gabor patches have been placed on high resolution slides, which have been trans-illuminated to prevent glare and reflection. Subjects were instructed not to guess during the test and subject responses were recorded on a scoring marker. The test was carried out under four simulated conditions: 1. mesopic (3.0 candela per meter square [cd/m2]), 2. photopic (85 cd/m2), 3. mesopic with glare (28 Lux, mesopic GD), 4. photopic with glare (135 Lux, photopic GD).

4.2.14.7 Retinal Straylight

Using the compensation comparison method, the C-Quant straylight meter (Oculus GmbH, Wetzler, Germany; see Appendix N) [209-211] measures the amount of retinal straylight. In a dark room, a suitable range setting was chosen for each subject The subject wore corrective lens if required. Subjects fixated on two flickering hemifields in the central 14° (one field with counter-phase compensation and the other without counter-phase compensation) and determined which half flickers more strongly, by a two alternative forced-choice comparison.

Subjects were advised to react on first impression and promptly, pressing the corresponding push button to register their response. The test was preceded by a training session to ensure understanding of test administration. During the test, a predetermined number of stimuli were presented to subjects. Straylight measurements were recorded in logarithmic form and judged reliable when the standard deviation (ESD) is ≤ 0.08, and the reliability coefficient (Q) is ≥ 1.

4.2.14.8 Photostress recovery

Photostress recovery time (PRT) was measured by assessing CS and investigating the impact of a light stress using a 300-watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions, GmbH, Germany) with a low-pass glass dichroic filter (see Appendix N). Subjects view the lamp directly with the study eye (the other eye is covered with an eye patch) at a distance of one metre for 10 seconds while limiting blinking. After 10 seconds, the lamp is extinguished and removed from the subject's field of view. Subjects' viewed the letter size 6/24 (LogMAR 0.6) on the LogMAR test chart (Test Chart 2000 PRO; Thomson Software Solutions), and viewed at 4m. A CS value of 0.30 log units (i.e. two lines) above the individual's contrast threshold is used. The time (in seconds) taken for the subject's eye to recover and see all five letters on the chart after the 10 second exposure was taken as the PRT.

4.2.14.9 Reading Performance

Reading acuity and reading speed was assessed using the English version of the standardized Radner reading chart at 40cm from the spectacle plane (see

Appendix P).[212] The Radner reading chart consists of a series of standardized sentences which are comparable in terms of number of words (14), word length, number of syllables, position of words, lexical difficulty and syntactical complexity. Subjects wear their habitual reading correction for this test. Sentences were covered with a piece of paper prior to testing. Subjects uncovered the chart, sentence by sentence, and read only one sentence at a time. Subjects were instructed to read each sentence as quickly and as accurately as possible without correcting reading errors. Subjects were also advised not to alter reading distance during the test, however, the examiner (I) ensured that the correct test distance is maintained. Any reading errors were noted. The reading acuity was recorded in logarithm of the reading acuity determination (LogRAD). The formula (logRAD + total number of incorrectly read syllables × 0.005) is used to calculate the LogRAD-score. LogRAD is the LogMAR equivalent for reading. Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14words × 60seconds divided by reading time in seconds). The mean reading speed was calculated as the average of the reading speed scores recorded for each of the standardized sentences. Also, the maximum reading speed was noted.

4.2.14.10 Pupillary dilation

Subjects' pupils were dilated using a drop each of 0.5% proxymetacaine hydrochloride, 2.5% phenylephrine hydrochloride, and 1% tropicamide prior to performing stereo fundus photography, optical coherence tomography, MP measurement using dual-wavelength autofluorescence and cataract grading (see Appendix Q).

4.2.14.11 Macular pigment measurement by customized heterochromatic flicker photometry

MP was measured using the Macular Densitometer™ (Macular Metrics, Corp., Providence, Rhode Island, USA) at 0.25º, 0.5º, 1.0º and 1.75º of retinal eccentricity (see Chapter 3, Section 3.2.3.1 for detailed description), with a reference point at 7º.[181] This protocol has been described in detail elsewhere and has been validated for subjects with non-advanced AMD.[182]

4.2.14.12 Retinal photography and Grading

Retinal photography was performed by a trained and certified photographer (K.O.A.; see Appendix R). Stereo colour fundus photographs are taken using the Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany) at a 45º magnification setting. The stereo photography technique used was the modified 3-standard stereoscopic fields (Field 1: optic disc; Field 2: macula; Field 3: temporal to macula). In addition, fundus reflex photographs of the external eye were taken in order to document any media opacities. The photographs were transferred to the MEHRC, London, UK, via an encrypted system (see Appendix M). Retinal grading followed the AREDS 11-step severity scale (see Chapter 1; Section 1.2.3).[15]

4.2.14.13 Optical coherence tomography

Optical coherence tomography (OCT) was performed using the Spectralis ® MultiColor HRA + OCT (Heidelberg Engineering GmbH, Heidelberg, Germany)[213] The device produces non-invasive retinal histological tomographs by integrating Spectral (Fourier) domain OCT technology with confocal scanning

laser ophthalmoscopy. The following scan acquisition protocol was used: a volume scan (20º×20º) of macular area, 193 B-scans each spaced 30µm apart at high speed with ART of 9 per frame rate; enhanced depth imaging (20º×20º), 193 B-scans at high speed with ART of 9 per frame rate; a cross scan (20º×20º) at high resolution with an ART of 10 per frame rate. Foveal thickness (mean) was recorded following analysis using Heidelberg Eye Explorer software (HEYEX, version 1.9.10.0).

4.2.14.14 Serum carotenoid analysis

Non-fasting blood samples were collected at each study visit by standard venepunture techniques in 9 mL vacuette tubes (BD Vacutainer® SST™ Serum Separation Tubes) containing a "Z Serum Sep Clot Activator". All collection tubes were inverted a minimum of five times to ensure appropriate mixing of the clot activator. The blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged for 10 minutes at 2700 rpm in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. After centrifugation, serum was transferred to light-resistant micro-tubes and stored at -80ºC until time of analysis. Serum analysis of L, Z and MZ was carried out by the CREST Senior Scientist (D.K.) using a procedure described elsewhere.[204]

4.2.15 Statistical Analysis

One eye (the study eye) of each subject comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0.

Armonk, NY. All analyses were conducted as per protocol. However, intention-to-treat (ITT) analysis [214-216] was also performed and discrepancies between ITT analyses and per protocol are reported below. No interim analyses were conducted over the course of the study.

Baseline characteristics in the intervention groups were reported using mean ± standard deviation for interval data and frequency distribution (percentages) for categorical data. Baseline differences between intervention groups were assessed using independent samples t-test for interval variables and contingency table analyses using the chi-squared test for categorical variables.

Most of the outcome variables in this study were changes (over time) in interval variables (e.g. CS, MP, L, Z, MZ). In order to compare the effects of the two intervention groups (on each interval outcome measure, over time), we used Repeated Measures Analysis of Variance, with time as a within-participants factor and intervention group as a between-participants factor. In the ITT analysis, the Last Observation Carried Forward (LOCF) was used when participant data were missing.

Within-group analyses of changes, over time, in interval outcome variables, were based on paired t-tests.

Tests of significance, for all comparisons of intervention groups on interval outcome measures, were two-tailed, and the 5% level of significance was used throughout. We did not correct for multiple tests. Multiple testing increases the probability of a Type I error, but it increases the probability of a Type II error. Our approach is – using the 5% level of significance but drawing attention to possibly spurious significance arising from multiple tests. We believe that the Type II error (in this instance, failing to report a statistically significant

observation), when, in fact, there really is a statistically significant observation is the one to be avoided. In this regard, we have clarified this approach in the statistical analysis section.

4.3 Results

Figure 11 shows the Consolidated Standards of Reporting Trials (CONSORT) diagram,[217] summarizing the CREST study design, participant enrolment, randomization, follow-up and the number of participant included in study analyses. 121 participants were enrolled at baseline with 98 participants completing final assessment at 24 months. Table 14 presents the demographic, lifestyle, vision and AMD grades of participants enrolled at baseline. Eligibility was initially determined clinically by an ophthalmologist (S.B.) during the CREST screening visit and this was subsequently confirmed by a non-detailed grading of retinal photographs at this eligibility visit. However, following detailed grading of retinal photographs at baseline, 3 participants were found to have AREDS grade 9. These participants were included in the ITT analyses, but not in the per protocol analyses. Baseline characteristics were statistically comparable across the two intervention groups with the exception of Letter CS (1.2 and 2.4cpd) and photopic CS at 3cpd (see Table 14). Losses to follow-up after two years of antioxidant supplementation were statistically comparable between the two intervention groups (p=0.680, Pearson Chi-square).

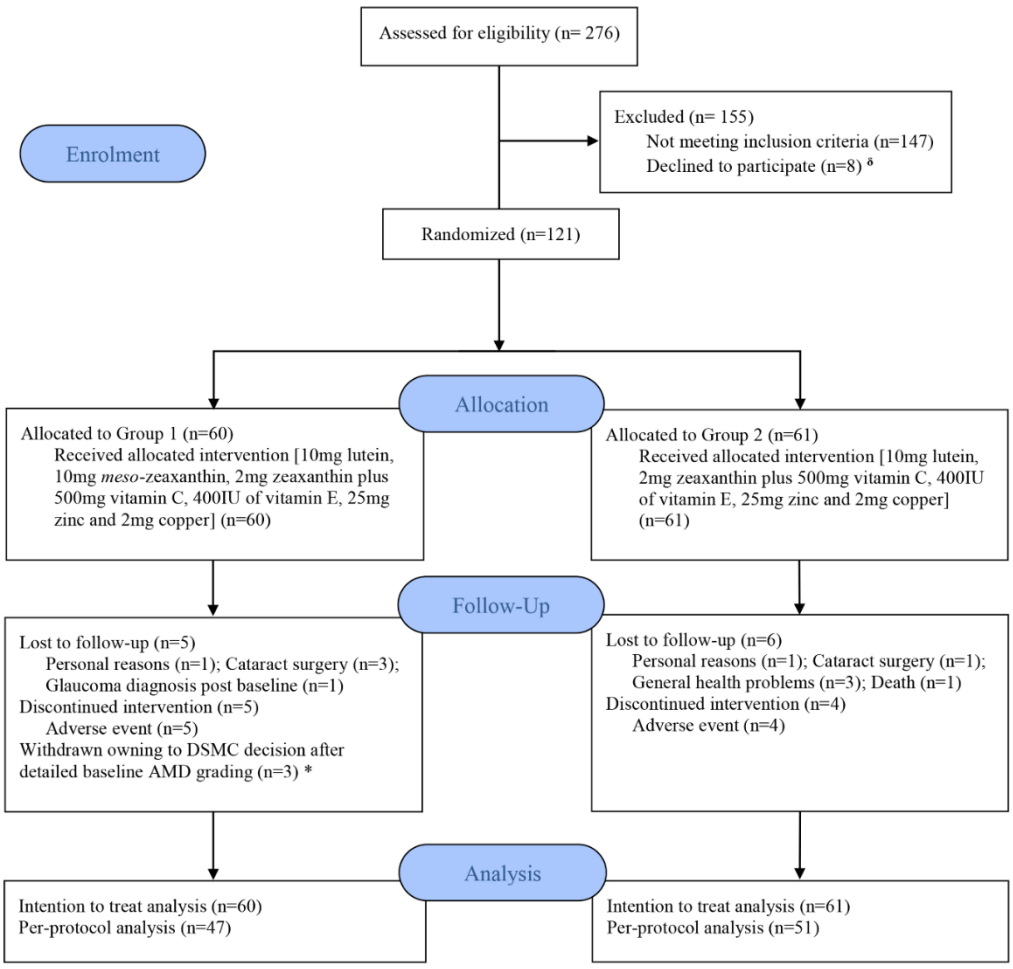


Figure 11: Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) consolidated standards of reporting trials (CONSORT) flow diagram. DSMC, Data and Safety Monitoring Committee; δ, Participants declined to participate either due to personal reasons, transportation difficulties, or cataract surgery; *, Participants were initially enrolled based on non-detail grading of retinal photographs obtained at screening visit, confirming eligibility by the Moorfields Eye Hospital Reading Centre (MEHRC). However, detailed grading of baseline retinal photographs showed some participants had AMD grades > 8 on the AREDS 11-step severity scale and therefore these participants were excluded based on a decision by the Data and Safety Monitoring Committee.

Table 14: Baseline characteristics by intervention group in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study (Per Protocol)

Variables	Group 1 (n=57)	Group 2 (n=61)	Sig.
Demographic, lifestyle and health			
Age (years)	65.09 ± 8.59	64.34 ± 9.50	0.657
Body mass index (kg/m^2)	28.27 ± 4.30	27.78 ± 4.57	0.551
Blood pressure (mmHg)			
Systolic	142.07 ± 20.98	138.00 ± 24.35	0.334
Diastolic	82.65 ± 11.21	79.12 ± 9.81	0.070
Sex			
Male	18 (45.0)	22 (55.0)	0.607
Female	39 (50.0)	39 (50.0)	
Education			
Primary	7 (43.8)	9 (56.3)	0.766
Secondary	29 (51.8)	27 (48.2)	
Tertiary	21 (45.7)	25 (54.3)	
Smoking			
Never	29 (50.0)	29 (50.0)	0.933
Past	23 (46.9)	26 (53.1)	
Current	5 (45.5)	6 (54.5)	
AMD family history			
Yes	16 (53.3)	14 (46.7)	0.406
No	31 (44.3)	39 (55.7)	
Cardiovascular disease			
Yes	5 (50.0)	5 (50.0)	0.804
No	50 (47.6)	55 (52.4)	
Hypertension			
Yes	17 (48.6)	18 (51.4)	0.970
No	40 (48.2)	43 (51.8)	
AMD grades			
1-3	13 (43.3)	17 (56.7)	0.528
4-8	44 (50.0)	44 (50.0)	
Diet score	26.90 ± 12.00	26.26 ± 12.03	0.776
Serum carotenoids*			
Serum L (µmol/l)	0.35 ± 0.20	0.34 ± 0.22	0.710
Serum Z (µmol/l)	0.07 ± 0.05	0.07 ± 0.05	0.639
Serum MZ (µmol/l)	0.00 ± 0.01	0.01 ± 0.02	0.205
Macular pigment			
Densitometer*			
0.25°	0.79 ± 0.24	0.72 ± 0.26	0.179
0.5°	0.65 ± 0.22	0.60 ± 0.21	0.204
1.0°	0.45 ± 0.16	0.45 ± 0.17	0.927
1.75°	0.32 ± 0.12	0.31 ± 0.15	0.933

Variables	Group 1 (n=57)	Group 2 (n=61)	Sig.
Vision			
Best corrected visual acuity (VAR)			
Study eye	100.04 ± 5.83	100.08 ± 5.62	0.965
Fellow eye	94.63 ± 10.95	95.92 ± 12.20	0.549
Letter contrast sensitivity (LogCS)			
1.2cpd	1.77 ± 0.17	1.85 ± 0.16	0.007
2.4cpd	1.76 ± 0.21	1.83 ± 0.18	0.045
6cpd	1.49 ± 0.25	1.56 ± 0.21	0.108
9.6cpd	1.23 ± 0.30	1.32 ± 0.25	0.082
15.15cpd*	0.86 ± 0.35	0.94 ± 0.29	0.160
Mesopic contrast sensitivity (LogCS)			
1.5cpd	1.53 ± 0.22	1.61 ± 0.21	0.065
3cpd	1.62 ± 0.23	1.68 ± 0.18	0.106
6cpd	1.21 ± 0.35	1.33 ± 0.35	0.065
12cpd	0.78 ± 0.27	0.85 ± 0.28	0.132
18cpd	0.33 ± 0.12	0.32 ± 0.11	0.749
Photopic contrast sensitivity (LogCS)			
1.5cpd	1.46 ± 0.19	1.52 ± 0.16	0.061
3cpd	1.72 ± 0.22	1.80 ± 0.19	0.047
6cpd	1.58 ± 0.31	1.68 ± 0.31	0.079
12cpd	1.19 ± 0.38	1.27 ± 0.35	0.279
18cpd	0.51 ± 0.34	0.62 ± 0.34	0.081
Mesopic glare disability (LogCS)			
1.5cpd	0.91 ± 0.32	0.99 ± 0.29	0.193
3cpd	1.11 ± 0.37	1.19 ± 0.32	0.241
6cpd	0.93 ± 0.25	0.93 ± 0.23	0.977
12cpd	0.66 ± 0.15	0.63 ± 0.11	0.355
18cpd	0.30 ± 0.00	0.31 ± 0.04	0.336
Photopic glare disability (LogCS)			
1.5cpd	1.40 ± 0.21	1.46 ± 0.17	0.082
3cpd	1.67 ± 0.22	1.73 ± 0.18	0.130
6cpd	1.51 ± 0.32	1.58 ± 0.31	0.210
12cpd	1.11 ± 0.36	1.19 ± 0.36	0.206
18cpd	0.52 ± 0.35	0.56 ± 0.31	0.583
Retinal Straylight	1.30 ± 0.18	1.33 ± 0.25	0.381
Photostress recovery time (seconds)	15.98 ± 8.72	15.97 ± 7.99	0.996
Reading performance			
Reading acuity (LogRAD)	0.12 ± 0.13	0.09 ± 0.12	0.165
Mean reading speed (seconds)	154.48 ± 26.82	156.45 ± 27.53	0.694
Maximum reading speed (seconds)	199.61 ± 31.58	201.56 ± 34.44	0.749
National Eye Institute Questionnaire -25			
Overall vision score	87.80 ± 9.96	90.38 ± 9.22	0.147

Data displayed are mean ± standard deviation for interval data and percentages [n(%)] for categorical data; Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day

copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Education, highest level of education; Smoking: Never (< 100 cigarettes in lifetime), Past (smoked ≥ 100 cigarettes in lifetime and none in past year), Current (smoked ≥ 100 cigarettes in lifetime and at least one in the last year); *, n≠57 in Group 1 and/or n≠61 in Group 2 as certain tests/measures were not obtained; VAR, visual acuity rating; LogCS, logarithm of contrast sensitivity units; cpd, cycles per degrees; POM, Primary outcome measure; Family history of AMD means having a first degree relative, i.e. parent or sibling, with age-related macular degeneration; AMD grades based Age-related Eye Disease (AREDS) 11step scale; Diet score, estimated dietary intake of lutein and zeaxanthin using the "L/Z screener" developed by Professor Elizabeth Johnson, Tufts University, USA; Macular pigment measured using Macular Densitometer™ (Macular Metrics, Corp., Providence, Rhode Island, USA); Serum macular carotenoids analyzed by high performance liquid chromatography (HPLC); Best corrected visual acuity measured with Test Chart 2000 Xpert (Thomson Software Solutions, Hatfield, UK); Letter contrast sensitivity measured using Test Chart 2000 PRO™ (Thomson Software Solutions, Hatfield, UK); Mesopic and photopic contrast sensitivity measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Mesopic and photopic glare disability measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Retinal straylight measured using Oculus C-Quant (Oculus GmbH, Wetzler, Germany) and recorded in Logarithms (judged reliable when ESD ≤ 0.08 and Q ≥ 1); Photostress recovery time measured by assessing the time of recovery after a 10-second exposure to a 300 watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions, GmbH, Germany) with a low-pass glass dichroic filter; Reading performance assessed using the English version of the standardized Radner reading chart at a distance of 40cm with reading correction; Reading acuity recorded in logarithm of the reading acuity determination (LogRAD); The formula (logRAD + total number of incorrectly read syllables × 0.005) was used to calculate the LogRAD-score; Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14words × 60seconds divided by reading time in seconds); National Eye Institute Visual Function Questionnaire–25 overall vision scores range from zero (worst) to 100 (best).

Table 15: Repeated measures analysis of visual function outcomes from baseline to 24 months in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study by Intervention groups

Variable	Group 1					Group 2					Time Effect	Time × Group Interaction
		Baseline		24 months			Baseline		24 months			
	N	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD	Sig.	Sig.
Vision												
Best corrected visual acuity (VAR)	46	101.22	5.16	100.91	5.8	51	100.78	5.08	101.31	5.2	0.746	0.233
Letter contrast sensitivity (LogCS)												
1.2cpd	46	1.79	0.17	1.89	0.2	51	1.86	0.14	1.91	0.16	<0.0005	0.058
2.4cpd	46	1.78	0.22	1.86	0.22	51	1.85	0.16	1.91	0.18	<0.0005	0.582
6cpd	46	1.53	0.24	1.57	0.29	51	1.58	0.18	1.61	0.23	0.013	0.881
9.6cpd	46	1.29	0.28	1.31	0.3	51	1.36	0.21	1.38	0.26	0.154	0.925
15.15cpd	46	0.92	0.33	0.95	0.34	51	0.96	0.27	1.01	0.33	0.082	0.747
Mesopic contrast sensitivity (LogCS)												
1.5cpd	46	1.55	0.22	1.62	0.24	51	1.63	0.21	1.7	0.23	0.007	0.982
3cpd	46	1.63	0.24	1.76	0.27	51	1.69	0.18	1.84	0.27	<0.0005	0.523
6cpd	46	1.25	0.35	1.48	0.45	51	1.34	0.34	1.49	0.42	<0.0005	0.228
12cpd	46	0.81	0.29	0.94	0.36	51	0.87	0.28	0.96	0.35	0.002	0.605
18cpd	46	0.33	0.13	0.39	0.23	51	0.31	0.08	0.41	0.25	<0.0005	0.369
Photopic contrast sensitivity (LogCS)												
1.5cpd	46	1.47	0.19	1.6	0.23	51	1.53	0.16	1.64	0.21	<0.0005	0.862
3cpd	46	1.75	0.23	1.84	0.23	51	1.82	0.18	1.91	0.21	<0.0005	0.986
6cpd	46	1.63	0.28	1.74	0.39	51	1.7	0.29	1.81	0.34	<0.0005	0.934
12cpd	46	1.25	0.37	1.34	0.43	51	1.3	0.33	1.34	0.37	0.015	0.468
18cpd	46	0.56	0.36	0.71	0.44	51	0.65	0.34	0.69	0.36	0.008	0.174
Mesopic glare disability (LogCS)												
1.5cpd	46	0.98	0.32	1.08	0.44	51	1.01	0.29	1.2	0.45	<0.0005	0.172
3cpd	46	1.19	0.36	1.22	0.43	51	1.22	0.3	1.38	0.41	0.001	0.04
6cpd	46	0.97	0.27	1.05	0.35	51	0.94	0.23	1.09	0.35	<0.0005	0.222
12cpd	46	0.67	0.16	0.68	0.22	51	0.64	0.12	0.69	0.18	0.133	0.412
18cpd	46	0.3	0	0.32	0.1	51	0.31	0.04	0.31	0.08	0.197	0.486

Variable	Group 1					Group 2					Time Effect	Time × Group Interaction
		Baseline		24 months			Baseline		24 months			
	N	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD	Sig.	Sig.
Photopic glare disability (LogCS)												
1.5cpd	46	1.43	0.21	1.55	0.26	51	1.47	0.18	1.55	0.24	<0.0005	0.364
3cpd	46	1.7	0.22	1.82	0.25	51	1.74	0.18	1.83	0.24	<0.0005	0.542
6cpd	46	1.56	0.31	1.65	0.4	51	1.61	0.29	1.7	0.34	0.001	0.987
12cpd	46	1.18	0.34	1.26	0.41	51	1.23	0.33	1.31	0.38	0.011	0.913
18cpd	46	0.58	0.37	0.6	0.39	51	0.57	0.31	0.62	0.33	0.179	0.646
Retinal Straylight (Logs)	41	1.29	0.18	1.25	0.19	43	1.33	0.2	1.26	0.16	0.004	0.359
Photostress recovery time (seconds)	46	16.93	9.19	12.47	6.79	51	16	8.51	10.96	6.05	<0.0005	0.757
Reading performance												
Reading acuity (LogRAD)	46	0.09	0.12	0.09	0.08	51	0.07	0.1	0.06	0.1	0.637	0.759
Mean reading speed (seconds)	46	154.61	27.11	189.89	26.53	51	158.75	27	192.82	28.54	<0.0005	0.765
Maximum reading speed (seconds)	46	200.44	32.25	244	35.02	51	204.74	33.4	245.38	37.9	<0.0005	0.606
National Eye Institute Questionnaire -25												
Overall vision score	46	89.24	7.95	89.27	9.61	50	90.83	9.66	91.93	7.01	0.408	0.434

Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; N, participants with data at all study visits; SD, standard deviation; cpd, cycles per degree; Sig., time group interaction effect obtained from Repeated Measures Analysis of Variance; Best corrected visual acuity measured with Test Chart 2000 Xpert (Thomson Software Solutions, Hatfield, UK); Letter contrast sensitivity measured using Test Chart 2000 PRO™ (Thomson Software Solutions, Hatfield, UK); Mesopic and photopic contrast sensitivity measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Mesopic and photopic glare disability measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Retinal straylight measured using Oculus C-Quant (Oculus GmbH, Wetzler, Germany) and recorded in Logarithms (judged reliable when ESD ≤ 0.08 and Q ≥ 1); Photostress recovery time measured by assessing the time of recovery after a 10-second exposure to a 300 watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions, GmbH, Germany) with a low-pass glass dichroic filter; Reading performance assessed using the English version of the standardized Radner reading chart at a distance of 40cm with reading correction; Reading acuity recorded in logarithm of the reading acuity determination (LogRAD); The formula (logRAD + total number of incorrectly read syllables × 0.005) was used to calculate the LogRAD-score; Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14words × 60seconds divided by reading time in seconds); National Eye Institute Visual Function Questionnaire–25 overall vision scores range from zero (worst) to 100 (best).

Table 16: Change in visual function over the course of the study in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study by Intervention groups

Variable	Group 1					Sig.	Group 2					Sig.	All groups					Sig.
		Baseline		24 months				Baseline		24 months				Baseline		24 months		
	N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD	
Best corrected visual acuity (VAR)	46	101.22	5.16	100.91	5.8	0.547	51	100.78	5.08	101.31	5.2	0.275	97	100.99	5.1	101.12	5.47	0.7
Letter contrast sensitivity (LogCS)																		
1.2cpd	46	1.79	0.17	1.89	0.2	<0.0005	51	1.86	0.14	1.91	0.16	0.003	97	1.83	0.16	1.9	0.18	<0.0005
2.4cpd	46	1.78	0.22	1.86	0.22	0.002	51	1.85	0.16	1.91	0.18	<0.0005	97	1.82	0.2	1.88	0.2	<0.0005
6cpd	46	1.53	0.24	1.57	0.29	0.092	51	1.58	0.18	1.61	0.23	0.07	97	1.56	0.21	1.59	0.26	0.013
9.6cpd	46	1.29	0.28	1.31	0.3	0.324	51	1.36	0.21	1.38	0.26	0.299	97	1.32	0.25	1.35	0.28	0.15
15.15cpd	46	0.92	0.33	0.95	0.34	0.255	51	0.96	0.27	1.01	0.33	0.179	97	0.94	0.3	0.98	0.34	0.077
Mesopic contrast sensitivity (LogCS)																		
1.5cpd	46	1.55	0.22	1.62	0.24	0.039	51	1.63	0.21	1.7	0.23	0.069	97	1.59	0.22	1.66	0.24	0.006
3cpd	46	1.63	0.24	1.76	0.27	<0.0005	51	1.69	0.18	1.84	0.27	<0.0005	97	1.66	0.21	1.8	0.27	<0.0005
6cpd	46	1.25	0.35	1.48	0.45	<0.0005	51	1.34	0.34	1.49	0.42	0.003	97	1.3	0.35	1.49	0.43	<0.0005
12cpd	46	0.81	0.29	0.94	0.36	0.011	51	0.87	0.28	0.96	0.35	0.064	97	0.84	0.28	0.95	0.36	0.002
18cpd	46	0.33	0.13	0.39	0.23	0.034	51	0.31	0.08	0.41	0.25	0.005	97	0.32	0.11	0.41	0.24	<0.0005
Photopic contrast sensitivity (LogCS)																		
1.5cpd	46	1.47	0.19	1.6	0.23	<0.0005	51	1.53	0.16	1.64	0.21	<0.0005	97	1.5	0.18	1.62	0.22	<0.0005
3cpd	46	1.75	0.23	1.84	0.23	0.003	51	1.82	0.18	1.91	0.21	<0.0005	97	1.78	0.21	1.88	0.22	<0.0005
6cpd	46	1.63	0.28	1.74	0.39	0.003	51	1.7	0.29	1.81	0.34	0.008	97	1.67	0.29	1.77	0.37	<0.0005
12cpd	46	1.25	0.37	1.34	0.43	0.044	51	1.3	0.33	1.34	0.37	0.181	97	1.27	0.35	1.34	0.4	0.016
18cpd	46	0.56	0.36	0.71	0.44	0.008	51	0.65	0.34	0.69	0.36	0.322	97	0.61	0.35	0.7	0.4	0.01
Mesopic glare disability (LogCS)																		
1.5cpd	46	0.98	0.32	1.08	0.44	0.016	51	1.01	0.29	1.2	0.45	0.001	97	1	0.3	1.14	0.45	<0.0005
3cpd	46	1.19	0.36	1.22	0.43	0.401	51	1.22	0.3	1.38	0.41	<0.0005	97	1.2	0.33	1.3	0.42	0.001
6cpd	46	0.97	0.27	1.05	0.35	0.058	51	0.94	0.23	1.09	0.35	0.002	97	0.95	0.25	1.07	0.35	<0.0005
12cpd	46	0.67	0.16	0.68	0.22	0.637	51	0.64	0.12	0.69	0.18	0.095	97	0.65	0.14	0.68	0.2	0.122
18cpd	46	0.3	0	0.32	0.1	0.183	51	0.31	0.04	0.31	0.08	0.659	97	0.3	0.03	0.32	0.09	0.208

Variable	Group 1					Sig.	Group 2					Sig.	All groups					Sig.
	Baseline			24 months			Baseline			24 months			Baseline			24 months		
	N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD	
Photopic glare disability (LogCS)																		
1.5cpd	46	1.43	0.21	1.55	0.26	<0.0005	51	1.47	0.18	1.55	0.24	0.024	97	1.45	0.19	1.55	0.24	<0.0005
3cpd	46	1.7	0.22	1.82	0.25	<0.0005	51	1.74	0.18	1.83	0.24	0.001	97	1.72	0.2	1.83	0.24	<0.0005
6cpd	46	1.56	0.31	1.65	0.4	0.018	51	1.61	0.29	1.7	0.34	0.017	97	1.58	0.3	1.68	0.37	0.001
12cpd	46	1.18	0.34	1.26	0.41	0.084	51	1.23	0.33	1.31	0.38	0.061	97	1.21	0.34	1.28	0.39	0.01
18cpd	46	0.58	0.37	0.6	0.39	0.554	51	0.57	0.31	0.62	0.33	0.179	97	0.57	0.34	0.61	0.35	0.169
Retinal straylight (Logs)	41	1.29	0.18	1.25	0.19	0.196	43	1.33	0.2	1.26	0.16	0.003	84	1.31	0.19	1.26	0.17	0.003
Photostress recovery time (seconds)	46	16.93	9.19	12.47	6.79	0.001	51	16	8.51	10.96	6.05	<0.0005	97	16.45	8.8	11.67	6.42	<0.0005
Reading performance																		
Reading acuity (LogRAD)	46	0.09	0.12	0.09	0.08	0.905	51	0.07	0.1	0.06	0.1	0.589	97	0.08	0.11	0.08	0.09	0.623
Mean reading speed (seconds)	46	154.61	27.11	189.89	26.53	<0.0005	51	158.75	27	192.82	28.54	<0.0005	97	156.79	26.99	191.43	27.5	<0.0005
Maximum reading speed (seconds)	46	200.44	32.25	244	35.02	<0.0005	51	204.74	33.4	245.38	37.9	<0.0005	97	202.7	32.76	244.72	36.38	<0.0005
National Eye Institute Questionnaire -25																		
Overall vision score	46	89.24	7.95	89.27	9.61	0.976	50	90.83	9.66	91.93	7.01	0.245	96	90.07	8.87	90.66	8.42	0.398

Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Sig., statistical significance (p values) obtained from paired t-tests; Best corrected visual acuity measured with Test Chart 2000 Xpert (Thomson Software Solutions, Hatfield, UK); Letter contrast sensitivity measured using Test Chart 2000 PRO^{TM} (Thomson Software Solutions, Hatfield, UK); Mesopic and photopic contrast sensitivity measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Mesopic and photopic glare disability measured using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA); Retinal straylight measured using Oculus C-Quant (Oculus GmbH, Wetzler, Germany) and recorded in Logarithms (judged reliable when ESD ≤ 0.08 and Q ≥ 1); Photostress recovery time measured by assessing the time of recovery after a 10-second exposure to a 300 watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions, GmbH, Germany) with a low-pass glass dichroic filter; Reading performance assessed using the English version of the standardized Radner reading chart at a distance of 40cm with reading correction; Reading acuity recorded in logarithm of the reading acuity determination (LogRAD); The formula (logRAD + total number of incorrectly read syllables × 0.005) was used to calculate the LogRAD-score; Reading speed (the time taken to read the number of words in a sentence) was measured in words per minute (w/min) with a stop watch for each standardized sentence (14words × 60seconds divided by reading time in seconds); National Eye Institute Visual Function Questionnaire–25 overall vision scores range from zero (worst) to 100 (best).

4.3.1 Primary Outcome Analysis

The repeated measures analysis of change in letter CS at 6cpd (POM) is shown in Table 15 (as per protocol). There was a statistically significant improvement in the POM over the study period (p=0.013 for time effect), but there was no statistically significant difference between the intervention groups (p=0.881 for the time × group interaction effect). Thus, there is no evidence that the two intervention groups are different with respect to improvement in this measure. Figure 12 graphically illustrate these findings.

4.3.2 Secondary Outcome Analysis

4.3.2.1 Other visual function outcomes at baseline and after 24 months

Results from the repeated measures analysis, for other visual function variables, are shown in Table 15. There was a statistically significant improvement ($p<0.05$, for time effect) in many measures of visual function over the study period including CS, PRT, retinal straylight and GD, and again these improvements were statistically comparable between intervention groups ($p>0.05$), with the exception of mesopic GD at 3cpd (p=0.040 for the time × group interaction effect), which improved to a borderline significantly greater extent in Group 2. However, ITT analysis results in a non-significant difference between the groups for change in this outcome variable (p=0.132 for the time × group interaction effect). Figures 12, 13, 14, and 15 graphically illustrate these findings.

The repeated measures analysis of change in NEI VFQ-25 overall score did not show significant differences between intervention groups over the course of the study (p=0.434 for the time × group interaction effect; see Table 15).

Furthermore, changes in NEI VFQ-25 overall score were not statistically significant within any of the intervention groups ($p>0.05$, for all; see Table 16).

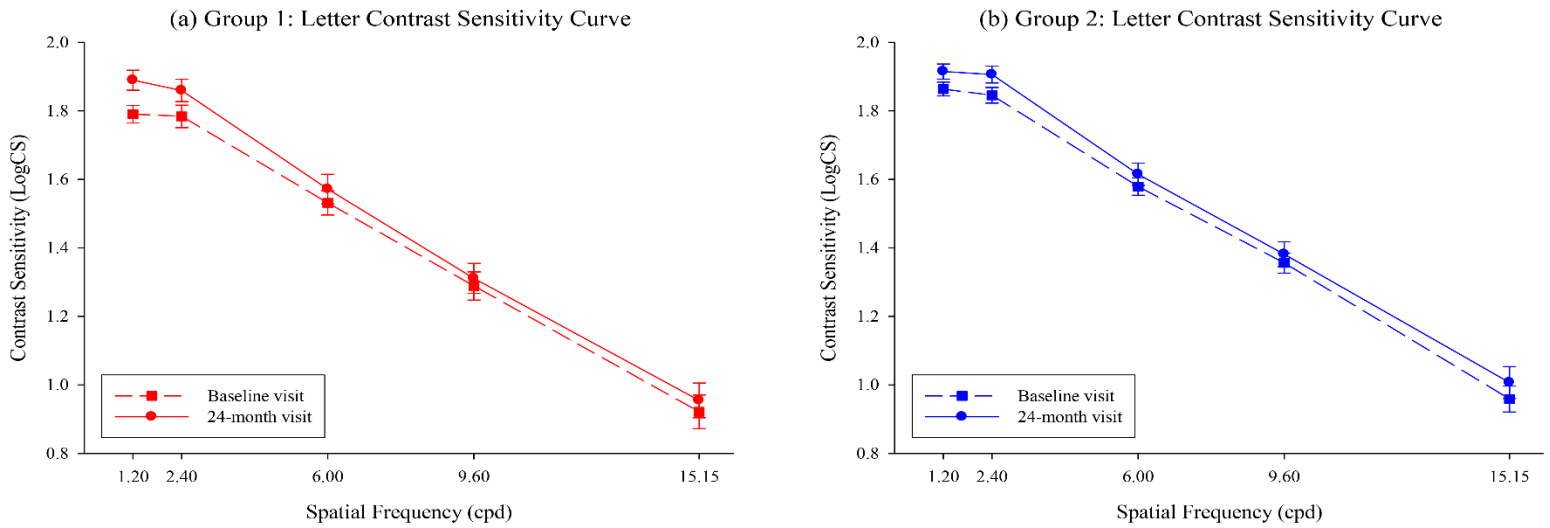


Figure 12: Letter contrast sensitivity function using the Test Chart 2000 PRO™ (Thomson Software Solutions, Hatfield, UK) in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units [IU]/day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Error bars represent standard error of mean.

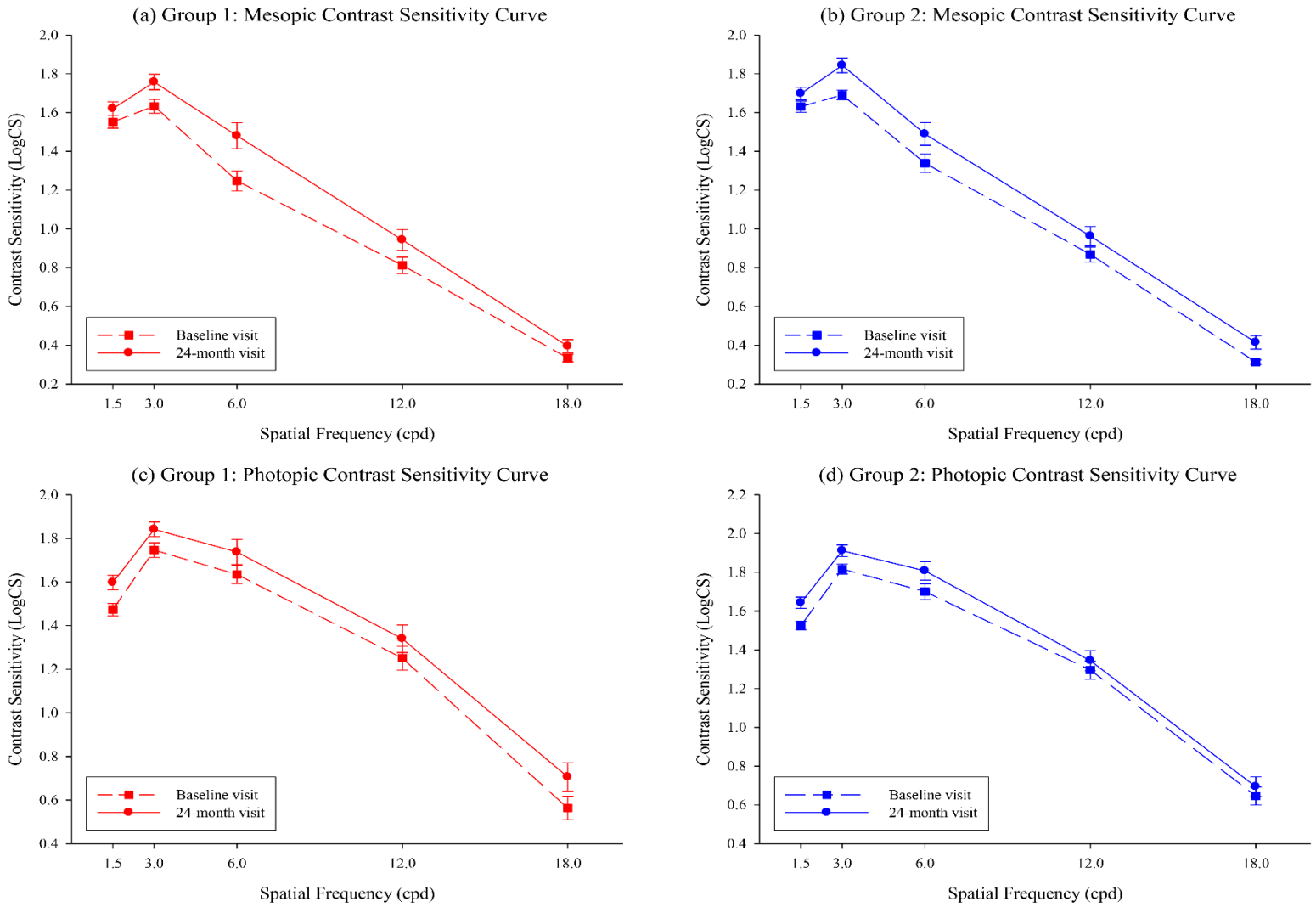


Figure 13: Mesopic and photopic contrast sensitivity function using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA) in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Error bars represent standard error of mean.

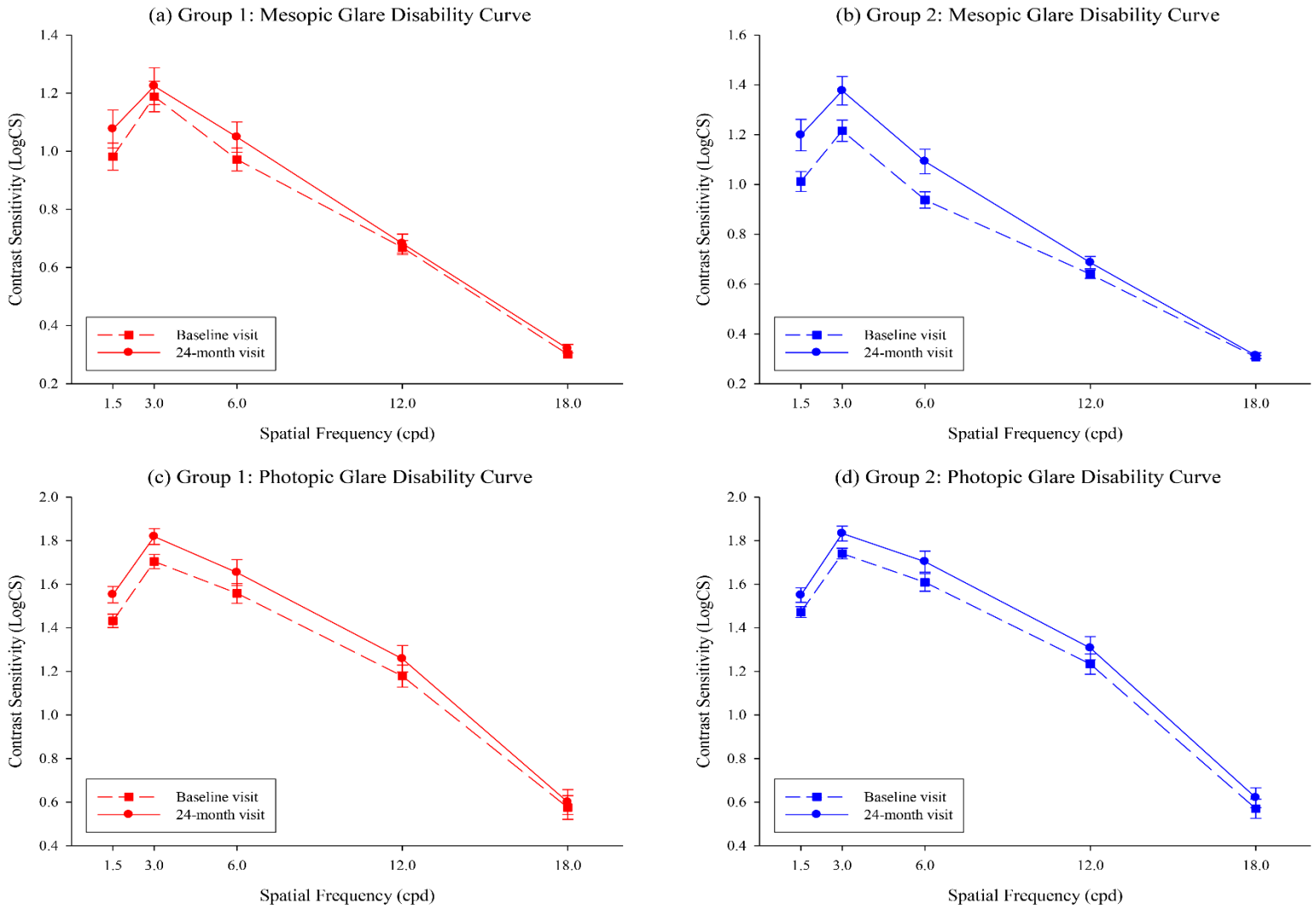


Figure 14: Mesopic and photopic glare disability using the Functional Vision Analyzer™ (Stereo Optical Co., Chicago, Illinois, USA) in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Error bars represent standard error of mean.

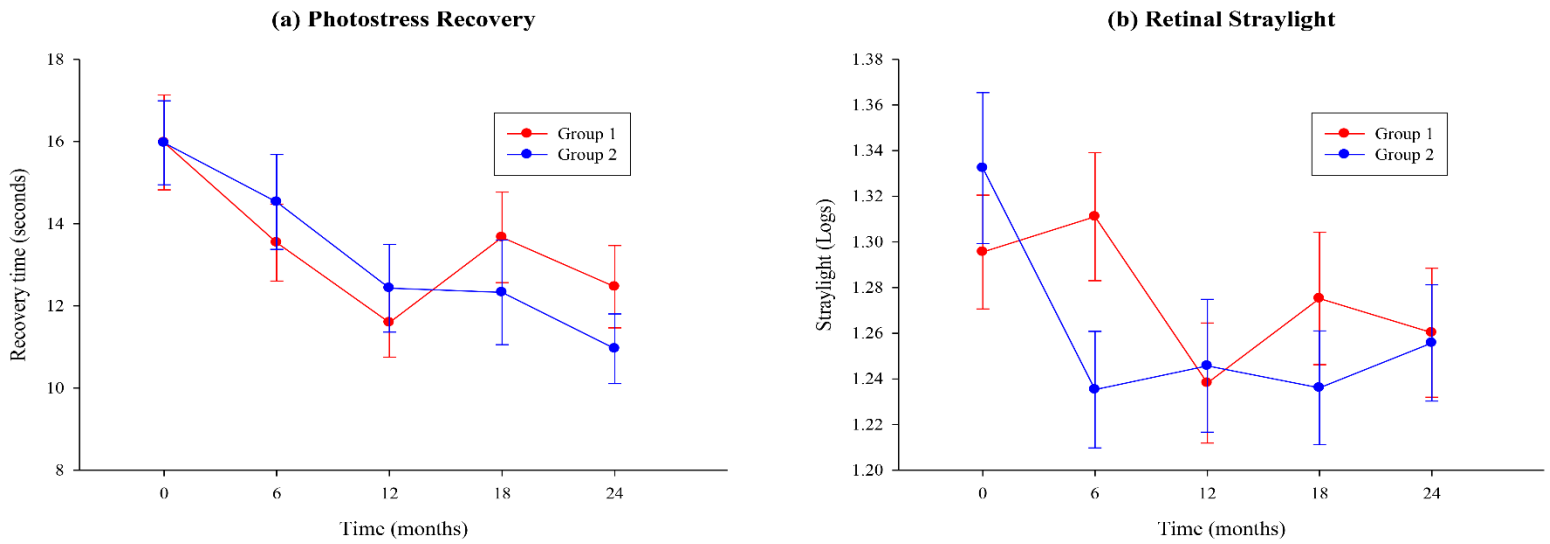


Figure 15: (a) Photostress recovery response in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. (b) Retinal straylight response using Oculus C-Quant (Oculus GmbH, Wetzler, Germany) in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Error bars represent standard error of mean.

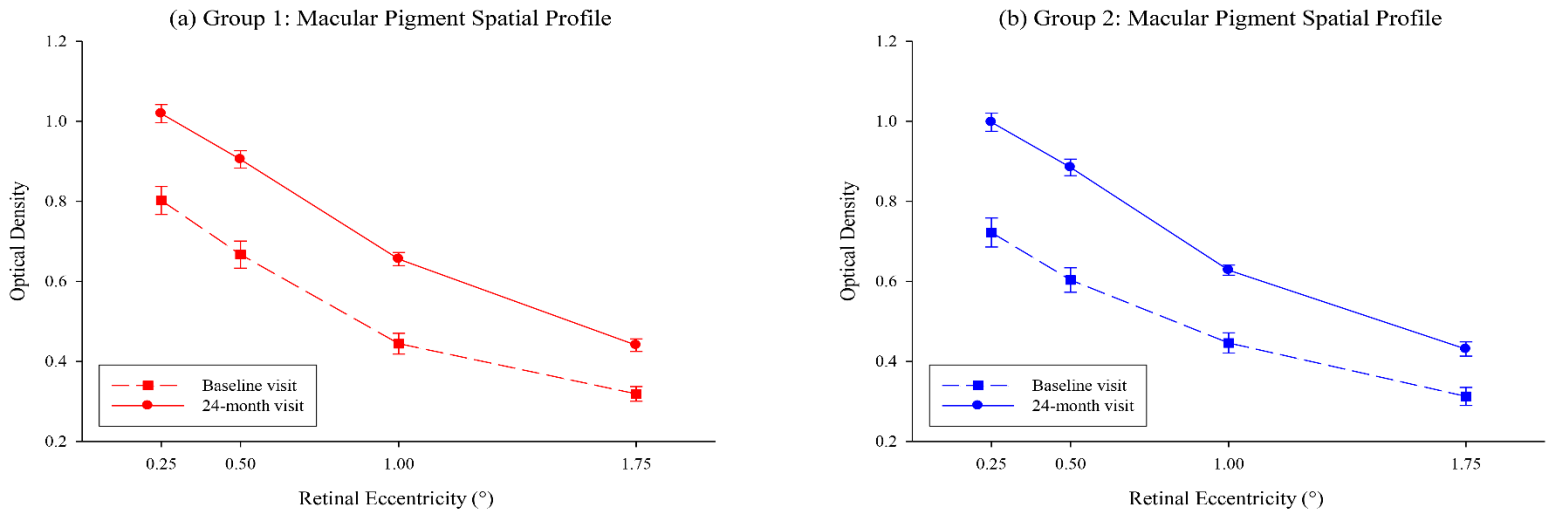


Figure 16 Macular pigment response in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Macular pigment measured using Macular Densitometer^{TM} (Macular Metrics, Corp., Providence, Rhode Island, USA); Error bars represent standard error of mean.

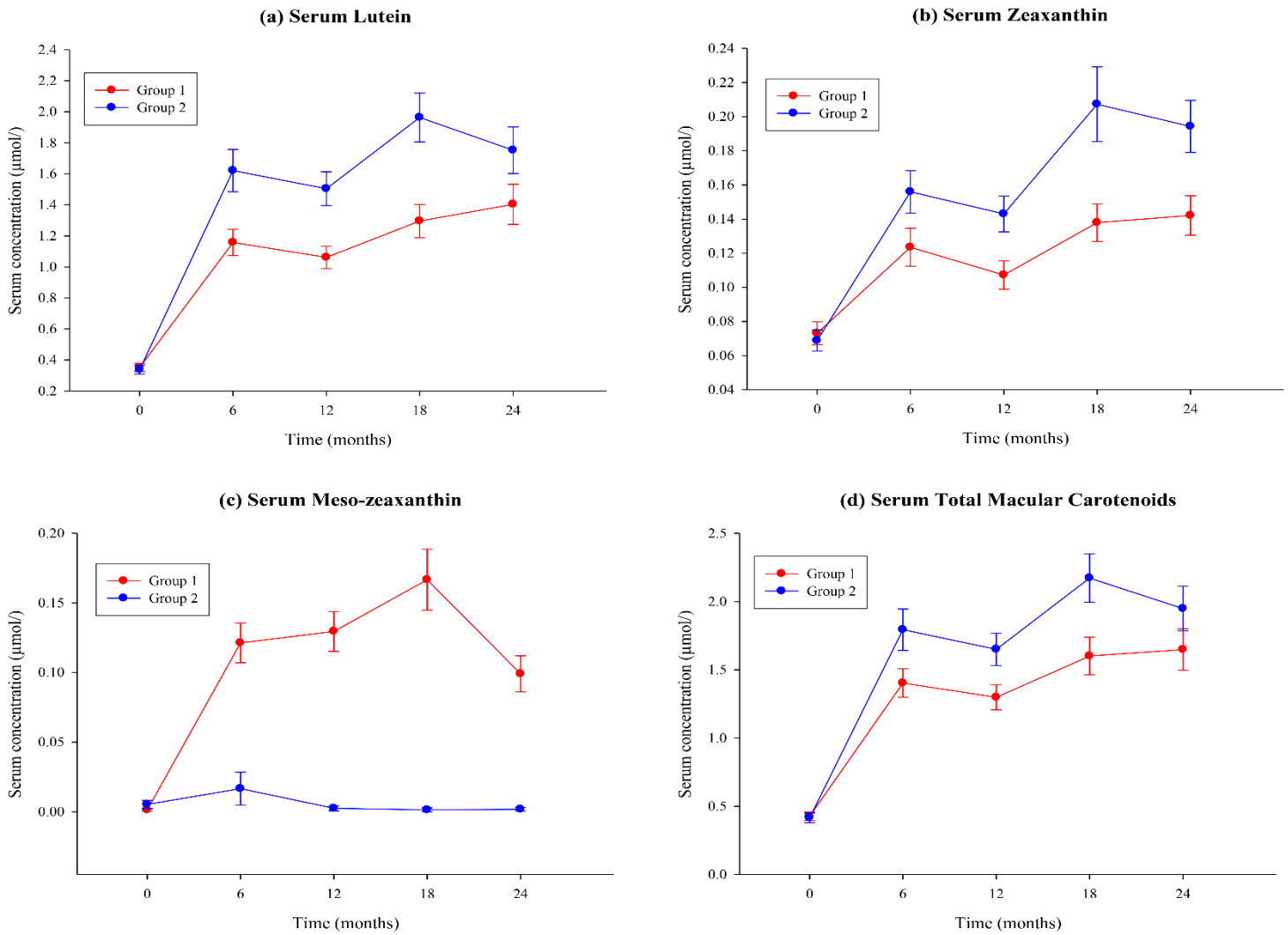


Figure 17 Serum carotenoid response in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study. Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Serum macular carotenoids analyzed by high performance liquid chromatography (HPLC); Serum total macular carotenoids represent the addition of serum lutein, zeaxanthin and *meso*-zeaxanthin concentrations obtained at each study visit; Error bars represent standard error of mean.

4.3.2.2 Change in visual function over time

While it clear from Table 15 that each intervention group was equally efficacious in terms of the observed improvements in parameters of visual function, it is evident from Table 16 that many improvements ($p<0.05$) were significant *within* each intervention group following two years of continuous supplementation. Table 16 presents the results of paired t-tests for all visual function variables, for each group separately, and for the two groups combined. It appears therefore that merging the two intervention groups is justified, given the statistically comparable observations in respect of these outcomes (Table 15), thereby lending greater statistical power to our findings reflected in the final column of Table 16, which reveals a greater number of significant results than in unmerged and individual intervention groups. The paired t-test results in Table 16 were obtained by comparing visual function scores at baseline and final visit at 24 months.

4.3.2.3 Macular pigment at baseline and after 24 months

There was a statistically significant increase in MP for all eccentricities over the course of the study ($p<0.0005$, for all time effects), but this increase was statistically comparable between intervention groups ($p>0.05$ for time × group interaction effect at all retinal eccentricities). Figure 16 graphically illustrate these findings.

4.3.2.4 Serum carotenoids at baseline and after 24 months

There was a statistically significant increase in serum concentrations of L, Z, and MZ over the course of the study ($p<0.0005$, for all time effect). The repeated measures analysis of change in serum L concentrations over time did not show

significant differences between intervention groups (p=0.111 for the time × group interaction effect). Observed increases in serum Z concentrations were significantly greater in Group 2 when compared to Group 1 (p=0.005 for the time × group interaction effect). Significant increases in serum MZ concentrations were observed in Group 1, but not in Group 2 (p<0.0005 for the time × group interaction effect). In terms of observed increases in total (composite) serum macular carotenoid concentrations (i.e. L, Z and MZ combined), no significant difference between intervention groups (p=0.241 for the time × group interaction effect) was observed. Figure 17 graphically illustrate these findings.

4.3.2.5 Relationship between Change in MP and Change in CS, GD, PRT, Retinal Straylight and Reading Speed

We investigated the relationship between change in CS, GD, PRT and change in MP, measured from baseline to final visit at 24-months. For these analyses, we merged the two intervention groups and employed Pearson correlation analyses. The following relationships were positive and statistically significant: between change in MP at 0.25° and change in CS at 6cpd (POM, r=0.219, p=0.033); between change in MP at 0.25° and mesopic CS at 1.5cpd (r=0.216, p=0.036). Thus, increases in MP over time were associated with increases in CS at 6cpd and mesopic CS at 1.5cpd. However, the following relationships were negative and statistically significant: between change in MP at 1.75° and mesopic GD at 6cpd (r=-0.220, p=0.032); between change in MP at 1.0° and photopic GD at 12cpd (r=-0.245, p=0.017); between change in MP at 1.75° and photopic GD at 12cpd (r=-0.230, p=0.025).

4.3.2.6 Relationship between Serum Concentrations of L, Z, MZ, and Change in CS, GD, PRT, Retinal Straylight and Reading Speed

With respect to change in serum carotenoids (L and Z), there were no statistically significant relationships between change in serum carotenoids and change in CS, GD, PRT, retinal straylight or reading speed ($p>0.05$, for all). Correlational analyses using serum MZ were restricted to Group 1 because serum MZ did not significantly change in Group 2. No statistically significant relationships were observed between serum MZ and change in CS, GD PRT, retinal straylight or reading speed ($p>0.05$, for all), with the exception of photopic CS at 1.5cpd ($r=-0.355$, $p=0.025$).

4.3.2.7 Relationship between Serum Concentrations of L, Z, MZ, and Change in MP

There was no statistically significant relationship between Change in MP (at any eccentricities) and change in each of the serum macular carotenoids ($p>0.05$, for all).

4.3.2.8 Grade of AMD at baseline and after 24 months

Participants were graded for AMD at baseline and final study visit at 24 months using the AREDS 11-step severity scale. Gradable retinal photographs were available in 121 participants at baseline, and in 96 of these participants at final visit. In Group 1, 37 participants (80%) at final visit (24 months) exhibited no change in AMD grade when compared with baseline, 9 participants (20%) exhibited disease progression. The corresponding figures in Group 2 were 45 participants (90%) and 5 participants (10%). These between-group differences were not statistically significant ($p=0.185$, Pearson chi-square test).

Notwithstanding that this study was not designed to assess the impact of supplementation on disease progression, and because of the small number of participants recruited, we collapsed AMD grades as follows: AREDS grades 1-3 (low risk of progression to advanced AMD), and AREDS grades 4-8 (high risk of progression to advanced AMD). Table 17 shows, within each intervention group, the transition between these grades from baseline to final study visit at 24 months. Only one participant (in Group 2) progressed to advanced AMD over the study period.

We also investigated clinically meaningful change in AMD grade along the AREDS 11-step scale, defined as a change of at least two steps along this scale. Thus, an increase of two steps between baseline and final visit at 24 months was considered clinically meaningful disease progression and a decrease of two steps was considered a clinically meaningful disease regression. On this basis, there was no clinically meaningful change in AMD grade in 94 (98%) study eyes, while 2 (2%) study eyes (both participants in Group 2) exhibited a clinically meaningful progression along the AREDS 11-step scale.

Table 17: Change in age-related macular degeneration disease status

	Intervention	Low risk	High Risk	Advanced AMD	Total
Baseline	**Group 1**	13	44	0	57
	Group 2	17	44	0	61
24-months	**Group 1**	11	35	0	46
	Group 2	11	38	1	50

Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units [IU]/day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Low risk: AMD grades 1-3 on Age-related Eye Disease (AREDS) 11step scale; High risk: AMD grades 4-8 on Age-related Eye Disease (AREDS) 11step scale; Advanced AMD: AMD grades 9-11 on Age-related Eye Disease (AREDS) 11step scale.

4.3.3 Compliance by Capsule Count

The compliance to study intervention (as measured by capsule counting [see Table 18]) was not significantly different between intervention groups over the course of the study (p=0.342 for the time × group interaction effect).

Table 18: Compliance by total capsule count

Intervention	n	6-month (%)	24-month (%)	Sig.$^{\alpha}$	Sig.$^{\beta}$
Group 1	25	95.17 ± 5.30	95.18 ± 6.10	0.997	0.342
Group 2	34	93.79 ± 5.55	91.57 ± 9.30	0.197	

Group 1, 10mg/day *meso*-zeaxanthin [MZ], 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units [IU]/day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Sig.$^{\alpha}$, statistical significance (p values) obtained from paired t-tests; Sig.$^{\beta}$, statistical significance (p value) obtained from repeated measures analysis of variance time group interaction effect

4.3.4 Adverse Events

The distribution of potential or perceived adverse events reported over the course of the study is shown in Table 19. Some participants reported more than one adverse event. The proportion of participants experiencing any adverse event was statistically similar between groups: 15 (26%) of 57 Group 1 and 10 (16%) of 61 Group 2 (p=0.187, Pearson chi-squared test). No serious adverse event relating to the study intervention was reported over the course of the study.

Table 19: Distribution of adverse events in the Central Retinal Enrichment Supplementation Trials (CREST) age-related macular degeneration (AMD) study by Intervention groups

Adverse Events	Group 1 (n=57)	Group 2 (n=61)
Any Adverse Event	15	10
Ocular		
Watery eyes	1	1
Transient blurred vision	1	0
Gritty eyes	1	0
Ocular pain	1	
Bloodshot eyes	1	0
Nonocular		
Nausea	2	3
Tiredness	2	1
Vomiting	3	0
Itchy skin	1	1
Metallic taste in mouth	1	1
Heat rash	0	2
Irritable bowel syndrome	1	0
Night-time urination	1	0
Headaches	1	0
Weight gain	1	0
Overactive kidney	0	1
Leg cramps	1	0
Knee ache	1	0
Red and swollen arms and legs	0	1
Dizziness	1	0
Neck stiffness	1	0
Abdominal pains	0	1
Pancreatitis	0	1
Palpitations	1	0
Sleep disturbance	1	0
Swollen face	0	1
Hallucinations	0	1
Swollen ankle	0	1
Loss of appetite	0	1

Data expressed as number of participants; Some participants reported more than one adverse event; Group 1, 10mg/day *meso*-zeaxanthin, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper; Group 2, 10mg/day lutein, 2mg/day zeaxanthin plus 500mg/day vitamin C, 400 international units /day of vitamin E, 25mg/day zinc and 2mg/day copper.

4.4 Discussion

This RCT was designed to compare the impact of two different macular carotenoid formulations, in combination with co-antioxidants, on visual function in patients with non-advanced AMD. Baseline (cross-sectional) data from the current study demonstrated that MP relates to many measures of visual function, even after controlling for age, sex and cataract grade.[218] In brief, the two interventions (AREDS2 formula with lower dose of zinc [25mg] versus same formulation but with the inclusion of 10mg MZ) were comparably efficacious in terms of observed improvements in visual function and in terms of MP augmentation. However, no comment can be made on the comparability of these two interventions in terms of AMD progression, as this study was neither designed nor powered to do so.

In the current study, AMD disease status of participants was graded using the AREDS 11-step severity scale (see Chapter 1, Section 1.1 for detailed description),[15] and included only eyes classed as grade 1 to 8 at baseline (referred to as non-advanced AMD for the purpose of the current study). We did not include eyes with non-central GA (AMD grade 9 on the AREDS 11-step severity scale). Given the biologically plausible rationale that benefits in terms of vision and in terms of MP augmentation are likely to extend to participants with earlier disease (before irreversible damage has occurred, such as in non-central geographic atrophy [grade 9 AREDS 11-step severity scale]), we have purposely recruited eyes with earlier stage disease. We report improvements in a range of measures of visual function (i.e. CS, GD, PRT, reading speed) following supplementation with macular carotenoids in combination with co-antioxidants. A

brief discussion on the mechanisms whereby the observed improvements in visual performance were realized as a result of supplementation is merited.

CS can be described as our ability to discriminate an object from its background and is determined by measuring the contrast threshold between visible and invisible at given spatial frequency.[185] CS is adversely affected in non-advanced AMD,[219-222] and in a way that relates to the severity of non-advanced AMD.[219, 221, 222] Furthermore, CS is adversely affected in non-advanced AMD to a greater extent than is VA affected in non-advanced AMD.[219, 221, 222] It is believed that CS is adversely affected in non-advanced AMD because of reduced efficiency in the lateral inhibitory mechanisms mediated by the horizontal and amacrine cells.[220, 223, 224] Mesopic CS is adversely affected to a greater extent than photopic CS because there is greater fall out of rods in non-advanced AMD than of cones.[225] A possible explanation for the role that MP plays in optimizing CS may rest on the visibility hypothesis of MP, which posits that this prereceptorial pigment enhances visualization of a target's detail by the absorption of blue haze.[145] Blue haze is a subjective experience and it is caused by scattered short-wavelength dominant air light (blue light) that results in a veiling luminance when we view objects at a distance.[145] MP accentuates the luminance of an object relative to its background by attenuating the impact of this scattered (veiling) short-wavelength visible blue light on the just noticeable differences of luminance required for discernability and, by consequence, extends the visual range.[226] Indeed, the visibility hypothesis has been tested empirically, and is supported by two studies, which have demonstrated the beneficial effect of MP in this respect under simulated blue haze conditions.[227, 228] Beyond this optical effect, the macular carotenoids may also influence lateral inhibitory mechanisms,[229] which

may also explain, at least in part, the observed improvements in CS following augmentation of MP. In the visual system, ganglion cells subserve receptive fields of different sizes with a centre-surround configuration.[229] Of note, for an on-centre ganglion receptive field, the centre field corresponds to excitation (i.e. increases ganglion cell response when stimulated) and the surround field correspond to inhibition (i.e. decreases ganglion cell response when stimulated).[229] Thus, light falling on the centre has a differential impact on the ganglion cell when compared to light falling on the surround. This antagonistic arrangement of receptive fields, and the resulting differential response of the centre versus the surround, increases the detection of edges (i.e. CS) in the visual system.[230] The macular carotenoids could enhance lateral inhibition mechanisms by increasing the signal to noise in the ganglion cell receptive fields and consequently enhance the detection of edges (CS) in the visual system. [229] Of note, these lateral inhibitory mechanisms subserve CS under both photopic and mesopic conditions,[231, 232] consistent with our observations of improved CS following MP augmentation under photopic and mesopic conditions. Finally, the macular carotenoids have been shown to inhibit the formation and oxidation of A2E *in vivo*[233] as well as inhibit the photooxidation of A2E *in vitro*,[234] and A2E (a key component of lipofuscin) inhibits RPE65 isomerohydrolase, a visual cycle enzyme responsible for the isomerization of all-trans retinyl ester to 11-cis retinol (a limiting step in the visual cycle).[235] Therefore, a reduction in production and/or oxidation in A2E could conceivably lead to a more efficient visual cycle and consequentially improved CS. However, these latter two mechanisms proposed to explain the observed contributions of MP augmentation to the observed

improvements in CS in eyes with non-advanced AMD warrant further investigation.

GD is defined as reduction in visual function caused by a glare source, resulting in retinal contrast loss secondary to retinal straylight.[236, 237] In other words, glare that causes vision loss. Clinically, GD can be measured by assessing the impact of a glare source on visual function (VA or CS) or by measurement of retinal straylight.[237] Of note, the *Commission Internationale de l'Eclairage* (CIE) defines GD as retinal straylight.[236] For the purposes of this study, GD was measured using the two aforementioned clinical assessment methods (i.e. by assessing CS under conditions of glare [in both mesopic and photopic conditions] using the Functional Vision Analyser [see Section 4.2.14.6] and by measuring retinal straylight using the Oculus C-Quant [see Section 4.2.14.7]). GD is adversely affected in non-advanced AMD,[238] and this adverse effect of GD is believed to be attributable to the loss of photoreceptors in non-advanced AMD.[225] Accordingly, mechanisms put forward to explain the observed improvements in CS following MP augmentation in patients with non-advanced AMD equally apply to the observed improvements in GD in this population, but with the possibility of an additional element, which relates the glare hypothesis of MP.[146] The absorption spectrum of MP[118] accounts for one third of the visible spectrum (see Section 1.6.1 and Figure 5), and wavelengths of light responsible for GD are those in the MP absorption range.[146] MP filters short-wavelength light at a prereceptorial level, thereby reducing the adverse impact of light scatter (caused by the glare source) that casts a veiling luminance on the retina, and consequentially improves GD.[146]

PRT measures the time of recovery (the dynamic response of the retina) required to perform predefined visual tasks (either VA or CS) after exposure to a dazzling (bright) light source.[239] PRT is adversely affected in non-advanced AMD because of the loss of photoreceptors and the disruption of the Bruch's membrane and RPE, leading to dysfunction in the visual cycle.[225, 240, 241] PRT is known to be adversely affected in non-advanced AMD,[219, 242, 243] and in a way that relates to the severity of the non-advanced AMD.[219, 242] Improvements in PRT that we observed as a result of supplementation may also be explained, at least in part, by the glare hypothesis of MP.[146] Macular pigment attenuates short-wavelength light from the glare source before it reaches the photoreceptors, thereby reducing its impact on photopigment bleaching, and consequently, reducing the recovery time (i.e. the time it takes for vision to be restored). Furthermore, the aforementioned effect of MP on attenuation of production and/or oxidation of A2E[233] may also result in faster regeneration of photopigments and consequential faster recovery times following photostress. In terms of the practical implications of the observed improvements in PRT, taking the example of a 60-year-old patient with non-advanced AMD driving a car at 100km/h, the patient's car will cover approximately 500m in 18 seconds. However, following exposure to a debilitating bright light source from an oncoming car, and given an improvement in PRT of approximately 5 seconds, the supplemented patient's vision will be restored approximately 140m before the unsupplemented patient with non-advanced AMD. These improvements in PRT will have implications on driving safety.

The observed improvement in reading speed may be explained by the impact of MP augmentation on visual and/or non-visual (neurocognitive) factors. Visual factors that could enhance reading speed include those already mentioned,

and which impact favorably upon CS and GD. Reading speed is a function of both spatial and temporal CS.[244] It has been shown that MP is positively related to critical flicker fusion frequency (CFF) and the full temporal CS function measured at the fovea but not the parafovea.[245] Furthermore, supplemental macular carotenoids have been shown to increase CFF thresholds and visual motor reaction time in young healthy subjects.[246, 247] Thus, MP could improve reading speed by its effects on temporal vision (i.e. increasing temporal processing speeds). Stringham and Stringham have suggested that temporal visual mechanisms compensate for MP's optical filtration properties by reducing temporal input from the short-wavelength-cone system and increasing temporal processing by the middle-/ long-wavelength cone system.[248] These temporal visual mechanisms may be enhanced following MP augmentation, and may lead to improvements in reading speed. Neurocognitive factors may also contribute to the observed improvements in reading speed following supplementation and consequential augmentation of MP, as a result of the emerging role of carotenoids in brain health[12] and cognition.[249] L and Z concentrations at the macula correlate with their respective concentrations in the occipital cortex in non-human and human primates.[250, 251] Indeed, several studies have shown that MP is positively related to measures of cognitive function.[252-255] For instance, a population-based study including 4,453 participants reveal that 11 tests of cognition in the 50 years and over age group were positively related to MP, suggesting that, at the very least, MP may represent a useful biomarker for cognitive health.[253] Furthermore, MP can be augmented in subjects with Alzheimer's disease, and such augmentation of MP results in visual improvements.[256] In other words and beyond the visual impact of MP augmentation, already discussed, there could be

neurocognitive benefits which may have also contributed to the observations of increased reading speed.

In the current study, MP increased significantly, at all eccentricities measured, in each intervention group over the study period, consistent with previous studies following supplementation with at least two of MP's constituent carotenoids.[149, 157, 162, 198] Ancillary analyses (not pre-specified) investigating the relationship between change in MP (at retinal eccentricities: 0.25°, 0.5°, 1.0°, and 1.75°) and change in visual function measures was also performed (see Section 4.3.2.5). Only 5 out of the 128 paired correlations (between MP and visual function parameters) were statistically significant and these include: the relationship between change in MP at 0.25° and change in letter CS at 6cpd (POM, r=0.219, p=0.033); the relationship between change in MP at 0.25° and mesopic CS at 1.5cpd (r=0.216, p=0.036); the relationship between change in MP at 1.75° and mesopic GD at 6cpd (r=-0.220, p=0.032); the relationship between change in MP at 1.0° and photopic GD at 12cpd (r=-0.245, p=0.017); the relationship between change in MP at 1.75° and photopic GD at 12cpd (r=-0.230, p=0.025). Of these statistically significant relationships between observed changes in MP and visual function, two were positive (mesopic CS at 1.5cpd and letter CS at 6cpd) and three were negative (mesopic GD at 6cpd and photopic GD at 12cpd). Given the small number of statistically significant paired correlations between observed changes in MP and observed improvements in measures of visual function (2%), these findings cannot be attributed solely to an optical effect, and benefits at a cellular level are implicated. Furthermore, given the general absence of significant relationships between change in MP and change in visual function, these findings should be treated circumspectly, and may be as a

consequence of the multiple testing (increased likelihood of Type 1 errors [rejecting the null hypothesis]) which can occasionally generate spurious results. For multiple tests, the recommendation is sometimes made that the level of significance be reduced well below 5%, so as to reduce the probability of a Type I error – rejecting null hypotheses when it should be accepted – arising from the multiplicity of tests. This approach, however, also greatly increases the risk of a Type II error –accepting null hypotheses when it should be rejected. In particular, Bonferroni adjustment is often advocated for multiple tests. This would entail, for this ancillary analyses investigating the relationship between change in MP and change in visual function measures, reducing the significance level from 0.05 to about 0.0004 (which is 0.05 divided by 128). Of note, none of the relationships reported between change in MP and change in visual function measures will be considered statistically significant after adjusting for multiple testing.

Serum response to supplementation was also assessed, and as anticipated, MZ increased significantly in Group 1 (AREDS2 formula with lower dose of zinc [25mg] but with the inclusion of MZ) only, but not in Group 2 (AREDS2 formula with lower dose of zinc [25mg], but not including MZ) and observed serum response in terms of total macular carotenoids (L, Z, and MZ) were comparable between interventions. Interestingly, serum Z increased to a significantly greater extent after two years in Group 2 than in Group 1, even though each formulation contained equal concentrations of Z (i.e. 2mg). In terms of mean ± SD increases (from baseline to 24 months), serum Z increased from 0.07 ± 0.04 µmol/l to 0.14 ± 0.07 µmol/l in Group 1, whiles serum Z increased from 0.07 ± 0.05 µmol/l to 0.19 ± 0.11 µmol/l in Group 2. In terms of percentage increases, serum Z increased by 100% ± 75% in Group 1, and by 171% ± 120% in Group 2. The

differential increases in serum Z between interventions could possibly suggest competition for gastrointestinal absorption between the isomers of Z (3R,3'R-zeaxanthin and 3R,3'S-zeaxanthin) as suggested by previous investigators.[179, 184] Given that the observed increases in MP and improvements in visual function were statistically comparable between interventions and given that the aim of supplementation is augmentation of MP and consequential visual improvements and photoprotection, the statistically significant greater rise in serum Z concentration in Group 2 (and since serum Z increased to a statistically significant degree in each group) are unlikely to be clinically meaningful.

The clinical meaningfulness of the observed visual benefits in patients with non-advanced AMD following supplementation with macular carotenoids (in combination of co-antioxidants) warrants discussion. Quality of life questionnaires relate to subjectively perceived visual function (e.g. as a result of reduced CS, GD, and reading speed), and therefore, it is likely that improvements in these parameters will result in improved quality of life. Scilley *et al* reported that persons with non-advanced AMD have good VA, but are more likely to have problems with night driving, near vision tasks, and GD, compared to persons with no retinal disease (age-matched controls with normal retinal health).[238] VA, CS and reading speed are associated with vision-related quality of life in non-advanced AMD.[257] In the Los Angeles Latino Eye Study, non-advanced AMD lesions (i.e. soft indistinct drusen and pigmentary abnormalities) were associated with a lower self-reported vision-related quality of life.[258] In the Melbourne Collaborative Cohort Study, after adjusting for age, sex, BMI, smoking and country of birth, intermediate AMD (non-advanced AMD) was significantly associated with total hip replacement for osteoarthritis (OR=1.22; 95% CI 1.00-

1.49) whereas advanced AMD was significantly associated with total hip replacement due to fractured neck of femur (OR=5.21; 95% CI 2.25-12.02).[259] In a hospital-based case-control study, 96 patients diagnosed with hip fracture were compared to 103 age-matched controls (patients with no diagnosis of hip fracture) in a cohort of patients aged 60 years and over. The presence of AMD (type of AMD unspecified) was significantly associated with increased risk of hip fractures (OR=10.65; 95% CI 1.57-20.18).[260] Newly diagnosed patients with (any) AMD were more likely to have experienced a hip fracture when compared to controls without AMD during a 10-year longitudinal retrospective study using Medicare claims data.[261] VA and CS are associated with greater fear of falling among patients with (any) AMD, and AMD disease status is also associated with greater fear of falling.[19] Persons with AMD (type unspecified), glaucoma and Fuchs corneal dystrophy are more likely to report activity limitation due to a fear of falling when compared to controls, and interestingly, CS largely explained the relationship between eye disease and fear of falling.[262] In patients with (any) AMD, 0.1 log decrease in CS is associated with a 11% reduction in moderate-to-vigorous physical activity per day (demonstrating the contributions of CS in mediating physical activity among persons with AMD).[263] Scott *et al* investigated the impact of visual function parameters on computer task performance in patients with AMD (any type), and reported that CS was the most significant contributor to computer task accuracy.[264] Therefore, the observed improvements in visual function parameters (in the current study) are likely to impact favourably on quality of life by increasing mobility, reducing the risk of falls and fractures, reducing the need to be accompanied for social events (better social independence), enhancing driving vision (even through glare, foggy and misty

conditions) and reducing difficulty in near-vision related activities. However, our vision-related quality of life instrument (NEI VFQ-25) did not show any statistically significant improvements following supplementation with macular carotenoids (in combination with co-antioxidants), and we suspect that a larger number of participants will be required to do so with such an instrument. For instance, in order to detect a two-point difference between interventions in NEI VFQ-25 overall score (assuming a 5% level of significance, 80% power and two-tailed test), the required sample size would be 3136 participants (1568 per intervention group).[203] Eye care professionals should be aware of the observed visual benefits afforded to patients with non-advanced AMD as a result of supplementation with macular carotenoids (and co-antioxidants), and the indication for recommending such supplements be no longer limited to the risk reduction for disease progression.

Given that psychophysical function is compromised in non-advanced AMD in a way that is commensurate with the stage of non-advanced AMD, and given that AMD is a progressive disease, our findings of visual improvements in a condition where visual deterioration is expected is as interesting as it is welcome. If psychophysical visual function, which reflects disease morphology, can be improved in a progressive condition (such as AMD), it is possible that the underlying morphological changes can also be reversed. However, longer term studies with larger numbers of patients with non-advanced AMD, and with regular monitoring of MP and psychophysical function as well as morphological changes, are required to confirm or refute this hypothesis. Indeed, it is biologically plausible that improvements in psychophysical function herald improvements in the morphological changes that underpin them.

The strengths of this study include: its randomized, controlled and double-masked design, the range of parameters of visual function assessed, the fact that MP was measured and monitored using an established and validated technique, the determination of serological responses, and that AMD was graded in a masked fashion by an accredited reading center. Finally, the study was overseen by an independent DSMC. Limitations of the current study include: 1) The small number of participants represents a limitation of the current study; with 46 and 51 subjects in the two intervention groups at 24 months, the study was slightly underpowered for the comparison of the two interventions (time × group interaction effects), but more than adequately powered for the assessment of time effects. Although slightly underpowered, based on our pre-determined effect size for the POM, the study still had power of nearly 80% to detect a between-group difference of 0.5 standard deviations (a commonly-used "medium" effect size for interval variables) assuming a two-tailed test and the 5% level of significance. 2) Another limitation is the absence of a placebo arm. However, as already noted, the original study protocol had a true placebo, but that protocol had to be revised on ethical grounds, following publication of the AREDS2 findings. 3) In our statistical analyses, no correction for multiple comparisons was performed. It must be conceded, however, that some of the reported statistically significant effects in Table 15 and 16 may well, therefore, be spurious due to the increased likelihood of Type I errors (false positives). 4) Some participants forgot to bring their study supplements to study visits and therefore accurate calculations of capsule count were not obtained for all participants. This may be considered a limitation of our capsule count data. However, examining capsule count and serum carotenoid data in parallel, it seems safe to conclude that participants were compliant to the study

intervention regardless of intervention assignment. 5) We did not measure the serum concentrations of any of the co-antioxidants (vitamin C, E, zinc and copper) in this RCT because both intervention groups were given the same concentrations of these compounds in the supplement formula. We were more concerned with the serum response of the macular carotenoids since the only difference between the two interventions was the inclusion of MZ. Assessing the concentrations of these co-antioxidants would have given insights into the interrelationships/interactions between these compounds and the macular carotenoids (especially MZ). Future studies should consider examining these interactions.

In summary, formulations containing the macular carotenoids (with or without MZ) in combination with co-antioxidants are comparable in terms of beneficial effects on visual function and in terms of MP augmentation in patients with non-advanced AMD.

4.5 Conclusion

Antioxidant supplementation in patients with non-advanced AMD, irrespective of whether MZ is included in the formulation, results in significant increases in MP and hitherto unappreciated visual benefits. These findings may have important implications for vision-related quality of life for patients afflicted with this condition. The protocol for this study was published in the Ophthalmic Epidemiology (see Appendix T) under the title, "***Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials.***"[150] Baseline data was also published in the British Journal of Ophthalmology (see Appendix T) under the title, "***Relationship between macular pigment and visual function in subjects with early age-related macular degeneration.***"[218] Furthermore, the results of this RCT is currently in preparation for submission to an ophthalmology and vision science journal.

Chapter 5. Conclusions and Future Considerations

This PhD thesis answered three main research questions. Firstly, what is the prevalence of AMD in the ROI (Research Question 1; Chapter 2)? Secondly, what is the impact of supplementation using three different carotenoid formulations on MP and visual function in non-advanced AMD over a three-year period (Research Question 2; Chapter 3)? Finally, does the inclusion of MZ to the standard of care confer advantages or disadvantages to patients with non-advanced AMD (Research Question 3; Chapter 4)? We present below the main conclusions from this thesis and recommendations for future research.

In research question 1 (Chapter 2), the prevalence of AMD was examined in community-living persons aged 50 years and older. I report the prevalence of AMD following retinal photographic grading to be 7.2%, affecting about 86,000 people aged 50 years and older. This thesis provides prevalence estimates for the first time in the ROI and will inform eye care professionals, epidemiologists, and policy makers involved in the delivery of care for those diagnosed with AMD. For example, these estimates of AMD prevalence will guide the Irish government to appropriately allocate funds to help persons living with AMD. Also, these estimates are useful for preparing reports pertaining to cost associated with AMD disease (e.g. cost of providing supplemental antioxidants to persons with non-advanced AMD; the cost of treating persons with neovascular AMD; the cost of providing low vision aids to persons with central vision impairment as a result of AMD). It is noteworthy that elderly persons living in institutional care settings, such as nursing homes, were not included in the investigation of AMD prevalence and therefore the prevalence estimates presented in this thesis may be greater than what has been estimated. With aging and growing populations, the prevalence of

AMD (estimated in this thesis) is expected to rise, and thus, many more people will be affected by AMD in the ROI. There is therefore the need for informed planning, and I hope that this thesis assists in this way. Currently, data on the incidence of AMD is not available in the ROI, and therefore future studies should be designed to investigate the incidence of AMD.

To answer research question 2 (Chapter 3), the impact of supplementation with three distinct carotenoid formulations over a three-year period was investigated by measuring visual function, MP response, and serum concentrations of MP's constituent carotenoids in patients with non-advanced AMD. MP continues to increase after two years of sustained supplementation, which supports the notion that sustained supplementation appears necessary to augment MP and optimize CS over a three-year period. The MOST study (described in Chapter 3) was an exploratory clinical trial and its findings suggested that the inclusion of supplemental MZ appears to offer advantages in terms of MP response and CS enhancement. However, these observations needed confirmation/validation in the context of a double-blind, randomized controlled trial (Chapter 4; CREST).

In the CREST study, the inclusion of MZ to a supplement formulation containing L and Z, in combination with co-antioxidants (the AREDS2 formula but with lower dose of zinc [25mg]), had no additional benefits or deleterious effects for visual function and MP augmentation when compared to a supplement formulation containing only L and Z, in combination with co-antioxidants. Thus, in patients with non-advanced AMD, supplementation with the macular carotenoids and co-antioxidants (with or without MZ) for a two-year period

results in improvements in several parameters of visual function. These findings have important implications for vision-related quality of life.

This thesis supports the view that psychophysical tests of visual function, such as CS, PRT, GD and reading speed, should be considered in the vision assessment of patients with non-advanced AMD, and this endeavour may be facilitated by emerging and commercially available vision-testing techniques. Data from my thesis suggests that using these tests, eye care professionals will be able to quantify changes in visual function and demonstrate the visual benefits of supplemental macular carotenoids in patients with non-advanced AMD. I believe it is not adequate for patients with non-advanced AMD to be assessed using only BCVA, and therefore, eye care professionals should endeavour to incorporate these tests. The benefits of assessing visual function with a range of tests is that a holistic representation of a patient's visual performance and experience is achieved. Furthermore, as the disease progresses, measures of visual function such as CS and GD are expected to exhibit deterioration in patients with non-advanced AMD with the passage of time, and yet this thesis has demonstrated that, in fact, these measures of visual function (CS, PRT, GD and reading speed) can actually be improved upon from pre-supplementation measurements. Moreover, these non-BCVA measures of visual function (CS, PRT, GD, reading speed) better reflect a patient's real world visual experience than BCVA, as we do not live in a world restricted to high contrast targets.

Although the CREST study was not sufficiently powered to comment on disease progression, the finding that 98% of participants did not progress (by AREDS grading standards) are of particular significance. Of note, disease progression in a majority of the study population would have been expected in the

absence of antioxidant supplementation. Instead, visual function measures improve following two years of antioxidant supplementation. It can be inferred from the visual performance improvements that the disease state actually improved in most patients.

Dark adaptation is a measure of visual function that is affected long before clinical signs of AMD are evident. Vitamin A restriction occurs early in AMD (sub-clinically), and manifests as impaired dark adaptation. It is noteworthy that macular carotenoid supplementation improved nearly all visual function tests and it is possible/probable that if measurement of dark adaptation had been conducted, a significant improvement in this measure would have been reported given the observed improvements in GD and PRT. Since it may not be clinically feasible to perform all the visual function tests, I would suggest the following core set of tests: CS, PRT and GD.

Data from OCT were obtained as part of the CREST study. Due to financial constraints, it was not feasible to send OCT data for grading at an accredited reading centre. This data would be useful for future analyses. For example, recent studies have shown that pseudodrusen is an independent risk factor for progression to advanced AMD, and OCT is a more sensitive imaging technique than fundus photography for estimating prevalence of pseudodrusen. Following grading of OCT images using standardized protocols, the impact of antioxidant supplementation on pseudodrusen (and the different retinal layers especially the photoreceptor layer, the retinal pigment epithelium and choroid [which are the main sites implicated in the pathogenesis of AMD]) can be investigated for new clinical and therapeutic insights. Furthermore, this OCT data

analysis could help us understand the mechanisms and pathways whereby the antioxidant supplementation improves vision.

Another area of analyses is the impact of supplementation on MP's spatial profile characteristics among persons with non-advanced AMD following two years of antioxidant supplementation. In the general population, different spatial profile characteristics are evident and these are broadly classified as either typical [exponential profile] OR atypical [non-exponential profile, with ringlike structures and central dips]). An important question not addressed in this thesis is whether the inclusion of MZ to the AREDS 2 formula (but with a lower dose of zinc [25mg]) has any putative benefits in terms of rebuilding the MP spatial profile in persons with non-advanced AMD. Indeed, using published definitions of different spatial profile characteristics, data from this thesis could help answer this research question.

There are several techniques for measuring MP. In this thesis, MP measurement was conducted using two techniques – customized HFP (Densitometer) and fundus autofluorescence (Spectralis). The Heidelberg Spectralis®HRA+OCT MultiColor (Heidelberg Engineering GmbH, Heidelberg, Germany) is a new, commercially available device which utilizes the 2WAF technique to measure MP, whereas the Macular DensitometerTM (Macular Metrics, Corp., Providence, Rhode Island, USA) has been available for over a decade, with over 100 peer-reviewed scientific publications which have used this device. I have recently reported that measurement of MP using the Densitometer and Spectralis are not statistically comparable and are not interchangeable in a given study in the clinical and research setting, but also concluded that each device yielded reliable measures of MP (and changes in MP) within subjects over

time (see Appendix T). Moreover, MP measurement using the Spectralis are affected by cataract and recommended that cataract is graded when measuring MP with a device that utilizes dual-wavelength fundus autofluorescence. Furthermore, a correction factor was proposed to compensate for cataract when measuring MP using the Spectralis. Cataract grading data were obtained within the first year of the study, at 18 months, and at 24 months. Although cataract grades were obtained only at baseline due to logistical constraints, further analyses of the MP data could validate the employment of a correction factor to compensate for cataract when using the Spectralis. Furthermore, using the cataract grading data along with retinal grading data, future mathematical models could also be generated as an improvement of the already published models.

The evidence of the impact of supplementation for visual function in patients with non-advanced AMD is overall positive and beneficial. This thesis has demonstrated that AREDS2 formulation (but with low levels of zinc) performed well and comparably irrespective of whether MZ is included in the formulation in patients with non-advanced AMD in terms of MP augmentation and in terms of visual function. What are the clinical and societal implications for these findings? Firstly, if an eye care professional wants to improve vision in patients with non-advanced AMD, either formulation could be recommended. Secondly, the safety profile of supplements with or without MZ are comparable and therefore any concerns regarding the safety of including MZ can be allayed. However, there is a need for continued pharmacovigilance and monitoring in order to ensure the long term safety of using supplemental antioxidants. I believe eye care professionals now have an evidence base which they can invoke to inform their decision-making process and will now be aware that supplementation

with macular carotenoids (and co-antioxidants) results in visual improvements in patients with non-advanced AMD. In other words, an attempt to halt disease progression is no longer the only indication for supplemental macular carotenoids and co-antioxidants in patients with non-advanced AMD, and improvements in visual performance represents another and hither to unappreciated indication for supplementation in patients with this condition.

BIBLIOGRAPHY

1. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Survey of Ophthalmology*. 1995;39:367-374.

2. Slakter JS, Stur M. Quality of life in patients with age-related macular degeneration: impact of the condition and benefits of treatment. *Surv Ophthalmol*. 2005;50:263-273.

3. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Archives of Biochemistry and Biophysics*. 2001;385:28-40.

4. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802-1811.

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

6. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. Lutein supplementation leads to decreased soluble complement membrane attack complex sC5b-9 plasma levels. *Acta Ophthalmol*. 2015;93:141-145.

7. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a and C3d. *PLoS ONE*. 2013;8:e73387.

8. Li SY, Fung FK, Fu ZJ, Wong D, Chan HH, Lo AC. Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Invest Ophthalmol Vis Sci*. 2012;53:5976-5984.

9. Firdous AP, Kuttan G, Kuttan R. Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. *Pharm Biol*. 2015:1-7.

10. Vishwanathan R, Neuringer M, Snodderly DM, Schalch W, Johnson EJ. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutr Neurosci*. 2013;16:21-29.

11. Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly. *Am J Clin Nutr*. 2012;96:1161S-1165S.

12. Craft NE, Haitema TB, Garnett KM, Fitch KA, Dorey CK. Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *J Nutr Health Aging*. 2004;8:156-162.

13. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128-1134.

14. Age-Related Eye disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119:1417-1436.

15. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol*. 2005;123:1484-1498.

16. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch Ophthalmol*. 2005;123:1570-1574.

17. Ferris FL, III, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology*. 2013;120:844-851.

18. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global Health*. 2014;2:e106-e116.

19. van Landingham SW, Massof RW, Chan E, Friedman DS, Ramulu PY. Fear of falling in age-related macular degeneration. *BMC Ophthalmol*. 2014;14:10.

20. Williams RA, Brody BL, Thomas RG, Kaplan RM, Brown SI. The psychosocial impact of macular degeneration. *Arch Ophthalmol*. 1998;116:514-520.

21. Brown GC, Brown MM, Sharma S, et al. The burden of age-related macular degeneration: a value-based medicine analysis. *Trans Am Ophthalmol Soc*. 2005;103:173-184.

22. Friedman DS, Katz J, Bressler NM, Rahmani B, Tielsch JM. Racial differences in the prevalence of age-related macular degeneration - The Baltimore eye survey. *Ophthalmology*. 1999;106:1049-1055.

23. Klein R, Klein BEK, Linton KLP. Prevalence of Age-Related Maculopathy - the Beaver Dam Eye Study. *Ophthalmology*. 1992;99:933-943.

24. Vingerling JR, Dielemans I, Hofman A, et al. The Prevalence of Age-Related Maculopathy in the Rotterdam Study. *Ophthalmology*. 1995;102:205-210.

25. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1995;102:1450-1460.

26. VanNewkirk MR, Nanjan MB, Wang JJ, Mitchell P, Taylor HR, McCarty CA. The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology*. 2000;107:1593-1600.

27. Klein R, Clegg L, Cooper LS, et al. Prevalence of age-related maculopathy in the Atherosclerosis Risk in Communities Study. *Arch Ophthalmol*. 1999;117:1203-1210.

28. Jonasson F, Arnarsson A, Sasaki H, Peto T, Sasaki K, Bird AC. The prevalence of age-related maculopathy in iceland: Reykjavik eye study. *Arch Ophthalmol*. 2003;121:379-385.

29. Klein R, Klein BE, Knudtson MD, et al. Prevalence of age-related macular degeneration in 4 racial/ethnic groups in the multi-ethnic study of atherosclerosis. *Ophthalmology*. 2006;113:373-380.

30. Andersen MV, Rosenberg T, la CM, et al. Prevalence of age-related maculopathy and age-related macular degeneration among the inuit in Greenland. The Greenland Inuit Eye Study. *Ophthalmology*. 2008;115:700-707.

31. Jonasson F, Arnarsson A, Eiriksdottir G, et al. Prevalence of age-related macular degeneration in old persons: Age, Gene/environment Susceptibility Reykjavik Study. *Ophthalmology*. 2011;118:825-830.

32. Piermarocchi S, Segato T, Scopa P, et al. The prevalence of age-related macular degeneration in Italy (PAMDI) study: report 1. *Ophthalmic Epidemiol*. 2011;18:129-136.

33. Augood CA, Vingerling JR, de Jong PT, et al. Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE). *Arch Ophthalmol*. 2006;124:529-535.

34. Bjornsson OM, Syrdalen P, Bird AC, Peto T, Kinge B. The prevalence of age-related maculopathy (ARM) in an urban Norwegian population: the Oslo Macular study. *Acta Ophthalmol Scand*. 2006;84:636-641.

35. Klein R, Chou CF, Klein BE, Zhang X, Meuer SM, Saaddine JB. Prevalence of age-related macular degeneration in the US population. *Arch Ophthalmol*. 2011;129:75-80.

36. Klein R, Cruickshanks KJ, Nash SD, et al. The prevalence of age-related macular degeneration and associated risk factors. *Arch Ophthalmol*. 2010;128:750-758.

37. Erke MG, Bertelsen G, Peto T, Sjolie AK, Lindekleiv H, Njolstad I. Prevalence of age-related macular degeneration in elderly Caucasians: the Tromso Eye Study. *Ophthalmology*. 2012;119:1737-1743.

38. Spanish Eyes Epidemiological (SEE) Study Group. Prevalence of age-related macular degeneration in Spain. *Br J Ophthalmol*. 2011;95:931-936.

39. Akuffo KO, Nolan J, Stack J, et al. Prevalence of age-related macular degeneration in the Republic of Ireland. *Br J Ophthalmol*. 2015;99:1037-1044.

40. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2007;114:253-262.

41. Klein R, Klein BEK, Tomany SC, Moss SE. Ten-Year Incidence of Age-related Maculopathy and Smoking and Drinking: The Beaver Dam Eye Study. *Am J Epidemiol*. 2002;156:589-598.

42. Klein R, Klein BEK, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy - The Beaver Dam eye study. *Ophthalmology*. 1997;104:7-21.

43. Wang JJ, Rochtchina E, Lee AJ, et al. Ten-year incidence and progression of age-related maculopathy: the blue Mountains Eye Study. *Ophthalmology*. 2007;114:92-98.

44. Mitchell P, Wang JJ, Foran S, Smith W. Five-year incidence of age-related maculopathy lesions - The Blue Mountains Eye Study. *Ophthalmology*. 2002;109:1092-1097.

45. Joachim N, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology*. 2015;122:2482-2489.

46. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of Ophthalmology*. 2000;45:115-134.

47. Parmeggiani F, Sorrentino FS, Romano MR, et al. Mechanism of inflammation in age-related macular degeneration: an up-to-date on genetic landmarks. *Mediators Inflamm*. 2013;2013:435607.

48. Telander DG. Inflammation and age-related macular degeneration (AMD). *Semin Ophthalmol*. 2011;26:192-197.

49. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *American Journal of Ophthalmology*. 2002;134:411-431.

50. Kijlstra A, La HE, Hendrikse F. Immunological factors in the pathogenesis and treatment of age-related macular degeneration. *Ocul Immunol Inflamm*. 2005;13:3-11.

51. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res*. 2000;70:441-449.

52. Nozaki M, Raisler BJ, Sakurai E, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A*. 2006;103:2328-2333.

53. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J*. 2000;14:835-846.

54. Nishiguchi KM, Yokoyama Y, Fujii Y, et al. Analysis of Macular Drusen and Blood Test Results in 945 Macaca fascicularis. *PLoS ONE*. 2016;11:e0164899.

55. Sarks SH, Arnold JJ, Killingsworth MC, Sarks JP. Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br J Ophthalmol*. 1999;83:358-368.

56. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. *Prog Retin Eye Res*. 2001;20:385-414.

57. Grossniklaus HE, Ling JX, Wallace TM, et al. Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. *Mol Vis*. 2002;8:119-126.

58. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385-389.

59. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419-421.

60. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet*. 2006;38:458-462.

61. Yates JRW, Sepp T, Matharu BK, et al. Complement C3 Variant and the Risk of Age-Related Macular Degeneration. *N Engl J Med*. 2007;357:553-561.

62. Radu RA, Hu J, Jiang Z, Bok D. Bisretinoid-mediated complement activation on retinal pigment epithelial cells is dependent on complement factor H haplotype. *J Biol Chem*. 2014;289:9113-9120.

63. Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between C-reactive protein and age-related macular degeneration. *JAMA*. 2004;291:704-710.

64. Shankar A, Mitchell P, Rochtchina E, Tan J, Wang JJ. Association between circulating white blood cell count and long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Am J Epidemiol*. 2007;165:375-382.

65. Kurtul BE, Ozer PA. The Relationship between Neutrophil-to-lymphocyte Ratio and Age-related Macular Degeneration. *Korean J Ophthalmol*. 2016;30:377-381.

66. Lechner J, Chen M, Hogg RE, et al. Higher plasma levels of complement C3a, C4a and C5a increase the risk of subretinal fibrosis in neovascular age-related macular degeneration: Complement activation in AMD. *Immun Ageing*. 2016;13:4.

67. Scholl HP, Charbel IP, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS ONE*. 2008;3:e2593.

68. Nassar K, Grisanti S, Elfar E, Luke J, Luke M, Grisanti S. Serum cytokines as biomarkers for age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:699-704.

69. Kur J, Newman EA, Chan-Ling T. Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease. *Prog Retin Eye Res*. 2012;31:377-406.

70. Harris A, Chung HS, Ciulla TA, Kagemann L. Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog Retin Eye Res*. 1999;18:669-687.

71. Friedman E. A hemodynamic model of the pathogenesis of age-related macular degeneration. *American Journal of Ophthalmology*. 1997;124:677-682.

72. Ciulla TA, Harris A, Kagemann L, et al. Choroidal perfusion perturbations in non-neovascular age related macular degeneration. *Br J Ophthalmol*. 2002;86:209-213.

73. Uretmen O, Akkin C, Erakgun T, Killi R. Color Doppler imaging of choroidal circulation in patients with asymmetric age-related macular degeneration. *Ophthalmologica*. 2003;217:137-142.

74. Ciulla TA, Harris A, Chung HS, et al. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. *Am J Ophthalmol*. 1999;128:75-80.

75. Grunwald JE, Metelitsina TI, Dupont JC, Ying GS, Maguire MG. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. *Invest Ophthalmol Vis Sci*. 2005;46:1033-1038.

76. Metelitsina TI, Grunwald JE, Dupont JC, Ying GS, Brucker AJ, Dunaief JL. Foveolar choroidal circulation and choroidal neovascularization in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2008;49:358-363.

77. Boltz A, Luksch A, Wimpissinger B, et al. Choroidal blood flow and progression of age-related macular degeneration in the fellow eye in patients with unilateral choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2010;51:4220-4225.

78. Ramrattan RS, Vanderschaft TL, Mooy CM, Debruijn WC, Mulder PGH, Dejong PTVM. Morphometric Analysis of Bruchs Membrane, the Choriocapillaris, and the Choroid in Aging. *Investigative Ophthalmology & Visual Science*. 1994;35:2857-2864.

79. Manjunath V, Goren J, Fujimoto JG, Duker JS. Analysis of choroidal thickness in age-related macular degeneration using spectral-domain optical coherence tomography. *Am J Ophthalmol*. 2011;152:663-668.

80. Sigler EJ, Randolph JC. Comparison of macular choroidal thickness among patients older than age 65 with early atrophic age-related macular degeneration and normals. *Invest Ophthalmol Vis Sci*. 2013;54:6307-6313.

81. Jonas JB, Forster TM, Steinmetz P, Schlichtenbrede FC, Harder BC. Choroidal thickness in age-related macular degeneration. *Retina*. 2014;34:1149-1155.

82. Yiu G, Chiu SJ, Petrou PA, et al. Relationship of central choroidal thickness with age-related macular degeneration status. *Am J Ophthalmol*. 2015;159:617-626.

83. Ratnapriya R, Chew EY. Age-related macular degeneration-clinical review and genetics update. *Clin Genet*. 2013;84:160-166.

84. Sobrin L, Seddon JM. Nature and nurture- genes and environment- predict onset and progression of macular degeneration. *Prog Retin Eye Res*. 2014;40:1-15.

85. Smith W, Mitchell P. Family history and age-related maculopathy: the Blue Mountains Eye Study. *Aust N Z J Ophthalmol*. 1998;26:203-206.

86. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol*. 1997;123:199-206.

87. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol*. 2010;10:31.:31.

88. Vingerling JR, Hofman A, Grobbee DE, Dejong PTVM. Age-related macular degeneration and smoking - The Rotterdam study. *Arch Ophthalmol*. 1996;114:1193-1196.

89. Smith W, Mitchell P, Leeder SR. Smoking and age-related maculopathy - The Blue Mountains Eye Study. *Arch Ophthalmol*. 1996;114:1518-1523.

90. Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. Smoking and age-related macular degeneration: a review of association. *Eye (Lond)*. 2005;19:935-944.

91. Nolan JM, Stack J, O' DO, Loane E, Beatty S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res*. 2007;84:61-74.

92. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. *Ophthalmology*. 2000;107:2224-2232.

93. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL, III. Risk factors for the incidence of Advanced Age-Related Macular Degeneration in the Age-Related Eye Disease Study (AREDS) AREDS report no. 19. *Ophthalmology*. 2005;112:533-539.

94. Peeters A, Magliano DJ, Stevens J, Duncan BB, Klein R, Wong TY. Changes in abdominal obesity and age-related macular degeneration: the Atherosclerosis Risk in Communities Study. *Arch Ophthalmol*. 2008;126:1554-1560.

95. Adams MK, Simpson JA, Aung KZ, et al. Abdominal obesity and age-related macular degeneration. *Am J Epidemiol*. 2011;173:1246-1255.

96. Zhang QY, Tie LJ, Wu SS, et al. Overweight, Obesity, and Risk of Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*. 2016;57:1276-1283.

97. Bressler SB, Munoz B, Solomon SD, West SK. Racial differences in the prevalence of age-related macular degeneration: the Salisbury Eye Evaluation (SEE) Project. *Arch Ophthalmol*. 2008;126:241-245.

98. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 2003;110:1273-1280.

99. Tan JS, Mitchell P, Smith W, Wang JJ. Cardiovascular risk factors and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology*. 2007;114:1143-1150.

100. Coleman AL, Seitzman RL, Cummings SR, et al. The association of smoking and alcohol use with age-related macular degeneration in the oldest old: the Study of Osteoporotic Fractures. *Am J Ophthalmol*. 2010;149:160-169.

101. Adams MK, Chong EW, Williamson E, et al. 20/20--Alcohol and age-related macular degeneration: the Melbourne Collaborative Cohort Study. *Am J Epidemiol*. 2012;176:289-298.

102. Fraser-Bell S, Wu J, Klein R, Azen SP, Varma R. Smoking, alcohol intake, estrogen use, and age-related macular degeneration in Latinos: the Los Angeles Latino Eye Study. *Am J Ophthalmol*. 2006;141:79-87.

103. Chong EW, Kreis AJ, Wong TY, Simpson JA, Guymer RH. Alcohol consumption and the risk of age-related macular degeneration: a systematic review and meta-analysis. *Am J Ophthalmol*. 2008;145:707-715.

104. Ritter LL, Klein R, Klein BE, Mares-Perlman JA, Jensen SC. Alcohol use and age-related maculopathy in the Beaver Dam Eye Study. *Am J Ophthalmol*. 1995;120:190-196.

105. Knudtson MD, Klein R, Klein BE. Alcohol consumption and the 15-year cumulative incidence of age-related macular degeneration. *Am J Ophthalmol*. 2007;143:1026-1029.

106. Smith W, Mitchell P. Alcohol intake and age-related maculopathy. *Am J Ophthalmol*. 1996;122:743-745.

107. Cho E, Hankinson SE, Willett WC, et al. Prospective study of alcohol consumption and the risk of age-related macular degeneration. *Arch Ophthalmol*. 2000;118:681-688.

108. Ajani UA, Christen WG, Manson JE, et al. A prospective study of alcohol consumption and the risk of age related macular degeneration. *Annals of Epidemiology*. 1999;9:172-177.

109. Boekhoorn SS, Vingerling JR, Hofman A, de Jong PT. Alcohol consumption and risk of aging macula disorder in a general population: the Rotterdam Study. *Arch Ophthalmol*. 2008;126:834-839.

110. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994;272:1413-1420.

111. Ma L, Dou HL, Wu YQ, et al. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. *Br J Nutr*. 2012;107:350-359.

112. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology*. 2008;115:334-341.

113. SanGiovanni JP, Chew EY, Clemons TE, et al. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch Ophthalmol*. 2007;125:1225-1232.

114. Chew EY, Clemons T, SanGiovanni JP, et al. The Age-Related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 report number 1). *Ophthalmology*. 2012;119:2282-2289.

115. Age Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309:2005-2015.

116. Chew EY, Clemons TE, SanGiovanni JP, et al. Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression: AREDS2 Report No. 3. *JAMA Ophthalmol*. 2013.

117. Wu J, Cho E, Willett WC, Sastry SM, Schaumberg DA. Intakes of Lutein, Zeaxanthin, and Other Carotenoids and Age-Related Macular Degeneration During 2 Decades of Prospective Follow-up. *JAMA Ophthalmol*. 2015;133:1415-1424.

118. Snodderly DM, Brown PK, Delori FC, Auran JD. The Macular Pigment .1. Absorbance Spectra, Localization, and Discrimination from Other Yellow Pigments in Primate Retinas. *Investigative Ophthalmology & Visual Science*. 1984;25:660-673.

119. Trieschmann M, van Kuijk FJ, Alexander R, et al. Macular pigment in the human retina: histological evaluation of localization and distribution. *Eye (Lond)*. 2008;22:132-137.

120. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Investigative Ophthalmology & Visual Science*. 1984;25:674-685.

121. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC - Retinal distribution and age study. *Investigative Ophthalmology & Visual Science*. 1988;29:843-849.

122. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Experimental Eye Research*. 1997;64:211-218.

123. Nebeling LC, Forman MR, Graubard BI, Snyder RA. Changes in carotenoid intake in the United States: The 1987 and 1992 National Health Interview Surveys. *Journal of the American Dietetic Association*. 1997;97:991-996.

124. Nebeling LC, Forman MR, Graubard BI, Snyder RA. The impact of lifestyle characteristics on carotenoid intake in the United States: The 1987 National Health Interview Survey. *Am J Public Health*. 1997;87:268-271.

125. Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol*. 1998;82:907-910.

126. Perry A, Rasmussen H, Johnson EJ. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. *Journal of Food Composition and Analysis*. 2009;22:9-15.

127. Maoka T, Arai A, Shimizu M, Matsuno T. The first isolation of enantiomeric and meso-zeaxanthin in nature. *Comp Biochem Physiol B*. 1986;83:121-124.

128. Nolan JM, Beatty S, Meagher KA, Howard AN, Kelly D, Thurnham DI. Verification of -Zeaxanthin in Fish. *J Food Process Technol*. 2014;5:335.

129. Rasmussen HM, Muzhingi T, Eggert EMR, Johnson EJ. Lutein, zeaxanthin, meso-zeaxanthin content in egg yolk and their absence in fish and seafood. *Journal of Food Composition and Analysis*. 2012;27:139-144.

130. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Investigative Ophthalmology Visual Science*. 2005;46:692-702.

131. Bhosale P, Serban B, Zhao dY, Bernstein PS. Identification and metabolic transformations of carotenoids in ocular tissues of the Japanese quail Coturnix japonica. *Biochemistry*. 2007;46:9050-9057.

132. Gorusupudi A, Shyam R, Li B, et al. Developmentally Regulated Production of meso-Zeaxanthin in Chicken Retinal Pigment Epithelium/Choroid and Retinameso-Zeaxanthin Formation in the Chicken Eye. *Investigative Ophthalmology & Visual Science*. 2016;57:1853-1861.

133. Howells O, Eperjesi F, Bartlett H. Measuring macular pigment optical density in vivo: a review of techniques. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:315-347.

134. Junghans A, Sies H, Stahl W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch Biochem Biophys*. 2001;391:160-164.

135. Wu J, Seregard S, Algvere PV. Photochemical damage of the retina. *Surv Ophthalmol*. 2006;51:461-481.

136. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. *Archives of Biochemistry and Biophysics*. 1999;371:301-307.

137. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the Human Macular Carotenoids. *Investigative Ophthalmology & Visual Science*. 1993;34:2033-2040.

138. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010.

139. Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. *Invest Ophthalmol Vis Sci*. 2013;54:1781-1788.

140. Tian Y, Kijlstra A, Renes J, Wabitsch M, Webers CA, Berendschot TT. Lutein Leads to a Decrease of Factor D Secretion by Cultured Mature Human Adipocytes. *J Ophthalmol*. 2015;2015:430741.

141. Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. *Mol Nutr Food Res*. 2011:10.

142. Musch DC. Evidence for including lutein and zeaxanthin in oral supplements for age-related macular degeneration. *JAMA Ophthalmol*. 2014;132:139-141.

143. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. *Vision Res*. 1989;29:1095-1101.

144. Schultze M. *Ueber den gelben Fleck der Retina, seinen Einfluss auf normales Sehen und auf Farbenblindheit*: Cohen & Sohn; 1866.

145. Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Prog Retin Eye Res*. 2002;21:225-240.

146. Stringham JM, Hammond BR, Jr. The glare hypothesis of macular pigment function. *Optom Vis Sci*. 2007;84:859-864.

147. Reading VM, Weale RA. Macular Pigment and Chromatic Aberration. *J Opt Soc Am A*. 1974;64:231-234.

148. Loughman J, Davison PA, Nolan JM, Akkali MC, Beatty S. Macular pigment and its contribution to visual performance and experience. *Journal of Optometry*. 2010;3:74-90.

149. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902-912.

150. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111-123.

151. Liu R, Wang T, Zhang B, et al. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:252-258.

152. Richer S, Stiles W, Statkute L, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry*. 2004;75:216-230.

153. Bartlett HE, Eperjesi F. Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. *Eur J Clin Nutr*. 2007;61:1121-1127.

154. Weigert G, Kaya S, Pemp B, et al. Effects of lutein supplementation on macular pigment optical density and visual acuity in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:8174-8178.

155. Richer SP, Stiles W, Graham-Hoffman K, et al. Randomized, double-blind, placebo-controlled study of zeaxanthin and visual function in patients with atrophic age-related macular degeneration: the Zeaxanthin and Visual Function Study (ZVF) FDA IND #78, 973. *Optometry*. 2011;82:667-680.

156. Piermarocchi S, Saviano S, Parisi V, et al. Carotenoids in Age-related Maculopathy Italian Study (CARMIS): two-year results of a randomized study. *Eur J Ophthalmol*. 2011;22:216-225.

157. Ma L, Yan SF, Huang YM, et al. Effect of Lutein and Zeaxanthin on Macular Pigment and Visual Function in Patients with Early Age-Related Macular Degeneration. *Ophthalmology*. 2012;119:2290-2297.

158. Beatty S, Chakravarthy U, Nolan JM, et al. Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology*. 2013;120:600-606.

159. Berrow EJ, Bartlett HE, Eperjesi F, Gibson JM. The effects of a lutein-based supplement on objective and subjective measures of retinal and visual function in eyes with age-related maculopathy -- a randomised controlled trial. *Br J Nutr*. 2013;109:2008-2014.

160. Dawczynski J, Jentsch S, Schweitzer D, Hammer M, Lang GE, Strobel J. Long term effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical density of macular pigment in AMD patients: the LUTEGA study. *Graefes Arch Clin Exp Ophthalmol*. 2013;251:2711-2723.

161. Garcia-Layana A, Recalde S, Alaman AS, Robredo PF. Effects of lutein and docosahexaenoic Acid supplementation on macular pigment optical density in a randomized controlled trial. *Nutrients*. 2013;5:543-551.

162. Huang YM, Dou HL, Huang FF, Xu XR, Zou ZY, Lin XM. Effect of supplemental lutein and zeaxanthin on serum, macular pigmentation, and visual performance in patients with early age-related macular degeneration. *Biomed Res Int*. 2015;2015:564738.

163. Kearney PM, Cronin H, O'Regan C, et al. Cohort profile: the Irish Longitudinal Study on Ageing. *Int J Epidemiol*. 2011;40:877-884.

164. Klein R, Chou CF, Klein BE, Zhang X, Meuer SM, Saaddine JB. Prevalence of age-related macular degeneration in the US population. *Arch Ophthalmol*. 2011;129:75-80.

165. Cronin H, O'Regan C, Finucane C, Kearney P, Kenny RA. Health and aging: development of the Irish Longitudinal Study on Ageing health assessment. *J Am Geriatr Soc*. 2013;61 Suppl 2:S269-S278.

166. R Development Core Team. R: A Language and Environment for Statistical Computing. In: Vienna, Austria: R Foundation for Statistical Computing; 2008.

167. Central Statistics Office. Census 2011. In: 2012.

168. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33:159-174.

169. Rudnicka AR, Jarrar Z, Wormald R, Cook DG, Fletcher A, Owen CG. Age and gender variations in age-related macular degeneration prevalence in populations of European ancestry: a meta-analysis. *Ophthalmology*. 2012;119:571-580.

170. Andersen MV, Rosenberg T, la CM, et al. Prevalence of age-related maculopathy and age-related macular degeneration among the inuit in Greenland. The Greenland Inuit Eye Study. *Ophthalmology*. 2008;115:700-707.

171. Piermarocchi S, Segato T, Scopa P, et al. The prevalence of age-related macular degeneration in Italy (PAMDI) study: report 1. *Ophthalmic Epidemiol*. 2011;18:129-136.

172. Klein R, Myers CE, Lee KE, et al. Small Drusen and Age-Related Macular Degeneration: The Beaver Dam Eye Study. *J Clin Med*. 2015;4:424-440.

173. Bressler NM, Munoz B, Maguire MG, et al. 5-Year Incidence and Disappearance of Drusen and Retinal-Pigment Epithelial Abnormalities - Waterman Study. *Arch Ophthalmol*. 1995;113:301-308.

174. Jonasson F, Arnarsson A, Sasaki H, Peto T, Sasaki K, Bird AC. The prevalence of age-related maculopathy in iceland: Reykjavik eye study. *Arch Ophthalmol*. 2003;121:379-385.

175. Börsch-Supan A, Alcser KH. *Health, ageing and retirement in Europe : first results from the survey of health, ageing and retirement in Europe*: Mannheim: Mannheim Research Institute for the Economics of Aging (MEA); 2005.

176. Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res*. 2012.

177. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871-7880.

178. Sabour-Pickett S, Beatty S, Connolly E, et al. SUPPLEMENTATION WITH THREE DIFFERENT MACULAR CAROTENOID FORMULATIONS IN PATIENTS WITH EARLY AGE-RELATED MACULAR DEGENERATION. *Retina*. 2014.

179. Meagher KA, Thurnham DI, Beatty S, et al. Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr*. 2013;110:289-300.

180. Prado-Cabrero A, Beatty S, Howard A, Stack J, Bettin P, Nolan JM. Assessment of lutein, zeaxanthin and meso-zeaxanthin concentrations in dietary supplements by chiral high-performance liquid chromatography. *European Food Research and Technology*. 2015:1-10.

181. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Investigative Ophthalmology & Visual Science*. 1999;40:2481-2489.

182. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445-453.

183. Nolan JM, Loskutova E, Howard AN, et al. Macular Pigment, Visual Function, and Macular Disease among Subjects with Alzheimer's Disease: An Exploratory Study. *Journal of Alzheimer's Disease*. 2014.

184. Thurnham DI, Tremel A, Howard AN. A supplementation study in human subjects with a combination of meso-zeaxanthin, (3R,3'R)-zeaxanthin and (3R,3'R,6'R)-lutein. *Br J Nutr*. 2008;100:1307-1314.

185. Pelli DG, Bex P. Measuring contrast sensitivity. *Vision Res*. 2013;90:10-14.

186. Sparrow JM, Dickinson AJ, Duke AM. The Wisconsin Age-related Macular Degeneration grading system: performance in an independent centre. *Ophthalmic Epidemiol*. 1997;4:49-55.

187. O'Neill ME, Carroll Y, Corridan B, et al. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition*. 2001;85:499-507.

188. O'Connell ED, Nolan JM, Stack J, et al. Diet and risk factors for age-related maculopathy. *Am J Clin Nutr*. 2008;87:712-722.

189. Bhosale P, Zhao dY, Serban B, Bernstein PS. Identification of 3-methoxyzeaxanthin as a novel age-related carotenoid metabolite in the human macula. *Invest Ophthalmol Vis Sci*. 2007;48:1435-1440.

190. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S. Transport and Retinal Capture of Lutein and Zeaxanthin with Reference to Age-related Macular Degeneration. *Surv Ophthalmol*. 2008;53:68-81.

191. Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. *Invest Ophthalmol Vis Sci*. 2013;54:1781-1788.

192. Beatty S, Nolan JM, Muldrew KA, Woodside J, Stevenson MR, Chakravarthy U. Visual outcome after antioxidant supplementation. *Ophthalmology*. 2013;120:645.

193. Beatty S, Chakravarthy U, Nolan JM, et al. Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology*. 2013;120:600-606.

194. Hogg RE, Chakravarthy U. Visual function and dysfunction in early and late age-related maculopathy. *Prog Retin Eye Res*. 2006;25:249-276.

195. Garcia-Layana A, Recalde S, Alaman AS, Robredo PF. Effects of lutein and docosahexaenoic Acid supplementation on macular pigment optical density in a randomized controlled trial. *Nutrients*. 2013;5:543-551.

196. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology of the CREST Randomized Controlled Trials. *Ophthalmic Epidemiol*. 2014;21:111-123.

197. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retin Eye Res*. 2016;50:34-66.

198. Sabour-Pickett S, Beatty S, Connolly E, et al. Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina*. 2014;34:1757-1766.

199. Crosby-Nwaobi R, Hykin P, Peto T, Sivaprasad S. An exploratory study evaluating the effects of macular carotenoid supplementation in various retinal diseases. *Clin Ophthalmol*. 2016;10:835-844.

200. Kramer CFL, Theimann J. *How many subjects?: Statistical Power Analysis in Research*: Sage Publications; 1987.

201. Orr P, Rentz AM, Margolis MK, et al. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:3354-3359.

202. Revicki DA, Rentz AM, Harnam N, Thomas VS, Lanzetta P. Reliability and validity of the National Eye Institute Visual Function Questionnaire-25 in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2010;51:712-717.

203. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol*. 2001;119:1050-1058.

204. Meagher KA, Thurnham DI, Beatty S, et al. Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr*. 2013;110:289-300.

205. Charalampidou S, Nolan J, Loughman J, et al. Psychophysical impact and optical and morphological characteristics of symptomatic non-advanced cataract. *Eye (Lond)*. 2011;25:1147-1154.

206. Hohberger B, Laemmer R, Adler W, Juenemann AG, Horn FK. Measuring contrast sensitivity in normal subjects with OPTEC 6500: influence of age and glare. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1805-1814.

207. Hitchcock EM, Dick RB, Krieg EF. Visual contrast sensitivity testing: a comparison of two F.A.C.T. test types. *Neurotoxicol Teratol*. 2004;26:271-277.

208. Terzi E, Buhren J, Wesemann W, Kohnen T. [Frankfurt-Freiburg Contrast and Acuity Test System (FF-CATS). A new test to determine contrast sensitivity under variable ambient and glare luminance levels]. *Ophthalmologe*. 2005;102:507-513.

209. Coppens JE, Franssen L, Van Rijn LJ, van den Berg TJ. Reliability of the compensation comparison stray-light measurement method. *J Biomed Opt*. 2006;11:34027.

210. Franssen L, Coppens JE, van den Berg TJ. Compensation comparison method for assessment of retinal straylight. *Invest Ophthalmol Vis Sci*. 2006;47:768-776.

211. van den Berg TJ, Van Rijn LJ, Michael R, et al. Straylight effects with aging and lens extraction. *Am J Ophthalmol*. 2007;144:358-363.

212. Stifter E, Konig F, Lang T, et al. Reliability of a standardized reading chart system: variance component analysis, test-retest and inter-chart reliability. *Graefes Arch Clin Exp Ophthalmol*. 2004;242:31-39.

213. Wolf-Schnurrbusch UE, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci*. 2009;50:3432-3437.

214. Feinman RD. Intention-to-treat. What is the question? *Nutr Metab (Lond)*. 2009;6:1.

215. Gupta SK. Intention-to-treat concept: A review. *Perspect Clin Res*. 2011;2:109-112.

216. Newell DJ. Intention-to-treat analysis: implications for quantitative and qualitative research. *Int J Epidemiol*. 1992;21:837-841.

217. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials*. 2010;11:32.

218. Akuffo KO, Nolan JM, Peto T, et al. Relationship between macular pigment and visual function in subjects with early age-related macular degeneration. LID -

bjophthalmol-2016-308418 [pii] LID - 10.1136/bjophthalmol-2016-308418 [doi]. *Br J Ophthalmol*. 2016.

219. Midena E, Degli AC, Blarzino MC, Valenti M, Segato T. Macular function impairment in eyes with early age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1997;38:469-477.

220. Brown B, Lovie-Kitchin J. Contrast sensitivity in central and paracentral retina in age related maculopathy. *Clinical and Experimental Optometry*. 1987;70:145-148.

221. Kleiner RC, Enger C, Alexander MF, Fine SL. Contrast sensitivity in age-related macular degeneration. *Arch Ophthalmol*. 1988;106:55-57.

222. Stangos N, Voutas S, Topouzis F, Karampatakis V. Contrast sensitivity evaluation in eyes predisposed to age-related macular degeneration and presenting normal visual acuity. *Ophthalmologica*. 1995;209:194-198.

223. Brown B. Reaction times to edges of variable sharpness in age-related maculopathy. *Am J Optom Physiol Opt*. 1986;63:362-365.

224. Brown B, Garner LF. Effects of luminance on contrast sensitivity in senile macular degeneration. *Am J Optom Physiol Opt*. 1983;60:788-793.

225. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1236-1249.

226. Hammond BR, Jr., Fletcher LM. Influence of the dietary carotenoids lutein and zeaxanthin on visual performance: application to baseball. *Am J Clin Nutr*. 2012;96:1207S-1213S.

227. Fletcher LM, Engles M, Hammond BR, Jr. Visibility through atmospheric haze and its relation to macular pigment. *Optom Vis Sci*. 2014;91:1089-1096.

228. Hammond BR, Jr., Wooten BR, Engles M, Wong JC. The influence of filtering by the macular carotenoids on contrast sensitivity measured under simulated blue haze conditions. *Vision Res*. 2012;63:58-62.

229. KUFFLER SW. Discharge patterns and functional organization of mammalian retina. *J Neurophysiol*. 1953;16:37-68.

230. HUBEL DH, WIESEL TN. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J Physiol*. 1962;160:106-154.

231. WESTHEIMER G. Spatial interaction in human cone vision. *J Physiol*. 1967;190:139-154.

232. WESTHEIMER G. Spatial interaction in the human retina during scotopic vision. *J Physiol*. 1965;181:881-894.

233. Bhosale P, Serban BF, Bernstein PS. Retinal carotenoids can attenuate formation of A2E in the retinal pigment epithelium.

234. Kim SR, Nakanishi K, Itagaki Y, Sparrow JR. Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. *Exp Eye Res*. 2006;82:828-839.

235. Moiseyev G, Nikolaeva OF, Chen YF, Farjo KF, Takahashi YF, Ma JX. Inhibition of the visual cycle by A2E through direct interaction with RPE65 and implications in Stargardt disease.

236. Vos JJ. Disability glare - A state of the art report. *Commission Internationale de l'Eclairage Journal*. 1984;3:39-53.

237. Mainster MA, Turner PL. Glare's causes, consequences, and clinical challenges after a century of ophthalmic study. *Am J Ophthalmol*. 2012;153:587-593.

238. Scilley K, Jackson GR, Cideciyan AV, Maguire MG, Jacobson SG, Owsley C. Early age-related maculopathy and self-reported visual difficulty in daily life. *Ophthalmology*. 2002;109:1235-1242.

239. SEVERIN SL, HARPER JY, Jr., CULVER JF. PHOTOSTRESS TEST FOR THE EVALUATION OF MACULAR FUNCTION. *Arch Ophthalmol*. 1963;70:593-597.

240. Owsley C, McGwin G, Jr., Clark ME, et al. Delayed Rod-Mediated Dark Adaptation Is a Functional Biomarker for Incident Early Age-Related Macular Degeneration. *Ophthalmology*. 2016;123:344-351.

241. Curcio CA, Johnson M, Rudolf M, Huang JD. The oil spill in ageing Bruch membrane. *Br J Ophthalmol*. 2011;95:1638-1645.

242. Dhalla MS, Fantin A, Blinder KJ, Bakal JA. The macular automated photostress test. *Am J Ophthalmol*. 2007;143:596-600.

243. Wu G, Weiter JJ, Santos S, Ginsburg L, Villalobos R. The macular photostress test in diabetic retinopathy and age-related macular degeneration. *Arch Ophthalmol*. 1990;108:1556-1558.

244. Legge GE, Rubin GS, Luebker A. Psychophysics of reading--V. The role of contrast in normal vision. *Vision Res*. 1987;27:1165-1177.

245. Renzi LM, Hammond BR, Jr. The relation between the macular carotenoids, lutein and zeaxanthin, and temporal vision. *Ophthalmic Physiol Opt*. 2010;30:351-357.

246. Bovier ER, Hammond BR. A randomized placebo-controlled study on the effects of lutein and zeaxanthin on visual processing speed in young healthy subjects. *Arch Biochem Biophys*. 2015;572:54-57.

247. Bovier ER, Renzi LM, Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on neural processing speed and efficiency. *PLoS ONE*. 2014;9:e108178.

248. Stringham NT, Stringham JM. Temporal Visual Mechanisms May Mediate Compensation for Macular Pigment. *Perception*. 2015;44:1400-1415.

249. Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly. *Am J Clin Nutr*. 2012;96:1161S-1165S.

250. Vishwanathan R, Neuringer M, Snodderly DM, Schalch W, Johnson EJ. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutr Neurosci*. 2013;16:21-29.

251. Vishwanathan R, Schalch W, Johnson EJ. Macular pigment carotenoids in the retina and occipital cortex are related in humans. *Nutr Neurosci*. 2016;19:95-101.

252. Vishwanathan R, Iannaccone A, Scott TM, et al. Macular pigment optical density is related to cognitive function in older people. *Age Ageing*. 2014;43:271-275.

253. Feeney J, Finucane C, Savva GM, et al. Low macular pigment optical density is associated with lower cognitive performance in a large, population-based sample of older adults. *Neurobiol Aging*. 2013;34:2449-2456.

254. Renzi LM, Dengler MJ, Puente A, Miller LS, Hammond BR, Jr. Relationships between macular pigment optical density and cognitive function in unimpaired and mildly cognitively impaired older adults. *Neurobiol Aging*. 2014;35:1695-1699.

255. Kelly D, Coen RF, Akuffo KO, et al. Cognitive Function and Its Relationship with Macular Pigment Optical Density and Serum Concentrations of its Constituent Carotenoids. *J Alzheimers Dis*. 2015;48:261-277.

256. Nolan JM, Loskutova E, Howard A, et al. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. *J Alzheimers Dis*. 2015;44:1157-1169.

257. Maguire M. Baseline characteristics, the 25-Item National Eye Institute Visual Functioning Questionnaire, and their associations in the Complications of Age-Related Macular Degeneration Prevention Trial (CAPT). *Ophthalmology*. 2004;111:1307-1316.

258. Choudhury F, Varma R, Klein R, Gauderman WJ, Azen SP, McKean-Cowdin R. Age-Related Macular Degeneration and Quality of Life in Latinos: The Los Angeles Latino Eye Study. *JAMA Ophthalmol*. 2016;134:683-690.

259. Chong EW, Wang Y, Robman LD, et al. Age Related Macular Degeneration and Total Hip Replacement Due to Osteoarthritis or Fracture: Melbourne Collaborative Cohort Study. *PLoS ONE*. 2015;10:e0137322.

260. Loriaut P, Loriaut P, Boyer P, Massin P, Cochereau I. Visual impairment and hip fractures: a case-control study in elderly patients. *Ophthalmic Res*. 2014;52:212-216.

261. Wysong A, Lee PP, Sloan FA. Longitudinal incidence of adverse outcomes of age-related macular degeneration. *Arch Ophthalmol*. 2009;127:320-327.

262. Wang MY, Rousseau J, Boisjoly H, et al. Activity limitation due to a fear of falling in older adults with eye disease. *Invest Ophthalmol Vis Sci*. 2012;53:7967-7972.

263. Nguyen AM, Arora KS, Swenor BK, Friedman DS, Ramulu PY. Physical activity restriction in age-related eye disease: a cross-sectional study exploring fear of falling as a potential mediator. *BMC Geriatr*. 2015;15:64.

264. Scott IU, Feuer WJ, Jacko JA. Impact of visual function on computer task accuracy and reaction time in a cohort of patients with age-related macular degeneration. *Am J Ophthalmol*. 2002;133:350-357.

Publications and Presentations

Below, I present my published first author peer-reviewed scientific papers:

1. **Akuffo KO**, Nolan JM, Stack J, Power R, Kirwan C, Moran R, Corcoran L, Owens N, Beatty S. *The Impact of Cataract, and Its Surgical Removal, on Measures of Macular Pigment Using the Heidelberg Spectralis HRA+OCT MultiColor Device.* Invest Ophthalmol Vis Sci. 2016
2. **Akuffo KO,** Nolan JM, Peto T, Stack J, Leung I, Corcoran L, Beatty S. *Relationship between macular pigment and visual function in subjects with early age-related macular degeneration.* Br J Ophthalmol. 2016
3. **Akuffo KO**, Beatty S, Stack J, Peto T, Leung I, Corcoran L, Power R, Nolan JM. *Concordance of Macular Pigment Measurement Using Customized Heterochromatic Flicker Photometry and Fundus Autofluorescence in Age-Related Macular Degeneration.* Invest Ophthalmol Vis Sci. 2015
4. **Akuffo KO**, Nolan JM, Howard AN, Moran R, Stack J, Klein R, Klein BE, Meuer SM, Sabour-Pickett S, Thurnham DI, Beatty S. *Sustained supplementation and monitored response with differing carotenoid formulation in early age-related macular degeneration.* Eye (Lond). 2015
5. **Akuffo KO**, Nolan J, Stack J, Moran R, Feeney J, Kenny RA, Peto T, Dooley C, O'Halloran AM, Cronin H, Beatty S. *Prevalence of age-related macular degeneration in the Republic of Ireland.* Br J Ophthalmol. 2015
6. **Akuffo KO**, Beatty S, Stack J, Dennison J, O'Regan S, Meagher KA, Peto T, Nolan J. *Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials.* Ophthalmic Epidemiol. 2014

Below, I present my other published peer-reviewed scientific papers:

1. Moran R, Nolan JM, Stack J, O'Halloran AM, Feeney J, **Akuffo KO**, Kenny RA, Beatty S. *Non-dietary correlates and determinants of plasma lutein and zeaxanthin concentrations in the Irish population.* J Nutr Health Aging 2016
2. Ní Bhuachalla B, McGarrigle CA, **Akuffo KO**, Peto T, Beatty S, Kenny RA. *Phenotypes of orthostatic blood pressure behaviour and association with visual acuity.* Clin Auton Res. 2015
3. Kelly D, Coen RF, **Akuffo KO**, Beatty S, Dennison J, Moran R, Stack J, Howard AN, Mulcahy R, Nolan JM. *Cognitive function and its relationship with macular pigment optical density and serum concentrations of its constituent carotenoids.* J Alzheimers Dis. 2015
4. Nolan JM, Loskutova E, Howard A, Mulcahy R, Moran R, Stack J, Bolger M, Coen RF, Dennison J, **Akuffo KO**, Owens N, Power R, Thurnham D, and Beatty S., *The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial.* J Alzheimers Dis. 2015
5. Sabour-Pickett S, Beatty S, Connolly E, Loughman J, Stack J, Howard A, Klein R, Klein BE, Meuer SM, Myers CE, **Akuffo KO**, Nolan JM. *Supplementation with Three Different Macular Carotenoid Formulations in Patients with Early Age-Related Macular Degeneration.* Retina 2014
6. Nolan, J. M., Loskutova, E., Howard, A. N., Moran, R., Mulcahy, R., Stack, J., Bolger M., Dennison J., **Akuffo K.O.**, Owens N., Thurnham D.I., and Beatty, S., *Macular Pigment, Visual Function, and Macular*

Disease among Subjects with Alzheimer's Disease: An Exploratory Study. J Alzheimers Dis. 2014

7. Smolarek-Kasprzak P, Nolan JM, Beatty S, Dennison J, **Akuffo KO**, Kuchling R, Stack J, O'Regan G. *Measuring Visual Function Using the MultiQuity System: Comparison with an Established Device.* J Ophthalmol. 2014

Below, I present my scientific papers currently in preparation:

1. **Kwadwo Owusu Akuffo,** Stephen Beatty, Tunde Peto, Jim Stack, Jim Stringham, David Kelly, Irene Leung, Laura Corcoran, and John M. Nolan, *The impact of supplemental antioxidants on visual function in non-advanced age-related macular degeneration: a head-to-head randomized clinical trial of two formulations.*
2. **Kwadwo Owusu Akuffo,** Robert F. Coen, John M. Nolan, Tunde Peto, Jim Stack, Laura Corcoran, David Kelly, Irene Leung, Rebecca Power, and Stephen Beatty, *Macular carotenoid and co-antioxidant supplementation on cognitive function in early age-related macular degeneration: CREST randomized clinical trial*.

Below, I present my non-peer reviewed publications:

1. **Kwadwo Owusu Akuffo**, Stephen Beatty, John Nolan. Macular pigment and its role in the prevention and retardation of age-related macular degeneration [published in spring edition Oculus (magazine of the Partially Sighted Society)]

2. **Kwadwo Owusu Akuffo**, Stephen Beatty, John Nolan. The importance of nutrition in the prevention and retardation of age-related macular degeneration [NCBI (National Council for the Blind of Ireland) News published in spring edition]

Below, I present my contributions at scientific conferences in my research field:

1. **Kwadwo O. Akuffo**, Jessica L. Dennison, Sarah O'Regan, Stephen Beatty, John M. Nolan, *Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology*, Association for Research in Vision and Ophthalmology Annual Meeting 2013, Seattle Washington, USA
2. **Kwadwo Owusu Akuffo**, John Nolan, Rachel Moran, Tunde Peto, Stephen Beatty, *Prevalence of sight-threatening ocular pathology in Ireland: The Irish Longitudinal Study on Ageing*, TILDA Scientific Advisory Board Meeting 2013, Trinity College, Dublin
3. **Kwadwo Owusu Akuffo**, Rachel Moran, Stephen Beatty, Tunde Peto, John Nolan, Rose Anne Kenny, Hilary Cronin, *Prevalence of Sight-Threatening Ocular Pathology in Ireland: The Irish Longitudinal Study on Ageing*, Macular Carotenoid Conference 2013, Downing College, Cambridge, UK
4. Beatty S., **Akuffo KO.,** Stack J., Howard AN., Nolan JM., *Supplementation with Three Different Macular Carotenoid Formulations in Patients with Early AMD: Most Report 2*, Macular Carotenoid Conference 2013, Downing College, Cambridge, UK

5. Nolan J., Stack J., **Akuffo K,** Howard A., Beatty S., *Supplementation with three different macular carotenoid formulations in patients with early AMD: Results after 36 months of supplementation*, International Carotenoid Symposium 2014, Park City Resort, Salt Lake, Utah, USA
6. Moran R., Johnson E., Stack J., **Akuffo K.,** Loskutova E., Beatty S., Nolan J., *The relationship between dietary intake of lutein(L), zeaxanthin(Z) and their concentrations in serum: Introduction of a novel L/Z dietary screener*, International Carotenoid Symposium 2014, Park City Resort, Salt Lake, Utah, USA
7. **Kwadwo O. Akuffo**, Jim Stack, Stephen Beatty, Tunde Peto, Irene Leung, John M. Nolan, *Macular pigment and visual function in early age-related macular degeneration: CREST randomized clinical trial*, Waterford Institute of Technology Research Day 2015, Main Auditorium, Cork Road, Waterford, Ireland
8. **Akuffo, K.**, Stack, J., Nolan, J.M., Peto, T., Leung, I., Corcoran, L., Power, R., Beatty, S., *Comparison between macular pigment measurement using customized heterochromatic flicker photometry and dual-wave autofluorescence in early age-related macular degeneration*, Macular Carotenoid Conference 2015, Downing College, Cambridge, UK
9. Power, R., Kelly, D., Coen, R.F., **Akuffo, K.**, Beatty, S., Dennison, J., Moran, R., Stack, J., Howard, A.N., Mulcahy, R., Nolan, J.M. *The relationship between macular pigment optical density and cognitive function in the CREST study*, Macular Carotenoid Conference 2015, Downing College, Cambridge, UK

10. Moran, R., Stack, J., O'Halloran, A.M., **Akuffo, K.**, Kenny, R.A., Feeney, J., Cronin, H., Beatty, S., Nolan, J.M. *Non-dietary determinants of plasma lutein and zeaxanthin concentrations in the Irish population*, Macular Carotenoid Conference 2015, Downing College, Cambridge, UK
11. Kuchling, R., O'Regan, G., Nolan, J.M., Dennison, J., **Akuffo, K.**, Stack, J., Beatty, S. *A comparison of visual acuity and contrast sensitivity measurements obtained using a computer generated algorithmic test (Multiquity®) and a conventional computer display system (Thompson test chart 2000 Xpert)*, Macular Carotenoid Conference 2015, Downing College, Cambridge, UK
12. Nolan, J.M., Power, R., Stack, J., **Akuffo, K.**, Dennison, J., Kelly, D., Corcoran, L., Peto, T., Beatty, S., *Central Retinal Enrichment Supplementation Trials (CREST): REPORT 1*, Macular Carotenoid Conference 2015, Downing College, Cambridge, UK

APPENDICES

Appendix A: TILDA Ethical Approval

THE UNIVERSITY OF DUBLIN
TRINITY COLLEGE

SCHOOL OF MEDICINE
FACULTY OF HEALTH SCIENCES

Trinity College, Dublin 2, Ireland
Tel: +353 1 896 1476
Fax: +353 1 671 3956
email: medicine@tcd.ie
email: medschadmin@tcd.ie

Professor Dermot Kelleher, MD, FRCPI, FRCP, F Med Sci
Head of School of Medicine
Vice Provost for Medical Affairs

Ms Fedelma McNamara
School Administrator

Professor Rose Ann Kenny
Medical Gerontology,
Trinity Centre,
St James Hospital Campus,
James St, D 8

Friday, 02 May 2008

Study Title
The Irish longitudinal study on ageing

Dear Applicant

Further to a meeting of the Faculty of Health Sciences Research Ethics Committee 2007 - 2008, I am pleased to inform you that the above project has been approved without further audit.

Yours sincerely

Dr. Orla Sheils
Chairperson
Faculty of Health Sciences Ethics Committee

Appendix B: TILDA Retinal Grading Certification

The Reading Centre

Moorfields Eye Hospital NHS Foundation Trust

Kwadwo Akuffo

Certified for Grading of Retinal Images for the TILDA Study

On 18th June 2013

Dr Tunde Peto
Head of Reading Centre

Appendix C: MOST Ethical Approval

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Waterford Regional Hospital,
Dunmore Road,
Waterford,
Ireland.

Telephone 051 848000
Fax 051 848572

RESEARCH ETHICS COMMITTEE,
HEALTH SERVICE EXECUTIVE, SOUTH EASTERN AREA.

Ms. Eithne Connolly,
Clinical Research Technician,
Institute of Vision Research,
Suite 14,
Whitfield Clinic,
Butlerstown North,
Cork Road
Waterford

Study Title: Amendment:
"Functional and Morphological and Biochemical responses to supplementation with three different macular carotenoids formulations"

Date: 10th June 2010

Dear Ms. Connolly

The Research Ethics Committee Coordinator and the Chairperson, HSE, South East reviewed the above study on the 2nd June 2010.

Expedited approval has been granted in advance of the REC meeting and constitutes full ethical approval.

In addition this study will be outlined at the next planned Research Ethics Committee Meeting for the HSE, South Eastern Area by the Research Ethics Committee Coordinator on Monday 21st June 2010 and any comments made at this meeting in relation to your study shall be communicated to you in writing.

The following documents were reviewed and approved:

1. (✓) Ethics Application Form
2. ( ) Protocol/ Research Proposal
3. (✓) Amendment
4. (✓) Participant Information Leaflet
5. (✓) Participant Consent Form
6. (✓) Recruitment Literature
7. ( ) Indemnity Form
8. ( )Investigator brochure
9. (✓)Investigator(s) CV (s)
10. ( )Investigators MDU/Insurance
11. ( )Sponsor insurance
12. ( )Funding for the study
13. ( )Patient Perception Survey

It is a requirement of the REC, HSE, South East that you send copy of your study to the Research Ethics Office on completion.

Yours sincerely,

Caroline Lamb

Ms Caroline Lamb
Research Ethics Committee Coordinator
Health Service Executive, South Eastern Area

Cc:

The Research Ethics Committee, HSE, South East is a recognized Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human use) Regulations 2004 and as such is authorized to undertake ethical review of clinical trials of all descriptions and classes for the Republic of Ireland.

The Research Ethics Committee, HSE, South East issues ethical approval on the basis of information provided. It is the responsibility of the researcher to notify the Research Ethics Office of any changes to a study to ensure that the approval is still relevant.

Appendix D: CREST AMD Ethical Approval

Waterford Institute of Technology WIT

Institiúid Teicneolaíochta Phort Láirge
Waterford, Ireland
TEL: +353-51-302000
WEB: www.wit.ie
EMAIL: info@wit.ie

Ref: 12/CLS/02

5th October, 2011.

Dr. John Nolan,
Director,
Macular Pigment Research Group,
WIT.

Dear John,

Thank you for submitting your amended documentation in relation to your project *'Enrichment of Macular Pigment and its impact on vision and blindness. CREST (Central Retinal Enrichment Supplementation)'* to the WIT Research Ethics Committee.

I am pleased to inform you that we now grant you final approval for WIT's participation in this project and we will convey this to Academic Council.

We wish you well in the work ahead.

Yours sincerely,

Dr. John Wells,
Chairperson,
Research Ethics Committee.

cc: Professor Stephen Beatty
Dr. James Loughman

Institiúid Teicneolaíochta Phort Láirge Waterford Institute *of* Technology

Port Láirge, Éire.
T: +353-51-302000
info@wit.ie

Waterford, Ireland.
T: +353-51-302000
www.wit.ie

Ref: 12/CLS/02

24th May, 2013.

Dr. John Nolan,
Director,
Macular Pigment Research Group,
Vision Research Centre,
Carriganore House,
WIT West Campus – Carriganore,
Waterford.

Dear John,

Thank you for submitting the documentation in relation to *Central Retinal Enrichment Supplementation Trials (CREST) – DSMC recommendations'* to the WIT Research Ethics Committee.

I am pleased to inform you that we reviewed your revised protocol and we are happy to grant approval for same. We will convey this to Academic Council.

Yours sincerely,

Professor John Wells,
Chairperson,
Research Ethics Committee.

cc: Professor Stephen Beatty
Dr. James Loughman

Appendix E: CREST AMD poster/leaflet

Appendix F: CREST AMD Information leaflet

CREST AMD Patient Information Leaflet
(Central Retinal Enrichment Supplementation Trials: Age-related Macular Degeneration)

Aim
The aim of CREST AMD is to investigate whether supplementation with the macular carotenoids can improve contrast sensitivity and glare disability in people who have age-related macular degeneration (AMD). In addition, we will monitor any changes in the disease over the course of the study.

Background Information
There is a yellow pigment at the retina at the back of the eye called macular pigment which is believed to be important for protecting against AMD, and for improving visual performance and comfort. Macular pigment is of dietary origin, i.e. we are not born with macular pigment but we accumulate it from eating certain fruits and vegetables. The dietary supplements used in the CREST AMD study are also a major source of macular pigment.

Study Design
CREST AMD is a double-blind, randomised, controlled clinical trial. This means that neither the volunteers enrolled nor the study investigators will know which type of supplements patients are allocated. This study aims to recruit 150 volunteers. Each volunteer will attend the Waterford Institute of Technology, West Campus, Carriganore, Waterford on five occasions over a 24 month period (at month one, month six, month 12, month 18 and month 24). Each study visit will last approximately two and a half hours. The volunteers will be asked to take either a supplement containing 10mg lutein, 10mg *meso*-zeaxanthin and 2mg zeaxanthin plus 500 mg vitamin C, 400 IU of vitamin E, 25 mg zinc and 2 mg copper or a supplement containing 10mg lutein and 2mg zeaxanthin plus 500 mg vitamin C, 400 IU of vitamin E, 25 mg zinc and 2 mg copper once a day for 24 months. The supplements will be provided free of charge by the study investigators.

The study design means that every participant will be given a supplement.

Study Visits
Informed consent
The study investigator will explain all aspects of the study to you, in addition to this information leaflet. If you agree to volunteer, you will be asked to sign an informed consent document which states that you are happy to participate in the study and that all aspects of the study have been explained to you by the study investigator.

Blood sample
A blood sample will be taken by a trained professional and analysed to measure macular pigment levels in your blood. The blood is the means by which the dietary nutrients are transported around the body.

Demographic and lifestyle questionnaire
You will be asked to complete a brief questionnaire to gather information on your demographics and lifestyle. This questionnaire will collect your contact and lifestyle details, and medical history, for analysis.

Vision tests
Various aspects of your vision will be tested using the following tests: visual acuity, contrast sensitivity, photostress recovery, light scatter and macular pigment levels. It is important to note that all the vision tests are non-invasive. These tests will measure the overall visual quality of your retina. All tests will be performed using specialised optical devices, and the results will allow the investigators to assess the functional status of the macula and identify changes over time. Feedback will be given regarding your vision status and macular pigment levels.

Study Participation
This study is entirely voluntary. You will not be paid for your participation in this study. If you decide to take part you are free to withdraw at any time and without giving a reason, and you can request that data already collected from you is not used by the investigators. This will not affect the standard of care you receive. A person who does not wish to participate will not be discriminated against in any way. Participation in this study is not intended to replace standard medical care, and is therefore for research purposes only.

Risks and/or Discomforts
We foresee no major risks to subjects participating in this research. There is of course a risk that the status of your AMD will worsen given that you have AMD. There is a designated Data Safety and Monitoring Committee established, which will review data during the study to ensure that no unforeseen risk presents as a consequence of your participation in this study.

Benefits
It is anticipated that society may benefit from the results of this study. At the end of the study, the investigators will provide you with information on the measurements performed on you during your study visits. Where possible and appropriate, the investigators will comment on how these measurements (e.g. visual acuity, blood pressure) compare to the normal levels. You will gain knowledge of your macular pigment level; research has suggested that a person's macular pigment level is a good indicator of overall eye health. You will also gain knowledge of your visual function, which is a measure of the sensitivity of your retina (back of your eye). General information on general health and eye health will be provided at the study centre for your interest.

Data Confidentially
All the data collected in this study will be treated as strictly confidential and will be obtained and processed in keeping with the Data Protection Act 1988 and the amended Data Protection Act of 2003. All data will be analysed collectively as a group and coded by data link to ensure volunteers' confidentiality.

Insurance
The study and its investigators are covered by an insurance, which protects you in case of problems directly caused by this study.

Organisers and Sponsors
Researchers at Waterford Institute of Technology, under the direction of Professor John Nolan, Principal Investigator, will be conducting and managing this study. This study is funded by the European Research Council (Reference: 281096).

Ethical Approval
Ethical approval for this study has been obtained from the Research Ethics Committee, Waterford Institute of Technology (Reference: 12/CLS/02).

Questions
The CREST Research Assistant, Sarah O'Regan, will answer any questions you may have concerning the study. Please phone **051 845505** or email soregan@wit.ie

We hope that this information has answered most of your questions. Should you have further questions or do not fully understand the information given, please feel free to ask us. The investigators who are carrying out this research would like to thank you for taking the time to read this information.

Version date: 29th August 2013

Appendix G: CREST AMD consent form

CREST AMD

(Central Retinal Enrichment Supplementation Trials: Age-related Macular Degeneration)

Date: _____ **Subject Number:** _____

- I confirm I have read and understand the Information Leaflet regarding this study. I further attest that the relevant information has been discussed fully in non-technical terms, and all my questions have been replied to with full satisfaction. ☐
- I understand that my participation is voluntary and that I am free to withdraw at any time, without my medical care or legal rights being affected. ☐
- I understand that my data concerning this study will be entered on a computer in order to be analysed together with the data obtained from other patients. My identity will always be protected. I give permission for this analysis. ☐
- I understand that responsible authorities and researchers within the Institute at Waterford may look at my data collected for this study where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
- I agree to take part in the above study and hereby give my consent to have a blood sample collected for serum analysis. ☐
- I agree for my blood sample to be stored until time of analysis. My identity will always be protected. ☐
- I have no objection to being contacted by the Waterford Institute of Technology about future research studies. ☐
- I give permission for any data collected today to be used for other research studies carried out by the Institute. ☐

_____	_____	_____
Name of Volunteer	Date	Signature of Volunteer
_____	_____	_____
Name of Witness	Date	Signature of Witness

Appendix H: CREST AMD Press release – following AREDS 2 study

BRACKEN PR

Bracken Public Relations Limited
11 Inns Court Winetavern Street Dublin 8

Tel: +353 (0)1 677 3277
Mob: + 353 (0) 86 7784296
Web: www.brackenpr.com

Research has confirmed that eye supplements prevent age-related blindness: call for volunteers

The long-awaited Age-Related Eye Disease Study 2 (AREDS2) results were just published at the Association for Research in Vision and Ophthalmology (ARVO) conference, Seattle, USA. The landmark study, conducted by the National Eye Institute, examined the effects of eye supplements over a five-year period in over 4,000 patients with age-related macular degeneration (AMD), the leading cause of blindness in the developed world. AREDS2 confirmed the beneficial impact of using antioxidant eye supplements for AMD, and notably, highlighted the importance of including the macular pigments in the supplement.

The results also show that the supplement for AMD should not include omega-3 fatty acids or beta-carotene.

Fruits and vegetables contain naturally occurring yellow pigments known as carotenoids, and three of them (lutein, zeaxanthin and meso-zeaxanthin) are uniquely found at the back of the eye (retina), where they are referred to as macular pigment. Macular pigment is important because it filters damaging blue light and neutralises unstable molecules that are known to cause AMD.

Importantly, AREDS2 has now shown that AMD patients with low dietary intake of the pigment who were given macular pigment supplements were able to reduce their risk of progression to advanced AMD by 26%, when compared to patients not given the macular pigment supplements.

In attendance at the conference were Professors John Nolan and Stephen Beatty from the Macular Pigment Research Group (MPRG), Waterford Institute of Technology (WIT). Professors Nolan and Beatty have been studying the role of macular pigment for eye health for over 12 years, and are now considered the world-leading researchers in this field, with over 70 scientific publications on the topic. Commenting on the AREDS2 results, Professor Nolan, MPRG Principal Investigator, said,

"We have been researching the role of macular pigment for many years. I always believed that these nutrients had an important role to play for patients with AMD. Indeed, many of our published research studies have already shown that increasing macular pigment with supplements is very beneficial for AMD patients, and can actually improve their vision. Now that AREDS2 has confirmed that taking macular pigment supplements reduces progression of AMD, we now have a standard of care for patients with this condition. This is an extremely important message for the eye care communities."

"The results of AREDS2 confirm the importance of supplementation with antioxidants that include the constituents of macular pigment in subjects with AMD. This will have important implications for patients with AMD. In addition, given that the number of people developing this condition continues to increase, the cost of AMD for healthcare is not sustainable, and preventative action must be taken to help reduce these costs." Professor Beatty, MPRG Director, states.

These results have implications for current trials being undertaken in Waterford. Indeed, the CREST clinical trial, funded by the European Research Council, was originally designed to compare macular pigment supplements to placebo in patients with AMD. This trial has now been halted by the Data Safety and Monitoring Committee (DSMC), which is responsible for overseeing the trial. The DSMC Chair, Dr James Loughman, Dublin Institute of Technology, said, *"Given that an AREDS2 formulation, containing macular pigment, but without omega-3 or beta carotene, has been designated as the new standard of care for AMD patients, it would be difficult to justify the continuation of a trial including a placebo group. An investigation that compares an AREDS2 formulation to an alternate formulation containing meso-zeaxanthin is certainly of scientific interest given the AREDS2 findings."*

The researchers at Waterford are pleased to announce that the redesigned CREST trial has already been approved by the WIT Research Ethics Committee and the DSMC. In this way, patients with AMD who are recruited into the trial will be guaranteed to receive a supplement formulation that has been shown to reduce risk of disease progression.

Free vision screening is now available at the Vision Research Centre in Waterford. Eligibility criteria for the trial include individuals with early stage AMD who are not currently taking an eye supplement. This screening will include checks for AMD using specialised vision technology at the Centre, followed by a review by Consultant Ophthalmologist. For more information on the CREST trial and to arrange a screening visit please call Sarah O'Regan on 051 845505 or email info@mprg.ie

Appendix I: CREST AMD Intervention Leaflet

CREST AMD Information Leaflet: Intervention and Multivitamin tablets
(Central Retinal Enrichment Supplementation Trials: Age-related Macular Degeneration)

Thank you for participating in our research study. You have been given two boxes at your study visit.

White box

The white box contains 14 blister packs, each containing 15 red ("supplement") tablets. These tablets contain the following:

10mg lutein, 10mg *meso*-zeaxanthin and 2mg zeaxanthin
OR
10mg lutein and 2mg zeaxanthin

Because CREST AMD is a double-blind research study, we cannot tell you which supplement tablet you have received.

WHITE BOX: TAKE ONE RED TABLET EVERY DAY WITH A MEAL

Brown box

The brown box contains 28 blister packs, each containing 15 gold ("multivitamin") tablets. These tablets contain the following:

Vitamin C (250mg), vitamin E (200 IU), zinc (12.5 mg) and copper (1 mg)

BROWN BOX: TAKE TWO GOLD TABLETS EVERY DAY WITH A MEAL

In total you will be taking three tablets (one red, two gold), every day with a meal. You can take all three tablets at the same time. Please keep the empty blister packs in the boxes and bring them with you to your next study visit, as we will need to count them.

Questions
If you have any questions or concerns, please call Laura Corcoran (Research Assistant) on **051 845505** or email info@mprg.ie

Appendix J: CREST AMD Adverse Event Form

ADVERSE EVENT

Central Retinal Enrichment Supplementation Trials: Age-Related Macular Degeneration

CREST ID: **CAXXX**	
☐ Initial Report ☐ Follow up report	Date of Report:

1. PATIENT INFORMATION

Patient Initials	Date of Birth	Age	Sex

2. DESCRIPTION OF THE SERIOUS ADVERSE EVENT

Category of the event *(tick all that apply)*: ☐ Patient died ☐ Life threatening ☐ Required or prolonged in-patient hospitalisation ☐ Involved persistent or significant disability ☐ Medically significant ☐Congenital anomaly/birth defect ☐ N/A
Description of event: signs and symptoms, diagnosis, course, underline the main event, include relevant lab data and details of treatment administered. *(additional information may be provided on a separate page).*

3. INFORMATION ABOUT THE ADVERSE EVENT

Start date	Stop date/ongoing	Relationship to study drug	Study drug action	Recovery status
27/04/2014		☐ Not related ☐ Possible ☐ Probable ☐ Definite	☐ Permanently discontinued ☐ Temporarily discontinued ☐ Dose continued	☐ Recovered without sequelae ☐ Recovered with sequelae ☐ Unknown ☐ Not yet recovered ☐ Fatal

4. RELATIONSHIP TO STUDY MEDICATION

Did the event abate upon discontinuation of study medication?	Did the event reoccur upon the reintroduction of the study medication?
☐ Yes ☐ No ☐ N/A	☐ Yes ☐ No ☐ N/A

5. CONCOMITANT MEDICATION

Brand name	INN	Indication	Daily dose	Route	Start date	Stop date

Please tick if no concomitant medications were administered: ☐

6. OTHER RELEVANT HISTORY

(e.g. diagnostics, allergies, pregnancy with last menstrual period date etc.)

Description	Start date	End date

7. REPORTER INFORMATION

Name (signature)	Name (block capitals)	Date	Telephone No.

8. PRINCIPAL INVESTIGATOR

Name (signature)	Name (block capitals)	Telephone No.	Date	Was this event ☐ Expected ☐ Unexpected

Appendix K: CREST AMD referral form

Referral Questionnaire

Volunteer Contact Information

Name: .....

Address: .....

Tel: .....

As part of your participation in CREST (Central Retinal Enrichment Supplementation Trials), your retinal images will be frequently reviewed by an ophthalmologist.

Q1. Do you want onward referral should anything unusual be noted?

☐ Yes ☐ No

If yes to Q1, please continue to questions below.

In an event that onward referral is recommended, you will be informed prior to arranging such a referral.

Q2. If onward referral is recommended by the ophthalmologist, where do you like to be referred? Public ☐ Private ☐

Q3. Please specify the region you would like to be referred to.

.....

Volunteer's GP Contact Information

Name: .....

Address: .....

.....

.....

.....

Appendix L: CREST AMD Screening Form

Date: ..... Use for trial: Y / N

CREST AMD Screening

Code: .....

Contact Information

Forename: ..... Surname: .....

Address: .....

.....

.....

.....

Telephone number: .....

Mobile number: .....

Email: .....

Date of birth: ..... Age: .....

CREST AMD Eligibility

CREST AMD Inclusion Criteria Checklist

- ☐ Early stage non-visually consequential AMD in at least one eye (between one and eight on the AREDS severity scale) and no visually consequential (or late stage) AMD in the fellow eye
- ☐ Corrected distance visual acuity of ≥ 6/12
- ☐ Spectacle prescription of ≤ ±5D
- ☐ Have not taken eye-related dietary supplements containing the macular carotenoids (lutein, zeaxanthin and/or *meso*-zeaxanthin) in the previous twelve months
- ☐ No other ocular pathology
- ☐ No diabetes

Distance spectacle power

	RE	LE
Focimetry		

Visual acuity

	UA=1 CA=2	Snellen	1st	2nd	3rd	Average of 3	Extra letters	LogMAR	VAR	Total Score
RE		6/	/5	/5	/5	/5	+			
LE		6/	/5	/5	/5	/5	+			

Anterior segment

	RE		LE	
Cornea	☐ Normal	☐ Abnormal	☐ Normal	☐ Abnormal
Anterior chamber	☐ Normal	☐ Abnormal	☐ Normal	☐ Abnormal

Lens status

	RE	LE
Lens	☐ Clear ☐ Cataract ☐ Pseudophakia	☐ Clear ☐ Cataract ☐ Pseudophakia
Nuclear	☐ N1 ☐ N2 ☐ N3 ☐ N4 ☐ N5 ☐ N6	☐ N1 ☐ N2 ☐ N3 ☐ N4 ☐ N5 ☐ N6

Cortical	☐ C1 ☐ C2 ☐ C3 ☐ C4 ☐ C5	☐ C1 ☐ C2 ☐ C3 ☐ C4 ☐ C5
Posterior Subcapsular	☐ P1 ☐ P2 ☐ P3 ☐ P4 ☐ P5	☐ P1 ☐ P2 ☐ P3 ☐ P4 ☐ P5

Intraocular Pressure

	RE	LE
IOP	mmHg	mmHg

Macular Examination

Feature	RE	LE
Soft drusen	☐ Absent ☐ Present	☐ Absent ☐ Present
	Type:	**Type:**
Pigmentary irregularities	Hypopigmentation ☐	Hypopigmentation ☐
	Hyperpigmentation ☐	Hyperpigmentation ☐

Supplementation

Ocular Inclusion/Exclusion Criteria Met?	☐ No = Do not enrol patient for study ☐ **Yes = Enrol patient for study**

Fundus photography

Was a fundus photograph taken of each eye? ..... Yes / No

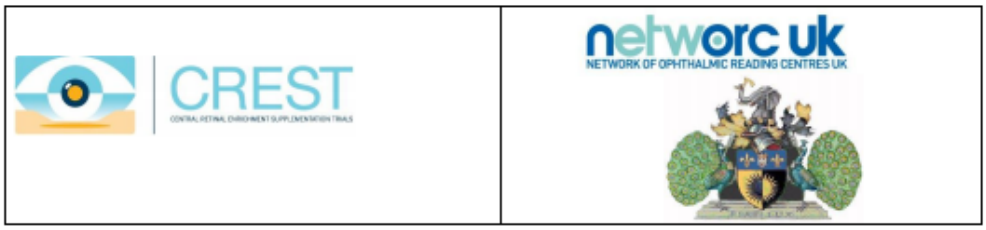
Ocular comorbidity (free text):

_____
_____
_____

_____ _____

Doctor Signature **Date**

Appendix M: CREST AMD Image transmittal logs



Transmittal Log for photographs for the CREST Study

Study name: CREST: Waterford

This transmittal log is to be completed for all images submitted to MEHRC. One transmittal log per patient per visit must be completed.

Subject number	Imaging modality and File name	Number of images	Study Visit
			☐ Eligibility ☐ V Baseline ☐ V 24 months

Please ensure that all ID's and dates on images match the ID's and dates entered on the transmittal log

Please submit one copy to MEHRC and retain the original for the site records.

Comments (any factors out of the photographers' control that resulted in poor image quality (e.g. Parkinson's disease or lack of patient co-operation, extreme photophobia):

.....

Dispatched by: _____ **Date :** _____ **MEHRC Notified of Dispatch by separate email:** ☐ (Please tick)

MEHRC USE ONLY

Date Received: _____

Type of imaging received	Date of receipt	Imported	Reading Centre decision

Appendix N: CREST AMD Case Report Form

Subject Number: .....**CAXXX**..... Subject Initials: ..... Date: ..... Study Eye: RE / LE

Case Report Form

Study Visit: CAV1

CRF Code: .....**CAXXXV1**.....

Central Retinal Enrichment Supplementation Trials (CREST)

CREST Study Procedures

Description	Approx. time (minutes)
A. Informed Consent	5
B. Cognitive Function Assessment	45
C. Visual Acuity	5
D. Reading Acuity (LogRAD)/Reading Speed	5
E. Letter Contrast Sensitivity	10
F. Functional Vision Analyser (FACT)	15
G. Measurement of Macular Pigment: Densitometer	20
H. Light Scatter	10
I. Photostress Recovery	5
J. Dilation	5
K. Blood Sample	5
L. Demographic, lifestyle, medical and visual experience	10
M. Ocular Coherence Tomography	10
N. Measurement of Macular Pigment: Autofluorescence	5
O. Fundus Photography	10
Total study visit time:	**2 hrs 40mins**

Informed Consent

Was the patient given a copy of his/her signed consent form? Yes/No

If yes:

Date of informed consent: .....

Obtained by: .....

Signature of person obtaining consent: .....

Cognitive Function Assessment

See separate cognitive function results sheet

Visual Acuity

	Eye	UA=1 CA=2	Snellen	1st	2nd	3rd	Average of 3	Extra letters	LogMAR	VAR
Study eye			6/	/5	/5	/5	/5	+		
Non-study eye			6/	/5	/5	/5	/5	+		

Reading Acuity/Reading Speed

See separate sheet attached

Letter Contrast Sensitivity

6/120 spatial frequency							
% Contrast	LogCS	1	2	3	4	5	Extra letters
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
CS score:							

6/60 spatial frequency							
% Contrast	LogCS	1	2	3	4	5	Extra letters
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
CS score:							

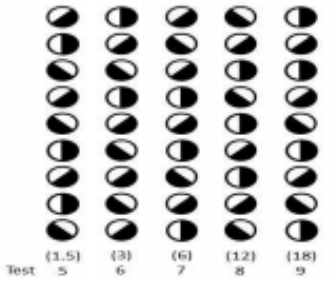
6/24 spatial frequency							
% Contrast	LogCS	1	2	3	4	5	Extra letters
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
CS score:							

6/15 spatial frequency							
% Contrast	LogCS	1	2	3	4	5	Extra letters
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
CS score:							

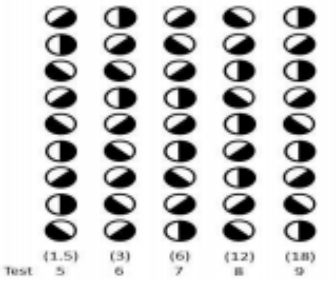
6/9.5 spatial frequency							
% Contrast	LogCS	1	2	3	4	5	Extra letters
100	0.00						
71.0	0.15						
50.1	0.30						
35.5	0.45						
25.1	0.60						
17.8	0.75						
12.6	0.90						
8.9	1.05						
6.3	1.20						
4.5	1.35						
3.2	1.50						
2.2	1.65						
1.6	1.80						
1.1	1.95						
0.8	2.10						
0.6	2.25						
CS score:							

Functional Vision Analyser (FACT)

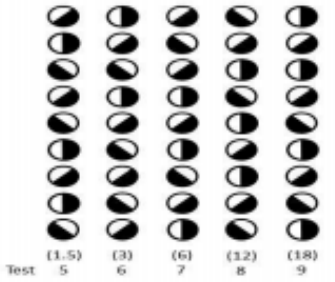
Night testing without glare



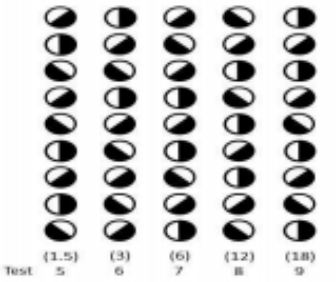
Night testing with glare



Day testing without glare



Day testing with glare



	1.5	3	6	12	18
Mesopic (CS)					
Mesopic (LogCS)					
Photopic(CS)					
Photopic(logCS)					
Mesopic with glare(CS)					
Mesopic with glare(logCS)					
Photopic with glare(CS)					
Photopic with glare(logCS)					

Light Scatter

	log(s)	estd	Q
1			
2			
3			
Best (lowest esd, greatest Q)			

Photostress Recovery (at Snellen 6/24)

Contrast value used: .....

Time (seconds) of adjustment: .....

Measurement of Macular Pigment: Densitometer

Eccentricity		Radiance Units	Optical Density
MPOD 0.25	1		.....
	2		
	3		
	4		
	5		
	6		
MPOD 0.50	1		.....
	2		
	3		
	4		
	5		
	6		
MPOD 1.0	1		.....
	2		
	3		
	4		
	5		
	6		

MPOD 1.75	1		.....
	2		
	3		
	4		
	5		
	6		
MPOD 7° Reference Point	1		
	2		
	3		
	4		
	5		
	6		

Dilation

No results required

Health Check

Height	cm	Body mass index (kg/m^2) _____
Weight	kg	
Blood pressure	_____ / _____ mmHg Systolic/Diastolic	

Food frequency questionnaire

SERVINGS:	Less than 1 per week	1 per week	2-3 per week	4-6 per week	1 per day	More than 1 per day
Eggs						
Broccoli						
Corn						
Dark green leafy vegetables						

This section for Investigator only:

TOTAL (use excel formulations):		
		Tick
Category 1	**0-15**	
Category 2	**16-30**	
Category 3	**31-75**	

Blood Sample

Was a blood sample taken from the patient? ..... Yes / No

If yes:

Time of blood extraction: .....

Time of subject's last meal: .....

Was the blood sample centrifuged, the serum extracted and stored in duplicate at -70 °C? Yes / No

If yes:

Time of centrifugation: .....

Name of person obtaining blood sample: .....

Signature of person obtaining blood sample: .....

Number of microtubes:

Ocular coherence tomography

See separate sheet attached

Measurement of macular pigment by Autofluorescence

Eccentricities	MPOD
0.00	
0.08	
0.16	
0.23	
0.27	
0.47	
0.59	
0.74	
0.78	
0.98	
1.02	
1.25	
1.48	
1.72	
1.76	
MPOD Vol.	

Fundus photography

Was a fundus photograph taken of each eye? ..... Yes / No

Photography comments:.....

.....

.....

Lens Opacities Classification System III (LOCS III) Grading

Have you had cataract surgery in your lifetime? ..... Yes / No. If yes, please indicate which eye? ..... RE / LE / BE

Pseudophakia patients

a) Posterior capsular opacification (could adversely affect vision)
b) YAG Laser capsulotomy (could improve vision)

Have you had cataract surgery/laser surgery before your study visit? Yes / No

RE				LE			
NO	NC	C	P	NO	NC	C	P

Other Comments: .....

.....

.....

.....

Subject Number: ..... Study Eye: RE / LE

Tablet Counting

Supplement containers	Number of Tablets Dispensed	Daily dose	Expected Number of Tablets to be taken	Number of Tablets Left	% Tablet Count
White tub	60	1			
White box	210	1			
Brown box	420	2			
Total					

White tub/box: 10mg lutein, 10mg *meso*-zeaxanthin and 2mg zeaxanthin **OR** 10mg lutein and 2mg zeaxanthin

Brown Box: Vitamin C (250mg), vitamin E (200 IU), zinc (12.5 mg) and copper (1 mg)

$$Expected\ number\ of\ tablets\ to\ be\ taken = Daily\ dose \times Number\ of\ days\ since\ dispensed$$

$$Number\ of\ days\ since\ dispensed = Number\ of\ days\ between\ V1\ and\ V2\ or\ Number\ of\ days\ between\ start\ date\ and\ V2$$

$$\%\ Tablet\ Count = \frac{Total\ tablets\ dispensed - Number\ of\ tablets\ left}{Expected\ number\ of\ tablets\ to\ be\ taken} \times 100\%$$

Other Comments: .....

.....

.....

.....

Appendix O: CREST AMD Questionnaires

Subject Number: ..... Subject Initials: ..... Date: ..... Study Eye RE / LE

Demographic, lifestyle and visual experience questionnaire

Demographic information

L.1 Contact Information

Forename: ..... Surname: .....

Address: .....

.....

.....

.....

Telephone number: .....

Mobile number: .....

Email: .....

Date of birth: ..... Age: .....

Please circle number corresponding to correct answer. All questions must be answered unless other specified.

L.2 Sex

Male 1

Female 2

L.3 Ethnicity

White 1

Black 2

Asian 3

Hispanic 4

Mixed race 5

Lifestyle Information

L.4 Education

What is your highest level of education?

Primary education 1

Secondary education 2

Higher (third level) education 3

L.5 Professional occupation

Please briefly describe your occupation:

_____
_____

L.6 Smoking habits

L.6.1. Which best describes your smoking habits (cigarette/cigarillo/cigar/pipe etc)?

Never smoked *(< 100 cigarettes in lifetime)*..... 1

Past smoker *(smoked ≥ 100 cigarettes in lifetime and none in past year)*.....2

Current smoker *(smoked ≥ 100 cigarettes in lifetime and at least one in the last year)*..... 3

L.6.2. Have you smoked at least 100 cigarettes in your life? ..... Yes / No

If no, skip to question ***L.6.6.***

L.6.3. How long has it been since you last smoked?

Less than one day 1

Less than one week 2

Less than one month 3

Less than 3 months 4

Less than 6 months 5

6 months to a year 6

Over a year 7

L.6.4. What is the average number of cigarettes you smoke (or smoked) on a daily basis?

_____

L.6.5. For how many years have you smoked (or did you smoke)? _____

L.6.6. Are you commonly exposed to second-hand smoke at home or in the work place?

Yes / No

L.7 Alcohol intake

L.7.1. Which of the following statements best describes the way you drink alcohol?

I never drink 1

I drink only on special occasions 2

I drink once or twice a month..... 3

I drink once or twice a week 4

I drink every day 5

I drink twice a day or more 6

L.7.2. What is your average alcohol consumption on a weekly basis?

*One unit ▪ Half pint of beer **or** 100cl glass of wine **or** single measure of sherry / spirit*

1 unit a week 1

2-5 units a week 2

6-10 units a week 3

> 10 units a week 4

M.8 Exercise

M.8.1. *Please complete the following table: how many times per week do you perform each physical activity, if any, and how long are each of these sessions in minutes?*

Exercise	**Number of sessions per week**	**Duration of each session (minutes)**	*CREST researcher only Total score*
Walking			
Running			
Cycling			
Swimming			
Gym-based work-out			
Team sport (please specify below)			
Other (please specify below)			
		Total (Investigator only)	

Please specify team sport/other sports, if applicable:

_____

M.9 Light exposure

M.9.1. How much time do you spend outside during your waking hours?

A lot *(I spend most of the day outside)* 1

Some *(50% outside, 50% inside)* 2

A little *(I spend most of the day inside)* 3

M.9.2. Do you tend to wear protective eyewear when you are exposed to light?

e.g. good quality sunglasses, photochromic lenses (lenses which darken when exposed to light)

Yes 1

No 2

M.10 Medical History

M.10.1. Non-ocular medical history

Medical history (including surgical procedures)	Yes	No	Date of diagnosis	Is this condition ongoing?
Cardiovascular disease				☐No ☐Yes
Hypertension				☐No ☐Yes
Angina				☐No ☐Yes
Stroke				☐No ☐Yes
Peripheral vascular disease				☐No ☐Yes
Diabetes				☐No ☐Yes
Malabsorption				☐No ☐Yes
Other (please specify)				☐No ☐Yes

M.10.2. Are you currently taking any medication?

Yes 1

No 2

If yes, please specify: .....

M.10.3. Ocular family history

Do you have a family history of any of the following eye conditions?

"Family history" means having a first degree relative, i.e. parent or sibling, with the condition

Age-related macular degeneration (AMD)

Yes 1

No 2

Cataract

Yes 1

No 2

Glaucoma

Yes 1

No 2

Other

Yes 1

No 2

If yes, please specify.....

Subject Number: ..... Study Eye: RE / LE

VISUAL FUNCTION QUESTIONNAIRE – 25

The following is a survey with statements about problems which involve your vision or feelings that you have about your vision condition. After each question please choose the response that best describes your situation.

Please answer all the questions as if you were wearing your glasses or contact lenses (if any). Please take as much time as you need to answer each question. All your answers are confidential. In order for this survey to improve our knowledge about vision problems and how they affect your quality of life, your answers must be as accurate as possible. Remember, if you wear glasses or contact lenses, please answer all of the following questions as though you were wearing them.

INSTRUCTIONS

1. In general we would like to have people try to complete these forms on their own.

2. Please answer every question (unless you are asked to skip questions because they don't apply to you).

3. Answer the questions by circling the appropriate number.

4. If you are unsure of how to answer a question, please give the best answer you can and make a comment in the left margin.

Subject Number: ..... Study Eye: RE / LE

Visual Function Questionnaire - 25

PART 1 - GENERAL HEALTH AND VISION

1. **In general, would you say your overall health is:**

(Circle One)

Excellent	**1**
Very Good	**2**
Good	**3**
Fair	**4**
Poor	**5**

2. **At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind?**

(Circle One)

Excellent	**1**
Good	**2**
Fair	**3**
Poor	**4**
Very Poor	**5**
Completely Blind	**6**

3. How much of the time do you worry about your eyesight?

(Circle One)

None of the time	1
A little of the time	2
Some of the time	3
Most of the time	4
All of the time?	5

4. How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)? Would you say it is:

(Circle One)

None	1
Mild	2
Moderate	3
Severe, or	4
Very severe?	5

PART 2 - DIFFICULTY WITH ACTIVITIES

The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.

5. How much difficulty do you have reading ordinary print in newspapers? Would you say you have:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

6. How much difficulty do you have doing work or hobbies that require you to see well up close, such as cooking, sewing, fixing things around the house, or using hand tools? Would you say:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

7. Because of your eyesight, how much difficulty do you have finding something on a crowded shelf?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

8. How much difficulty do you have reading street signs or the names of stores?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

9. Because of your eyesight, how much difficulty do you have going down steps, stairs, or curbs in dim light or at night?

(Circle One)

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

10. Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along?

(Circle One)

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

11. Because of your eyesight, how much difficulty do you have seeing how people react to things you say?

(Circle One)

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

12. Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

13. Because of your eyesight, how much difficulty do you have visiting with people in their homes, at parties, or in restaurants ?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

14. Because of your eyesight, how much difficulty do you have going out to see movies, plays, or sports events?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

15. Are you currently driving, at least once in a while?

(Circle One)

Yes	1	*Skip To Q 15c*
No	2	

15a. IF NO: Have you never driven a car or have you given up driving?

(Circle One)

Never drove	1	*Skip To Part 3, Q 17*
Gave up	2	

15b. IF YOU GAVE UP DRIVING: Was that mainly because of your eyesight, mainly for some other reason, or because of both your eyesight and other reasons?

(Circle One)

Mainly eyesight	1	*Skip To Part 3, Q 17*
Mainly other reasons	2	*Skip To Part 3, Q 17*
Both eyesight and other reasons ...	3	*Skip To Part 3, Q 17*

15c. IF CURRENTLY DRIVING: How much difficulty do you have driving during the daytime in familiar places? Would you say you have:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4

16. How much difficulty do you have driving at night? Would you say you have:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Have you stopped doing this because of your eyesight	5
Have you stopped doing this for other reasons or are you not interested in doing this	6

16A. How much difficulty do you have driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic? Would you say you have:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Have you stopped doing this because of your eyesight	5
Have you stopped doing this for other reasons or are you not interested in doing this	6

PART 3: RESPONSES TO VISION PROBLEMS

The next questions are about how things you do may be affected by your vision. For each one, please circle the number to indicate whether for you the statement is true for you all, most, some, a little, or none of the time.

READ CATEGORIES:	All of the time	Most of the time	Some of the time	A little of the time	None of the time
	(Circle One On Each Line)				
17. Do you accomplish less than you would like because of your vision?	1	2	3	4	5
18. Are you limited in how long you can work or do other activities because of your vision?	1	2	3	4	5
19. How much does pain or discomfort in or around your eyes, for example, burning, itching, or aching, keep you from doing what you'd like to be doing? Would you say:	1	2	3	4	5

For each of the following statements, please circle the number to indicate whether for you the statement is definitely true, mostly true, mostly false, or definitely false for you or you are not sure.

(Circle One On Each Line)

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
20. I stay home most of the time because of my eyesight.....	1	2	3	4	5
21. I feel frustrated a lot of the time because of my eyesight.....	1	2	3	4	5
22. I have much less control over what I do, because of my eyesight.	1	2	3	4	5
23. Because of my eyesight, I have to rely too much on what other people tell me..	1	2	3	4	5
24. I need a lot of help from others because of my eyesight.....	1	2	3	4	5
25. I worry about doing things that will embarrass myself or others, because of my eyesight.....	1	2	3	4	5

SUBSCALE: NEAR VISION

A3. Wearing glasses, how much difficulty do you have reading the small print in a telephone book, on a medicine bottle, or on legal forms? Would you say:

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

A4. Because of your eyesight, how much difficulty do you have figuring out whether bills you receive are accurate?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

A5. Because of your eyesight, how much difficulty do you have doing things like shaving, styling your hair, or putting on makeup?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

SUBSCALE: DISTANCE VISION

A6. Because of your eyesight, how much difficulty do you have recognizing people you know from across a room?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

A7. Because of your eyesight, how much difficulty do you have taking part in active sports or other outdoor activities that you enjoy (like golf, bowling, jogging, or walking)?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

A8. Because of your eyesight, how much difficulty do you have seeing and enjoying programs on TV?

(Circle One)

No difficulty at all	1
A little difficulty	2
Moderate difficulty	3
Extreme difficulty	4
Stopped doing this because of your eyesight	5
Stopped doing this for other reasons or not interested in doing this	6

NEI-VFQ SCORING SHEET

SUBSCALES	SCORE
General Health	
General Vision	
Ocular Pain	
Near activities	
Distance Activities	
Vision Specific	
Social Function	
Mental Health	
Role Difficulties	
Dependency	
Driving	
Color Vision	
Peripheral Vision	
Overall Vision Score	

Appendix P: Radner Reading Chart Scoring Sheet

RADNER READING CHARTS - SCORING SHEET:

logRAD 40cm/**32cm** 16" / **12,5"**	READING CHART 1	Time OD	 OS	 OU
1.1 / **1.2** M: 5	The tourists walked right by our restaurant, in which we served very expensive foods			
1.0/ **1.1** M: 4	Her players arrived late for the tournament, at which each team appeared very confident			
0.9 / **1.0** M: 3.2	Our father always hired this bad bricklayer, who did not come until Friday afternoon			
0.8 / **0.9** M: 2.5	The trainer soon replaced the old goalkeeper, who was once their most expensive player			
0.7 / **0.8** M: 2	Wet clothing produced most of her discomfort, for which she blamed many different things			
0.6 / **0.7** M: 1.6	Red curtains were bought for the apartment, which was not ready until twelve yesterday			
0.5 / **0.6** M: 1.25	The pirates never entered the old battleship, in which the prince kept powerful weapons			
0.4 / **0.5** M: 1	Her students rested close to the settlement, of which they took twenty excellent photos			
0.3 / **0.4** M: 0.8	His mother never looked for the sunglasses, which he lost near these flowers yesterday			
0.2 / **0.3** M: 0.63	The teacher started to wrap his sandwiches, for which he bought waxed paper yesterday			
0.1 / **0.2** M: 0.5	Our sister travelled here in his helicopter, for which he bought leather seats recently			
0.0 / **0.1** M: 0.4	Old farmers often gather at her restaurant, in which the guests wear casual clothing			
-0.1 / **0.0** M: 0.32	She phoned every officer of the government, whom she will meet this Thursday afternoon			
-0.2 / **-0.1** M: 0.25	Two pupils played games with his microphone, which he just received from the headmaster			

Name:

Date:

40cm o	32cm o	.....cm

Distance acuity:

OD:

OS:

Diagnosis:

logRAD-Score:

OD:	OS:

logRAD + ∑ of syllables of incorrectly read words x 0.005

Appendix Q: CREST AMD LOCS III Certification and Grading

CHYLACK INC.
A PARTNER IN VISION RESEARCH

COMMENT SHEET: LOCS II OR III CERTIFICATION/RE-CERTIFICATION

TEST RESULTS REPORTED 10/6/2014

CONFIDENTIAL: ONLY FOR USE IN COMMUNICATIONS WITH SPONSOR LISTED BELOW

Doctor's Name:	Dr. Kwadwo Akuffo		
Test Date (mm/dd/yy)	9/30/14	**Sponsor / Protocol Number**	Macular Pigment Research Group
Test / Retest	Test	**Initial Certification?**	Yes

Grade Session 1: Complete	83
Grade Session 2: Complete	83
Reconciled Grade by LTC	83

Comments on test results: PASS				
Errors in grading: Many: (++) ; Some (+)	**NO**	**NC**	**C +**	**P**
Excellent grading:	**NO X**	**NC X**	**C**	**P X**

X	When viewing retroillumination images of clear or minimally cataractous lenses, doctor rarely graded photographic artefact as C and P cataract.
X	Used wrong grading conventions. Inappropriate use of "CG" once when grading NC and missed appropriate use of "CG" once when grading P. Inappropriate use of "CG" when grading NC. It is almost always possible to grade NC accurately even in the presence of advanced cortical or nuclear opacification. Assigning "CG" when grading NC is rarely justified.
X	Occasionally missed small but gradable C opacities in retroillumination images.
X	Occasionally missed or ignored lightly shadowed C opacities. N.B. They are weighted the same as densely or darkly shadowed opacities in LOCS II/III.
X	NO grading errors were almost always too high. Be sure you are estimating average NO in the two nuclear zones and not the peak NO in the central oval zone.

Recommendations: Visit the https://locs.webex.com web site and view the following recorded sessions and/or take the indicated test session:

	LOCS III Webex Recording - Introduction	X	**LOCS III Self-Administered Training (Part 1)**
X	**LOCS Retro Image Artefacts**		**LOCS III Self-Administered Training (Part 2) (C+P)**
	LOCS II Web Review	X	**LOCS III Self-Administered Training (Part 3) (NO)**
	LOCS III Re-certification Test		**LOCS III Self-Administered Training (Part 4) (Certification Test)**

Contacts

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jenn@chylackinc.com
TEL: 413-230-3391 FAX: 413-230-3408

Consultant: Leo T. Chylack, Jr., M.D., President, Chylack Inc.

Reported	☐
Master File	☐
B.A. File	☐

File: CI_Acad_Akuffo_Kwadwo_C_CSLTC_100614

CHYLACK INC.
A PARTNER IN VISION RESEARCH

COMMENT SHEET: LOCS III CERTIFICATION/RE-CERTIFICATION

TEST RESULTS REPORTED 4/13/2015

CONFIDENTIAL: ONLY FOR USE IN COMMUNICATIONS WITH SPONSOR LISTED BELOW. CERTIFICATION IS PROTOCOL-SPECIFIC AND NON-TRANSFERABLE. CERTIFICATION MAY NOT BE APPLIED TO ANY CONTEXT OTHER THAN THE PROTOCOL LISTED BELOW.

Doctor's Name:	Dr. Kwadwo Akuffo		
Test Date (mm/dd/yy)	4/6/15	**Sponsor / Protocol Number**	Academic License
Test / Retest	Test	**Re-certification Number**	01

Grade Session 1: Complete	87
Grade Session 2: Complete	87
Reconciled Grade by LTC	87

Comments on test results: PASS				
Errors in grading: Many: (++) ; Some (+)		**NO +**	**NC**	**C**	**P**
Excellent grading:		**NO**	**NC X**	**C X**	**P X**
X	C and P grading errors, albeit rare, were consistently too low. Be sure you are carefully examining each test image for small but gradable opacities or features. You appear to be missing the smaller but real and gradable opacities. You need to review the use of the size-reference spot at 6 o'clock in the C 1.0 standard image; this gives the lower size limit of gradable opacities. Opacities as large, or larger, than this spot are graded on the LOCS C and P scales.			
X	Used wrong grading conventions. Missed appropriate use of "CG" once when grading NO.			
X	Rarely missed small but gradable C and P opacities in retroillumination images.			
X	Rarely missed or ignored lightly shadowed C and P opacities. N.B. They are weighted the same as densely or darkly shadowed opacities in LOCS II/III.			
X	**EXCELLENT LOCS III GRADING THROUGHOUT! CONGRATULATIONS!**			

Recommendations: Visit the https://locs.webex.com web site and view the following recorded sessions and/or take the indicated test session:

	LOCS III Webex Recording - Introduction	**X**	**LOCS III Self-Administered Training (Part 1)**
	LOCS Retro Image Artefacts	**X**	**LOCS III Self-Administered Training (Part 2) (C+P)**
	LOCS II Web Review		**LOCS III Self-Administered Training (Part 3) (NO/NC)**
	LOCS III Re-certification Test		**LOCS III Self-Administered Training (Part 4) Certification Test**

Contacts

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Reported	☐
Master File	☐
B.A. File	☐

File: CI_Acad_Akuffo_Kwadwo_R1_CSLTC_0413015

CHYLACK INC.
A PARTNER IN VISION RESEARCH

COMMENT SHEET: LOCS III CERTIFICATION/RE-CERTIFICATION

TEST RESULTS REPORTED 11/8/2015

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Doctor's Name:	Dr. Kwadwo Akuffo		
Test Date (mm/dd/yy)	10/21/15	**Sponsor / Protocol Number**	Academic License
Test / Retest	Test	**Re-certification Number**	02

Grade Session 1: Complete	80
Grade Session 2: Complete	80
Reconciled Grade by LTC	80

Comments on test results: PASS				
Errors in grading: Many: (++) ; Some (+)		**NO**	**NC**	**C +**	**P +**
Excellent grading:		**NO X**	**NC X**	**C**	**P**
X	C and P grades consistently too low: Be sure you are carefully examining each test image for small but gradable opacities or features. You appear to be missing the smaller but real and gradable opacities. You need to review the use of the size-reference spot at 6 o'clock in the C 1.0 standard image; this gives the lower size limit of gradable opacities. Opacities as large, or larger, than this spot are graded on the LOCS C and P scales.			
X	NO grading errors occasionally too high, and NC grading errors always too low. Be sure you are estimating average NO in the two nuclear zones and not the peak NO in the central oval zone. Also, when grading NC, be sure you are looking at the reflection on the posterior capsule and not at the adjacent Purkinje image, which always looks whiter than the reflection.			
X	Often missed small but gradable C and P opacities in retroillumination images.			
X	Often missed or ignored lightly shadowed C and P opacities. N.B. They are weighted the same as densely or darkly shadowed opacities in LOCS II/III.			

Recommendations: Visit the https://locs.webex.com web site and view the following recorded sessions and/or take the indicated test session:

	LOCS III Webex Recording - Introduction	**X**	**LOCS III Self-Administered Training (Part 1)**
	LOCS Retro Image Artefacts	**X**	**LOCS III Self-Administered Training (Part 2) (C+P)**
	LOCS II Web Review	**X**	**LOCS III Self-Administered Training (Part 3) (NO/NC)**

Contacts

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Consultant: Leo T. Chylack, Jr., M.D., President, Chylack Inc.

Reported	☐
Master File	☐
B.A. File	☐

File: CI_Acad_Akuffo_Kwadwo_R2_CSLTC_110815

LENS OPACITIES CLASSIFICATION SYSTEM III (LOCS III)
Central Retinal Enrichment Supplementation Trial: Age-related Macular Degeneration (CREST AMD)

PUPILLARY DILATION

1. Ascertain that the anterior chamber depth is sufficient to minimize the risk of angle closure glaucoma.
2. If the pupil is safe to dilate, dilate with tropicamide 1% and phenylephrine 2.5%.
3. The pupil must be dilated maximally for valid LOCS III grading. If the maximal size of the pupil is only 6mm, then valid LOCS III grading can be done. If the maximal size of the pupil is < 6mm, then valid LOCS III grading cannot be done.

SLITLAMP BIOMICROSCOPY CONFIGURATION

Nuclear Opalescence (NO) / Nuclear Color (NC) Grading

1. One may use any combination of slitlamp settings to find the cataract.
2. LOCS III standards on a light box are located near the patient's right or left shoulder.
3. Once the cataract has been located, then the slitlamp must be configured as follows:
   a) **Transformer setting**: 5 volts
   b) **Filter wheel:** 0
   c) **Slit height:** Just tall enough to overlap the pupil margin
   d) **Slit width:** 0.2mm (or 0.3mm if 0.2mm is too dim); whichever slit width is used, keep it constant throughout the protocol
   e) **Slit beam angle:** 45° to the angle of observation
   f) **Focus:** In center of nucleus (in central clear zone)
   g) **Room lights:** Out

Cortical (C) / Posterior (P) Grading

1. One may use any combination of slitlamp settings to find the cataract.
2. LOCS III standards on light box are located near the patient's right or left shoulder.
3. Once the cataract has been located, then the slitlamp must be configured as follows:
   a) **Transformer setting:** 5 volts
   b) **Filter wheel:** 0
   c) **Slit height:** variable, but 3-4mm is usually good
   d) **Slit width:** variable, but wide enough to create a bright retro image
   e) **Slit beam angle:** 3-5° to angle of observation
   f) **Focus:**
      i. **Anteriorly focussed:** Plane of pupil
      ii. **Posteriorly focussed:** Plane of posterior capsule
   g) **Room lights:** Out
   h) **Joystick:** Move to left or right to move position of the shadow in the retro image and allow one to create in the mind's eye a uniformly bright retro image.

Appendix R: CREST AMD Retinal Photography Certification

The Reading Centre

Moorfields Eye Hospital NHS Foundation Trust

Kwadwo Akuffo

Certified for Retinal Photography for the CREST STUDY - AMD

On 18th June 2013

Dr Tunde Peto
Head of Reading Centre

Appendix S: CREST AMD Unmasking of Data Recommendations

May 24, 2016 **[CREST DSMC RECOMMENDATIONS]**

Dear Prof. Nolan

The DSMC would like to thank the CREST Investigators for notifying the DSMC of the completion of testing on the CREST AMD study, and for making the formal request to unmask the data. The DSMC understand that the responsibility for the conduct of the trial, including unmasking of data, rest with the Principal Investigator. However, the DSMC have considered the information supplied thoroughly, and given the absolute priority that should be afforded to eliminating possible sources of bias, the DSMC have agreed the following recommendations with respect to the request that the study statistician can now obtain the code from pharmacy and proceed with unmasking the data:

1. The DSMC recommends that any data "cleaning" that is required should be undertaken prior to unmasking the data.
2. We recommend that a "Blind Review" of the CREST AMD data be conducted prior to unmasking the data. This pre-analysis review, masked to treatment, should cover, for example, decisions concerning the exclusion of subjects or data from the analysis sets, the checking of possible transformations and definitions of outliers, the addition to the model of important covariates identified in other recent research, and other factors that might be of relevance to the data analysis. Decisions made at this time should be described in a report and should be distinguished from those made after the statistician has had access to the treatment codes when final decisions can be adequately taken, as blind decisions will generally introduce less potential for bias.
3. We further recommend that the statistical methods to be employed be reviewed and where possible, fully defined, now that the dataset is largely complete. This should include, for example, aspects such as the treatment of missing data, treatment of outliers, etc. This statistical plan should only be finalised after the blind review of the final trial data to allow the statistical plan to be updated according to the fully complete dataset. The date of finalisation of the plan should be recorded prior to unmasking. The analysis set (i.e. set of subjects to be included in the main analyses) should be defined in the statistical plan. Each subject should receive equal scrutiny for eligibility violations or other reasons for exclusion from analysis prior to unmasking. By the time of the actual analysis, full plans should exist for all its aspects within the protocol and statistical plan

including subject selection, data selection and modification, data summary and tabulation, estimation, and hypothesis testing.

4. The DSMC should be provided with a copy of the final statistical plan prior to unmasking.

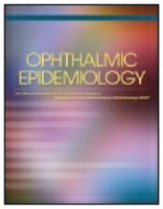
The investigators should note that this statistical plan is not binding – i.e. changes can occur during the unmasked analysis, but these deviations from the planned analysis should be fully documented and explained. Equally, new possibilities for analysis of data in relation to new research questions are possible, but should be treated entirely separately to the trial primary analyses.

Given that the serum analysis for meso-Z is yet to be included in the dataset, and this forms part of the analysis, the DSMC recommends that consideration be given to awaiting the inclusion of the "cleaned" serum data in the database prior to unmasking.

Should you have any queries in relation to the above recommendations, please don't hesitate to contact us.

Prof. James Loughman, on behalf of the CREST DSMC

Appendix T: Peer-reviewed scientific publications



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Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology of the CREST Randomized Controlled Trials

Kwadwo Owusu Akuffo, Stephen Beatty, Jim Stack, Jessica Dennison, Sarah O'Regan, Katherine A. Meagher, Tunde Peto & John Nolan

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ORIGINAL ARTICLE

Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology of the CREST Randomized Controlled Trials

Kwadwo Owusu Akuffo[1], Stephen Beatty[1], Jim Stack[1], Jessica Dennison[1], Sarah O'Regan[1], Katherine A. Meagher[1], Tunde Peto[2], and John Nolan[1]

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ABSTRACT

Purpose: The Central Retinal Enrichment Supplementation Trials (CREST) aim to investigate the potential impact of macular pigment (MP) enrichment, following supplementation with a formulation containing 10 mg lutein (L), 2 mg zeaxanthin (Z) and 10 mg *meso*-zeaxanthin (MZ), on visual function in normal subjects (Trial 1) and in subjects with early age-related macular degeneration (AMD; Trial 2).

Methods: CREST is a single center, double-blind, randomized clinical trial. Trial 1 (12-month follow-up) subjects are randomly assigned to a formulation containing 10 mg L, 10 mg MZ and 2 mg Z ($n=60$) or placebo ($n=60$). Trial 2 (24-month follow-up) subjects are randomly assigned to a formulation containing 10 mg L, 10 mg MZ, 2 mg Z plus 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc and 2 mg copper (Intervention A; $n=75$) or 10 mg L and 2 mg Z plus 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc and 2 mg copper (Intervention B; $n=75$). Contrast sensitivity (CS) at 6 cycles per degree represents the primary outcome measure in each trial. Secondary outcomes include: CS at other spatial frequencies, MP, best-corrected visual acuity, glare disability, photostress recovery, light scatter, cognitive function, foveal architecture, serum carotenoid concentrations, and subjective visual function. For Trial 2, AMD morphology, reading speed and reading acuity are also being recorded.

Conclusions: CREST is the first study to investigate the impact of supplementation with all three macular carotenoids in the context of a large, double-blind, randomized clinical trial.

Keywords: Age-related macular degeneration, lutein, macular pigment, *meso*-zeaxanthin, randomized clinical trial, visual performance, zeaxanthin

INTRODUCTION

A yellow pigment composed of the carotenoids lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ),[1] accumulates at the macula where it is known as macular pigment (MP). MP is a short-wavelength (blue) light filter[2] and a powerful antioxidant,[3] and is therefore believed to protect against age-related macular degeneration (AMD),[4] which is the commonest cause of blindness in the developed world.[5] In addition, MP is essential for optimal vision because of its optical properties.[6–8]

In July 2011, the European Research Council (ERC) awarded funding of €1,493,342 to support and conduct the Central Retinal Enrichment Supplementation Trials (CREST). The CREST project is funded under the ERC "Ideas" Framework 7 program. These trials were designed to investigate the impact of supplementation with a combined carotenoid formulation of L, Z, and MZ on visual function in normal subjects (Trial 1) and in subjects with early AMD (Trial 2).

A novel and important feature of the CREST trials is the inclusion of MZ in the study intervention. Recent data from our laboratory show that optimal

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enrichment of MP is dependent upon inclusion of MZ (along with L and Z) in the supplement formulation.[7,9,10] MZ is believed to be particularly important for the following reasons. First, MZ is the dominant macular carotenoid at the foveal epicenter,[8] and is therefore ideally located to exert optimal antioxidant activity and short-wavelength light filtration at the central macula, the specialized part of the retina responsible for color vision and high spatial resolution.[11] Second, it has been shown (*in vitro*) that the antioxidant properties of the macular carotenoids (L, Z, and MZ) are enhanced when all three carotenoids are present.[12] Third, the absorbance spectrum of MZ extends the range of pre-receptoral short-wavelength (blue) light filtration, and its orientation (compared to L) in the Henle fiber layer likely confers beneficial effects related to light polarization at the macula.[13] Last, it has been shown that individuals at increased risk of AMD (i.e. older subjects and cigarette smokers) are more likely to display atypical and undesirable central dips in their MP spatial profiles,[14] and it has been shown that these central dips can only be normalized when MZ is included in a study intervention.[9,15]

A novel and distinctive feature of the CREST trials is the variety of methods and outcome measures selected to assess visual function in both the normal and AMD populations under investigation. These methods, which are described fully below, are appropriate and sensitive to detect change in visual function, if present.[16,17] Indeed, previous clinical trials investigating the potential impact of carotenoid supplementation on visual function in normal subjects[6] and in patients with early AMD[18] were limited by the outcome measures (e.g. typically best-corrected visual acuity; BCVA) and in terms of interventions used (i.e. an intervention without MZ).

The study hypothesis of CREST is that MP will be uniquely and best enriched using a supplement formulation containing all three macular carotenoids, and that enrichment of MP centrally, and across its spatial profile, will enhance visual function via the optical properties of this pigment, by reducing the deleterious effects of chromatic aberration, light scatter, and veiling luminance in normal subjects (non-diseased retina, Trial 1, see below) and in patients with early AMD (diseased retina, Trial 2, see below). This article outlines the design and methodology of the CREST trials.

MATERIALS AND METHODS

Management, Design and Registration

The management of CREST, including its research team, supporting service providers, and research collaborators is summarized in Figure 1. CREST is a parallel group, double blind, randomized controlled trial studying two populations of interest. Trial 1 is investigating the impact of macular carotenoid nutrition on visual function in normal subjects, and Trial 2 is investigating the impact of macular carotenoid nutrition on visual function in patients with early AMD. These trials are registered on the current controlled trials register (Trial 1: ISRCTN68270512; Trial 2: ISRCTN13894787) and are being conducted as a single center study at the Macular Pigment Research Group (www.mprg.ie), Vision Research Centre, Waterford Institute of Technology, Ireland. Figures 2 and 3 show the consolidated standards of reporting trials diagram,[19] explaining the flow of subjects through Trial 1 and Trial 2, respectively.

Ethical Assessment and Approval

All subjects are required to provide written informed consent prior to enrolment into CREST. Ethical approval for the study was granted by the Research Ethics Committee of the Waterford Institute of Technology, Waterford, Ireland, and the Ethics Committee of the ERC. CREST adheres to the tenets of the Declaration of Helsinki, and will follow the full code of ethics with respect to subject recruitment, subject testing and data protection.

Research Questions

Trial 1: Does supplementation with all three macular carotenoids in a ratio (mg/day) of 10:10:2 (L:MZ:Z), for 12 months, enhance visual function in normal subjects (without retinal disease) when compared to placebo?

Trial 2: Does supplementation with all three macular carotenoids in a ratio (mg/day) of 10:10:2 (L:MZ:Z) plus 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc and 2 mg copper for 24 months, enhance visual function in patients with early AMD when compared to 10:2 (L:Z) plus 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc and 2 mg copper.

Primary Outcome Measure

Contrast sensitivity (CS) at 6 cycles per degree (cpd) is the primary outcome measure in both trials.

Secondary Outcome Measures

Secondary outcome measures include CS at other spatial frequencies, visual acuity, glare disability,

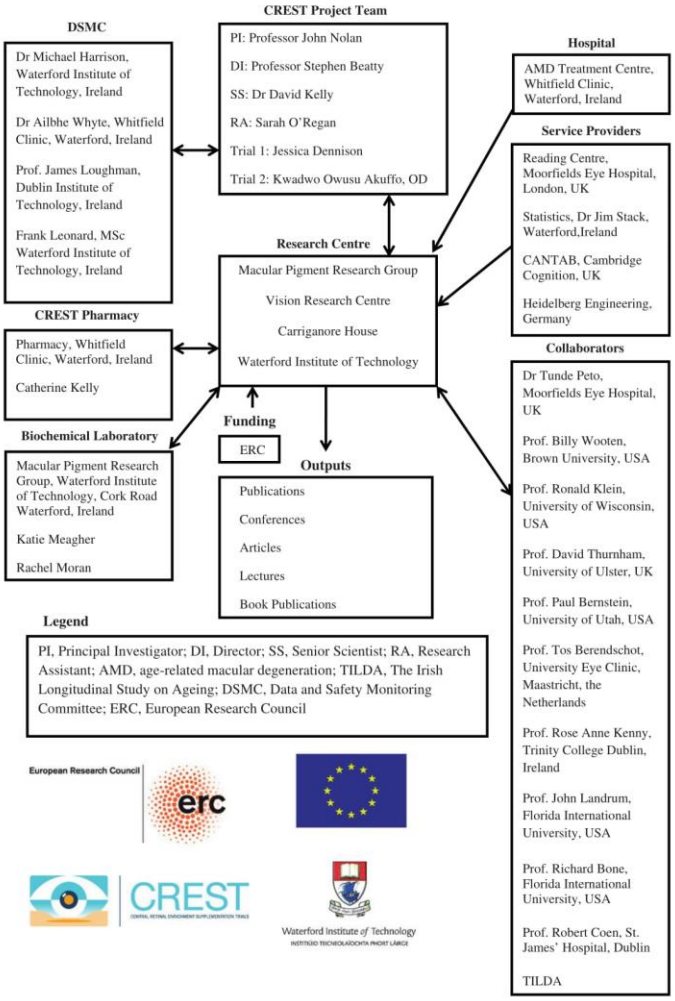

FIGURE 1. Structure of the Central Retinal Enrichment Supplementation Trials (CREST) management and collaboration.

photostress recovery, MP, light scatter, foveal architecture, serum carotenoid concentrations, subjective visual function, and cognitive function. In Trial 2, AMD morphology, reading acuity and reading speed are also being assessed.

Randomization and Intervention

Block randomization is used to assign subjects to intervention groups for both trials. The use of blocking is designed to ensure that an equal number

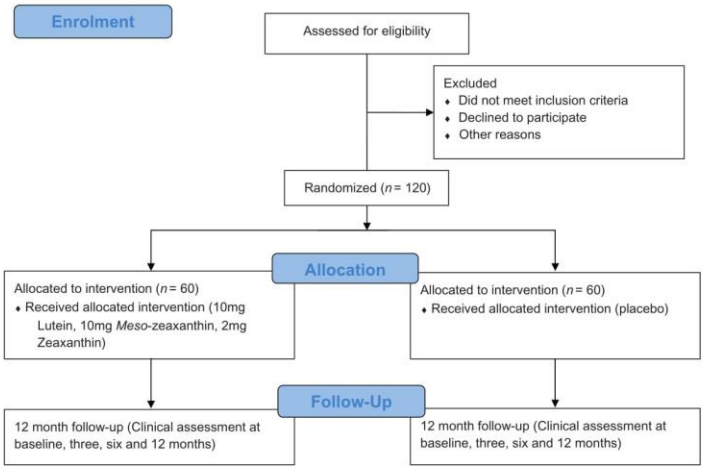

FIGURE 2. Central Retinal Enrichment Supplementation Trials (CREST) Trial 1 consolidated statement of reporting trials flow diagram.

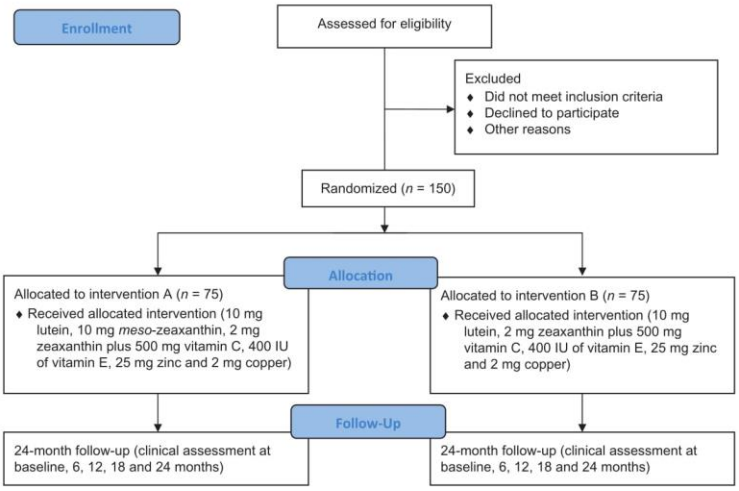

FIGURE 3. Central Retinal Enrichment Supplementation Trials (CREST) Trial 2 consolidated standards of reporting trials flow diagram.

of subjects are assigned to intervention groups. The random numbers were generated using Minitab 16 Statistical Software (Minitab Inc, State College, PA, USA) and the blocks generated by one of the methods described by Friedman and co-authors.[20] The randomization ratio is 1:1 with no stratification. The randomization code list was generated by the study statistician (JS) who has no contact with study subjects

and no access to data until study completion. Random allocation is carried out by a pharmacist (CK) at Whitfield Clinic, Waterford, who has no contact with study subjects. The pharmacist tosses a coin to assign subjects to intervention groups based on the randomization code list. Study researchers only receive a box of tablets with subject identification label. The code is revealed only at study completion.

The intervention for Trial 1 is a softgel capsule containing 10 mg L, 10 mg MZ and 2 mg Z in a sunflower oil suspension (commercially available as Macushield™, provided by Macuvision Europe Limited, Solihull, UK, and prepared by EuroCaps Limited, Tredegar, South Wales, UK). The placebo is a softgel capsule containing sunflower oil (provided by EuroCaps Limited, Tredegar, South Wales, UK). Trial 1 subjects are instructed to take one capsule daily with a meal. The intervention and placebo supplements are identical in external appearance and therefore the two treatments are indistinguishable from each other.

The interventions for Trial 2 consist of a softgel capsule containing 10 mg L, 10 mg MZ and 2 mg Z in a sunflower oil suspension plus two multivitamin capsules each containing 250 mg vitamin C, 200 IU vitamin E, 12.5 mg zinc, and 1 mg copper (provided by Macuvision Europe Limited, Solihull, UK, prepared by EuroCaps Limited, Tredegar, South Wales, UK; Intervention A) or a softgel capsule containing 10 mg L, 2 mg Z in a sunflower oil suspension plus two multivitamin capsules each containing 250 mg vitamin C, 200 IU vitamin E, 12.5 mg zinc, and 1 mg copper (provided by Macuvision Europe Limited, Solihull, UK, prepared by EuroCaps Limited, Tredegar, South Wales, UK; Intervention B). The macular carotenoid capsules are also indistinguishable from each other in external appearance. Trial 2 subjects are instructed to take one macular carotenoid capsule and two multivitamin capsules daily with a meal.

Compliance

Frequent phone calls and reminder text messages are sent to subjects to ensure compliance with consumption of the study intervention. Capsule counting is implemented at follow-up visits. In addition, compliance will be assessed at the end of the study (after the randomization code is broken) by analyzing serum carotenoid concentrations using high performance liquid chromatography (see method below).

Sample Size Calculations

The primary outcome measure in CREST is change in CS at 6cpd over the course of the study: Y = CS2 – CS1, where CS1 is CS at baseline, CS2 is CS at the end of the study. The appropriate statistical test is the independent samples t-test, comparing the mean change in Y in treatment groups.

Pilot studies were conducted to inform CREST with respect to power and sample size (trial ISRCTN81595685). From this pilot work, estimates of standard deviation of CS, and the correlation between CS pre- and post-intervention were available and were used in the sample size calculations. There was strong evidence from the pilot work of a positive effect (i.e. improvement in mean CS) of treatment on CS relative to placebo and therefore a one-tailed rather than a two-tailed test was deemed more appropriate for Trial 1. For Trial 2, since there was no such evidence for the two treatment groups, a two-tailed test was used.

Using a clinically significant effect size of 0.15 logarithm (log) CS units (an improvement of one line on a Thomson logarithm of the minimum angle of resolution, LogMAR, chart) and on standard assumptions (5% level of significance, 80% power, equal group sizes), the required minimum sample size in Trial 1 is 90 subjects (45 per treatment group) and Trial 2 is 112 (56 per treatment group).[21]

However, assuming a 25% dropout rate for both trials, we decided on a total sample size of 120 for Trial 1 and a total sample size of 150 for Trial 2.

Some power calculations for secondary outcome variables were also performed based on 45 subjects per group. These were based on Cohen's suggested classification[22] of effect sizes:

(a) Interval variables (independent samples t-tests), e.g. for comparing changes in MP in the two groups, with 45 subjects in each group, there is very high power (0.98) for detecting a large effect size (0.8 standard deviations on Cohen's definition) and close to acceptable power (0.76) for detecting a medium effect size (0.5 standard deviations). These power calculations, as with those for the principal outcome measure, assume a 5% level of significance and a one-tailed test.
(b) Categorical variables, e.g. comparing numbers (at the end of the study) in terms of AMD severity grade categories for intervention groups. The power in this case depends on the dimensions of the contingency table. If there are two rows in the table (two intervention groups) and three columns (e.g. mild, moderate, and severe AMD) then the power is 0.99 for detecting a large effect (W = 0.5 on Cohen's definition) and 0.72 for detecting a medium effect (W = 0.3). The W-statistic, devised by Cohen,[22] is derived from the χ^2 statistic used to test for independence in the contingency table. We have assumed a 5% level of significance.

In summary, for analysis of secondary outcome variables, interval or categorical, the power of this study is inadequate for small effect sizes by Cohen's

definition (e.g. 0.2 standard deviations for an interval variable), but the power is adequate/strong for medium/large effect sizes. The results for power presented here were obtained from the PASS 2008 software (NCSS LLC, Kaysville, Utah, USA).[23]

Eligibility Criteria

Trial 1

Inclusion criteria for Trial 1 include: (1) 18 years or older; (2) BCVA of 6/6 or better; (3) no more than five diopters spherical equivalence of refraction; (4) no previous consumption of supplements containing the macular carotenoids (L, Z and/or MZ); (5) no ocular pathology; and (6) MP at 0.25 degrees of eccentricity less than 0.5 optical density units. A subject is described as normal where he/she exhibits no vision-related abnormalities following a comprehensive battery of tests, including BCVA (better than or equal to 6/6), fundus photography (scrutinized by a retinal specialist), optical coherence tomography (OCT; scrutinized by a retinal specialist), and a general health questionnaire, with particular attention directed towards the possibility of diabetes mellitus or amblyopia.

Trial 2

Inclusion criteria for Trial 2 include: (1) early AMD in at least one eye, based on the grading of a fundus photograph from one (drusen absent or questionable or small hard drusen present, total drusen area $<125\,\mu m$ diameter, without retinal pigment abnormalities) to eight (drusen ≥ 0.5 disc area, DA, with retinal pigment epithelium depigmentation $\geq 350\,\mu m$ to <0.5 DA or any drusen with ≥ 0.5 DA retinal pigment epithelium depigmentation) on the Age-Related Eye Disease Study (AREDS) severity scale;[24] (2) BCVA of 6/12 or better; (3) no more than five diopters spherical equivalence of refraction; (4) no previous consumption of supplements containing the macular carotenoids (L, Z and/or MZ); (5) no other retinal pathology beyond AMD; and (6) no diabetes mellitus.

Screening Visits to Assess and Confirm Eligibility

For both trials, efforts are made to ensure that subjects enrolled into the study meet the inclusion criteria. This is achieved by conducting a screening visit on all subjects prior to enrollment into either CREST trial.

Trial 1

Subjects are recruited into this trial through an organized advertising campaign. National and local media were informed of the trial and many mainstream Irish newspapers published the call for volunteers. Radio and online adverts have also been carried out. In addition, flyers have been developed for distribution to the general public. Educational events for general practitioners, optometrists and ophthalmologists are held regularly to create awareness of the trial and to solicit help with recruitment. Interested subjects attend our Vision Research Centre and an initial assessment is performed to determine if the subject meets the eligibility criteria for inclusion. Clinical examination consisting of ocular and medical history, BCVA, MP measurement, OCT and fundus photography is carried out. The screening visit is conducted to confirm absence of ocular pathology and to satisfy all other criteria for inclusion.

Trial 2

Subjects are recruited from hospitals in the Republic of Ireland. This has been facilitated by raising awareness of the trial at each hospital. Also, as above, educational events for general practitioners, optometrists and ophthalmologists are held regularly to create awareness of the trial and to solicit help with recruitment. Interested and potential volunteers are invited to attend our Vision Research Centre for assessment to confirm eligibility (with particular emphasis placed on presence of early AMD). During the screening visit, demographic information is collected. This is followed by measurement of BCVA. In addition, anterior and posterior segment examination using the Haag-Streit BM 900 Slit lamp biomicroscope (Haag-Streit AG, Switzerland) is carried out by a consultant ophthalmologist with a special interest in AMD (SB). Subjects who are deemed suitable following the ophthalmological examination by SB then have stereo fundus photographs taken. These stereo fundus photographs are then sent to the Reading Centre at Moorfields Eye Hospital, London, for confirmation that the patient has early AMD. Only patients who have such confirmation by the Reading Centre are invited to participate in the study.

Study Visits

In Trial 1, study visits are conducted at baseline, 3 months, 6 months, and 12 months. At each visit, subjects undergo a series of tests and procedures, which are described in detail below. Table 1 summarizes the clinical procedures conducted in Trial 1 at each study visit. The duration of a typical study visit is approximately 120 minutes.

In Trial 2, study visits are conducted at baseline, 6 months, 12 months, 18 months and 24 months. Table 2 summarizes the clinical procedures conducted in Trial 2 at each study visit. A typical study visit in Trial 2 takes about 150 minutes.

TABLE 1. Central Retinal Enrichment Supplementation Trials (CREST) study procedures in Trial 1.

Study procedures	Baseline	3 months	6 months	12 months
Demographic and lifestyle questionnaire	•			
Subjective visual function questionnaire	•			•
Dietary carotenoid screener	•			•
Cognitive function assessment	•			•
Visual acuity assessment	•	•	•	•
Letter contrast sensitivity	•	•	•	•
Contrast sensitivity with functional vision analyzer	•	•	•	•
Light scatter	•	•	•	•
Photostress recovery	•	•	•	•
MP measurement by customized heterochromatic flicker photometry	•	•	•	•
MP measurement by dual-wave autofluorescence	•	•	•	•
Optical coherence tomography	•	•	•	•
Fundus photography	•	•	•	•
Serum carotenoid analysis	•	•	•	•

MP, macular pigment

TABLE 2. Central Retinal Enrichment Supplementation Trials (CREST) study procedures in Trial 2.

Study procedures	Baseline	6 months	12 months	18 months	24 months
Demographic and lifestyle questionnaire	•				
NEI VFQ-25	•				•
Dietary carotenoid screener	•				•
Cognitive function assessment	•				•
Visual acuity assessment	•	•	•	•	•
Reading acuity	•	•	•	•	•
Reading speed	•	•	•	•	•
Letter contrast sensitivity	•	•	•	•	•
Contrast sensitivity with functional vision analyzer	•	•	•	•	•
Light scatter	•	•	•	•	•
Photostress recovery	•	•	•	•	•
MP measurement by customized heterochromatic flicker photometry	•	•	•	•	•
MP measurement by dual-wave autofluorescence	•	•	•	•	•
Optical coherence tomography	•	•	•	•	•
Fundus photography	•	•	•	•	•
Fundus grading	•				•
Serum carotenoid analysis	•	•	•	•	•

MP, macular pigment; NEI VFQ-25, 25-item National Eye Institute Visual Functioning Questionnaire

Statistical Analysis

Baseline analysis

Placebo and intervention groups will be investigated for statistically significant differences in outcome measures, demographic variables etc, at baseline. This will be done using standard statistical analyses, e.g. independent sample t-tests for interval variables and contingency table analysis for categorical variables. It is expected that the randomization process will result in the intervention groups being statistically comparable. However, any between-group differences in variables, which are identified at baseline, will be controlled for in subsequent analyses.

Analysis of changes over time

If there is no need to control for baseline differences between placebo and intervention groups, then a straightforward independent sample t-test will suffice for the analysis of change over time in the primary outcome measure (CS at 6cpd). However, linear mixed models[20] may also be used, to control (if necessary) for baseline differences between groups and to analyze data from multiple time points. The principle of intention to treat[25–27] will not, in general, be followed (but will nevertheless be performed) in the statistical analysis, but wherever intention to treat-based analysis is found to yield substantially different findings to the main analysis, such discrepancies will be reported.

Most of the secondary outcome measures in this study (contrast sensitivity at other frequencies, MP, serum concentrations, etc) are also interval variables, and will be analyzed using the same methods as for CS at 6cpd. However, some outcome variables (in particular, in Trial 2, change in AMD severity grade

over time in the intervention groups), are ordinal rather than interval variables, and therefore logistic regression or contingency table analysis will be used. Statistical significance will be set at the standard $p < 0.05$ for all analyses. In order to reduce the risk of a type II error, there will be no adjustment for multiple comparisons, but this will be clearly stated when reporting study findings.

Questionnaires

Demographic and Lifestyle Questionnaire

The demographic and lifestyle questionnaire obtains the following details: contact details, ethnicity, education, occupation, smoking habits (history and frequency), alcohol intake (average consumption per week, frequency), exercise (number of sessions per week, duration of each session in minutes), light exposure (time spent outdoors, use of protective eyewear such as sunglasses, photochromic lenses), body mass index, blood pressure, medical history, and ocular medical history.

Subjective Visual Function Questionnaire

The subjective visual function questionnaire assesses visual function based on responses to closed-ended questions under four subscales, namely glare disability, acuity/spatial vision, light/dark adaptation and daily visual tasks. This questionnaire is administered in only Trial 1. All questions must be answered (forced-choice). In each of the four subscales, subjects respond to questions in three tiers. First, subjects rate their visual function in specified daily scenarios (situational analysis) using a five-point Likert scale (never, rarely, sometimes, often, always). Second, subjects compare their visual function to friends and family in a comparative analysis using a five-point Likert scale (significantly better than others, marginally better than others, equivalent to others, marginally worse than others, significantly worse than others). Last, subjects rate their overall visual performance on a scale from zero (worst) to 10 (best) known as subjective satisfaction score. Each tier analysis is computed to give a score out of 100 for each subscale. This questionnaire has been previously described.[6]

National Eye Institute Visual Functioning Questionnaire 25

Subjective visual function is assessed in Trial 2 using the validated[28,29] National Eye Institute Visual Functioning Questionnaire 25.[30]

Dietary Carotenoid Screener

The dietary carotenoid screener is a simplified questionnaire which assesses the dietary intake of four carotenoid-rich food substances (eggs, broccoli, corn and dark green leafy vegetables). Subjects indicate their serving size by ticking any of six categories (<1/week, 1/week, 2–3/week, 4–6/week, 1/day, >1/day) with respect to each of the food substances. Responses are entered into a computer program developed by Professor Elizabeth Johnson, Tufts University, USA, which weighs responses based on the frequency of food intake and the bioavailability of L and Z within these food substances, and calculates a dietary score. The dietary scores generated range from 0–75, and are further divided into three subgroups (low intake, category 1, 0–15: $\leq$2 mg/day; medium intake, category 2, 16–30: 3–13 mg/day; high intake, category 3, 31–75: >13 mg/day). This method has been used previously by our group.[9,31]

Cognitive Function Assessment

Cambridge Neuropsychological Test Automated Battery[32–34] (CANTAB, Cambridge Cognition, Cambridge, UK) assesses cognition using a computerized software program. A battery of tests consisting of the motor screening task,[35,36] verbal recognition memory,[37] attention switching task[38,39] and the paired associate learning[40] is used. The CANTAB protocol[41] is followed in the administration of these tests.

Best-corrected Visual Acuity

BCVA is measured with a computerized LogMAR Early Treatment Diabetic Retinopathy Study (ETDRS) test chart (Test Chart 2000 Xpert, Thomson Software Solutions, Hatfield, UK) viewed at 4 m. The Sloan ETDRS letterset is used for this test. At the first incompletely read line, the letters of the line are randomized three times using the testing software's randomization function and an average of three scores is taken. BCVA is recorded in visual acuity rating.

Contrast Sensitivity

Letter Contrast Sensitivity

Letter CS is assessed using the computerized LogMAR ETDRS test chart (Test Chart 2000 PRO, Thomson Software Solutions) at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, 15.15cpd).[42] The Sloan optotypes are chosen and subjects are asked to read the letters aloud while fixating on the chart at a distance of 4 m. The letter set is randomized during the test at each change of contrast. The percentage contrast of letter optotypes is decreased in 0.15 log CS steps until the lowest contrast value for which subjects see at least three letters is reached. The test is then repeated for the other spatial frequencies. Each letter

has a nominal log CS value of 0.03. Missed letters at any contrast level are noted. The resultant log CS value for the subject at a particular spatial frequency is calculated by adding any extra letter(s) and/or subtracting missed letters from best log CS value corresponding to the lowest percentage contrast.

Contrast Sensitivity with Functional Acuity Contrast Test

The Optec Functional Vision Analyzer[43] (Stereo Optical Co, Inc, Chicago, IL, USA) uses the functional acuity contrast test[44,45] to assess contrast sensitivity at five different spatial frequencies (1.5, 3, 6, 12, 18cpd). A detailed description of the method has been reported previously.[6,46]

Light Scatter

Using the compensation comparison method, the C-Quant Straylight Meter (Oculus GmbH, Wetzler, Germany)[48–50] measures light scatter by objectively determining the amount of intraocular straylight on the retina. Straylight measurements are reported in logarithmic form and judged reliable when standard deviation is $\leq$0.08, and the reliability coefficient is $\geq$1.

Photostress Recovery

Photostress recovery time is measured by assessing CS and investigating the impact of a light stress using a 300 watt tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions GmbH, Berlin, Germany) with a low-pass glass dichroic filter. Subjects view the lamp directly with the study eye (the other eye is covered with an eye patch) at a distance of 1 m for 10 seconds while limiting blinking. After 10 seconds, the lamp is extinguished and removed from the subject's field of view. A letter size of 6/24 (LogMAR 0.6) is displayed on the LogMAR test chart (Test Chart 2000 PRO, Thomson Software Solutions), and viewed at 4 m. A CS value of 0.30 log units (i.e. two lines) above the individual's contrast threshold, is used. The time taken for the subject's eye to recover and see all five letters on the chart after the 10-second exposure is taken as the photostress recovery time.

Macular Pigment Measurement by Customized Heterochromatic Flicker Photometry

Using the Macular Densitometer (Macular Metrics Corp, Providence, RI, USA),[51,52] MP is measured by customized heterochromatic flicker photometry. The spatial profile of MP is assessed by measuring MP at 0.25°, 0.5°, and 1.75° of retinal eccentricity, with a reference point at 7°. A detailed description of the protocol is reported elsewhere.[53,54]

Pupillary Dilation

Subjects' pupils are dilated prior to performing stereo fundus photography, OCT and MP measurement using dual-wavelength autofluorescence. A drop each of 0.5% proxymetacaine hydrochloride, 2.5% phenylephrine hydrochloride, and 1% tropicamide is used.

Macular Pigment Measurement by Dual-wavelength Autofluorescence

Using the Spectralis HRA + OCT MultiColor (Heidelberg Engineering GmbH, Heidelberg, Germany), MP is measured by dual-wavelength (488 nm and 518 nm) autofluorescence.[55–57] Subject details are input into the Heidelberg Eye Explorer (HEYEX version 1.7.1.0) software. Assessment is performed with the room lights off. The following acquisition parameters are used: high speed scan resolution, two seconds cyclic buffer size, internal fixation, 30 seconds movie and manual brightness control. Alignment, focus and illumination are first adjusted in infrared mode. Once the image is evenly illuminated, the laser mode is switched from infrared to blue plus green laser light autofluorescence. Focus and illumination are re-adjusted for optimal acquisition. A 30-second movie of the macula is acquired for subsequent MP analysis using the HEYEX software.

Optical Coherence Tomography

Using the Spectralis HRA + OCT MultiColor (software version 5.6, Heidelberg Engineering GmbH),[58] foveal architecture is assessed using OCT. The device produces non-invasive retinal histological tomographs by integrating spectral (Fourier) domain OCT technology with confocal scanning laser ophthalmoscopy. The following scan acquisition protocol is used for Trial 1: compact volume scan (20° × 20°) of the macular area, 97 B-scans each spaced 60 μm apart at high speed with automatic real-time mean (ART) of 9/frame rate; cross scan (20° × 20°) at high resolution with an ART of 10/frame rate. The following scan acquisition protocol is used for Trial 2: volume scan (20° × 20°) of the macular area, 193 B-scans each spaced 30 μm apart at high speed with ART of 9/frame rate; cross scan (20° × 20°) at high resolution with an ART of 10/frame rate.

Fundus Photography and Grading

All photography is performed by trained and certified photographers. For subjects in Trial 1, standard color

fundus photographs centered on the macula are taken using the Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany) at a 45° magnification setting. These fundus photographs are reviewed by SB in order to exclude any other ocular pathology.

For subjects in Trial 2, stereo color fundus photographs are taken using the Zeiss Visucam 200 (Carl Zeiss Meditec AG) at a 45° magnification setting. The stereo photography technique used is the modified 3-standard stereoscopic fields (Field 1: optic disc, Field 2: macula, Field 3: temporal to macula). In addition, fundus reflex photographs of the external eye are taken in order to document any media opacities. The anonymized photographs are then sent for grading at the Reading Centre, Moorfields Eye Hospital, London, UK, using a secure file transfer protocol. Fundus grading follows the AREDS 11-step severity scale.[24]

Reading Acuity and Reading Speed

This test is only being performed in Trial 2. Reading acuity and reading speed are assessed with the English version of the standardized Radner reading chart[59] at 40 cm. Reading acuity is recorded in logarithm of the reading acuity determination (LogRAD). Reading speed (the time taken to read the number of words in a sentence) is measured in words per minute.

Serum Carotenoid Analysis

Non-fasting blood samples are collected at each study visit by standard venepunture techniques in 9 mL vacuette tubes (BD Vacutainer SST Serum Separation Tubes, Becton, Dickinson and Company, Plymouth, United Kingdom) containing a "Z Serum Sep Clot Activator." All collection tubes are inverted a minimum of five times to ensure appropriate mixing of the clot activator. The blood samples are allowed to clot at room temperature for 30 minutes and then centrifuged for 10 minutes at 2700 rpm in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. After centrifugation, serum is transferred to light-resistant microtubes and stored at −80 °C until the time of analysis. Carotenoid analysis is carried out using a procedure described elsewhere.[31]

Data and Safety Monitoring Committee

An independent data and safety monitoring committee (DSMC) has been appointed to examine and review data collected during the CREST project. This committee scrutinizes the data for evidence of safety and efficacy each year. The CREST DSMC consists of a statistician, a medical ophthalmologist, a health science researcher and a vision scientist. The DSMC has full access to the randomization code for both trials and the authority to break the code if needed. The DSMC has the authority to recommend any of the following: continuation of the study uninterrupted, alteration of either trial or any arm of either trial, or termination of either trial or any arm of either trial.

DISCUSSION

CREST has been designed to investigate the impact of supplementation with a combined carotenoid formulation of L, Z and MZ on visual function in normal subjects (Trial 1) and in subjects with early AMD (Trial 2). Enhancement of visual function as a result of MP augmentation, if present, is likely the result of its attenuation of both chromatic aberration and veiling luminance, with consequential benefits in terms of CS and glare disability,[7] and rests on its anatomical (prereceptoral and central retinal)[1] and optical (short wavelength-filtering) properties.[2] The hypothesis that MP confers protection against AMD is premised on these same attributes of this pigment, as well as its antioxidant capacity,[3,12] as (photo)-oxidative stress is believed to be important in the pathogenesis of this condition.[60]

The landmark AREDS provided level 1 evidence that supplementation with a formulation of antioxidants and zinc, but which was devoid of the macular carotenoids, was associated with risk reduction for visual loss and disease progression in subjects with at least intermediate AMD.[61] The AREDS, therefore, furnished the scientific community with proof of principle that supplemental dietary antioxidants are of benefit in AMD, and somewhat paradoxically, generated interest in the role that MP might play, given its exquisite biological relevance to the tissue affected by this condition. As a consequence, studies were designed to investigate the putative benefits of supplementation with MP's constituent carotenoids on the course of AMD.

Indeed, the subsequent AREDS2 study which was recently published, was designed to assess the impact of supplemental L, Z and omega-3 fatty acids plus co-antioxidants on progression to advanced AMD in eyes with at least intermediate AMD.[62,63] In brief, AREDS2 has found that, controlling for baseline AMD status, none of the treatments were shown to significantly reduce risk of AMD progression relative to the group who received the "placebo" AREDS1 supplement only, although the trend was in favor of the treatments including L and Z (primary analysis). Also, there are many important secondary outcome variables available from this study. For example, there was a statistically significant ($p = 0.01$) reduction of 9% in risk of progression to advanced AMD for subjects

receiving L and Z when compared with subjects not receiving L and Z; participants with the lowest dietary intake of L and Z showed a statistically significant ($p = 0.01$) reduction of 26% in risk of progression to advanced AMD, when compared with subjects not receiving L and Z; and, there was a statistically significant ($p = 0.02$) reduction of 18% in risk of progression to advanced AMD for subjects receiving L and Z in the absence of beta carotene when compared with subjects receiving an AREDS formulation with beta carotene (and not receiving L and Z). However, it is important to point out the major differences between CREST and the AREDS (1 and 2) studies. AREDS was designed and powered to investigate change in AMD morphology following supplementation with antioxidants, whereas CREST (which is a much smaller sample) is designed and powered to investigate change in visual function (i.e. CS) following supplementation with the macular carotenoids. These fundamental differences between two randomized controlled trials need to be appreciated when either of these studies is under discussion.

Another published clinical trial that deserves mention is the Carotenoids in Age-Related Maculopathy (CARMA) study. CARMA was a randomized, double-blind, placebo-controlled clinical trial of L (12 mg) and Z (0.6 mg) supplementation with co-antioxidants versus placebo in patients with AMD.[64] The primary outcome measure, corrected distance visual acuity (CDVA) at one year, did not differ significantly between the placebo and the intervention arms of the study.[65] It was noted, however, that CDVA was significantly better in the intervention arm of the study at 36 months follow-up.[18] In addition, an increase in serum L was associated with significantly improved CDVA and slowing of progression along the AMD severity scale.[18] However, one clear difference between CARMA and CREST was the absence of MZ in the CARMA study intervention, the importance of which has been discussed.

In addition, as a secondary outcome measure, the impact of macular carotenoid supplementation on cognitive function is being assessed. Indeed, a recent study has shown that MP is related to brain carotenoid levels,[66] and there is a growing body of evidence that poor antioxidant status represents risk for age-related loss of cognitive function.[67–69] Therefore, as MP represents a readily accessible and a non-invasive biomarker of antioxidant status within the central nervous system (i.e. retina), we believe that it is important to investigate the relationship, if any, between MP and cognitive function in CREST.

CREST will ascertain, through sufficiently powered, double-blind, randomized controlled clinical trials, the impact of supplementation with all three macular carotenoids (uniquely including the centrally dominant macular carotenoid, MZ) on vision in normal subjects and subjects with AMD. CREST will inform and advance our understanding of the protective and optical hypotheses of MP, and potentially identify ways to optimize vision in the absence of ocular disease and prevent or delay blindness attributable to AMD.

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CREST Pharmacist
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DECLARATION OF INTEREST

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REFERENCES

1. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exper Eye Res* 1997;64:211–218.
2. Snodderly DM, Brown PK, Delori FC, Auran JD. The Macular Pigment.1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci* 1984;25:660–673.
3. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 1997;38: 1802–1811.
4. Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. *Mol Nutr Food Res* 2012;56:270–286.

5. Congdon NG, Friedman DS, Lietman T. Important causes of visual impairment in the world today. *JAMA* 2003;290: 2057–2060.
6. Nolan JM, Loughman J, Akkali MC, et al. The impact of macular pigment augmentation on visual performance in normal subjects: COMPASS. *Vision Res* 2011;51:459–469.
7. Loughman J, Nolan JM, Howard AN, et al. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci* 2012;53:7871–7880.
8. Stringham JM, Garcia PV, Smith PA, et al. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. *Invest Ophthalmol Vis Sci* 2011;52:7406–7415.
9. Nolan JM, Akkali MC, Loughman J, et al. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res* 2012;101: 9–15.
10. Meagher KA, Thurnham DI, Beatty S, et al. Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr* 2013;110:289–300.
11. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. *Vision Res* 1989;29:1095–1101.
12. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys* 2010;504:56–60.
13. Billsten HH, Bhosale P, Yemelyanov A, et al. Photophysical properties of xanthophylls in carotenoproteins from human retinas. *Photochem Photobiol* 2003;78:138–145.
14. Kirby ML, Beatty S, Loane E, et al. A central dip in the macular pigment spatial profile is associated with age and smoking. *Invest Ophthalmol Vis Sci* 2010;51: 6722–6728.
15. Connolly EE, Beatty S, Thurnham DI, et al. Augmentation of macular pigment following supplementation with all three macular carotenoids: an exploratory study. *Curr Eye Res* 2010;35:335–351.
16. Charalampidou S, Loughman J, Nolan J, et al. Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol* 2011; 129:1155–1161.
17. Neelam K, Nolan J, Chakravarthy U, Beatty S. Psychophysical function in age-related maculopathy. *Surv Ophthalmol* 2009;54:167–210.
18. Beatty S, Chakravarthy U, Nolan JM, et al. Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology* 2013;120:600–606.
19. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials* 2010;11:32.
20. Friedman LM, Furberg CD, Demets DL. *Fundamentals of clinical trials*. 4th ed. New York: Springer; 2010.
21. Kramer CFL, Theimann J. *How many subjects?: Statistical power analysis in research*. Thousand Oaks, CA: Sage Publications; 1987.
22. Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
23. Hintze J. PASS 2008. In: NCSS LLC. Kaysville, Utah; 2008.
24. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol* 2005;123:1484–1498.
25. Feinman RD. Intention-to-treat. What is the question? *Nutr Metab (Lond)* 2009;6:1.
26. Gupta SK. Intention-to-treat concept: a review. *Perspect Clin Res* 2011;2:109–112.
27. Newell DJ. Intention-to-treat analysis: implications for quantitative and qualitative research. *Int J Epidemiol* 1992; 21:837–841.
28. Orr P, Rentz AM, Margolis MK, et al. Validation of the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25) in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2011;52:3354–3359.
29. Revicki DA, Rentz AM, Harnam N, et al. Reliability and validity of the National Eye Institute Visual Function Questionnaire-25 in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2010;51:712–717.
30. Mangione CM, Lee PP, Gutierrez PR, et al. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* 2001;119:1050–1058.
31. Meagher KA, Thurnham DI, Beatty S, et al. Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr* 2013;110:289–300.
32. Lawrence AD, Sahakian BJ. The neuropsychology of frontostriatal dementias. In: Woods RT, editor. *Handbook of the clinical psychology of aging*. New York: Wiley; 1996:243–265.
33. Robbins TW, James M, Owen AM, et al. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia* 1994;5:266–281.
34. Wild K, Howieson D, Webbe F, et al. Status of computerized cognitive testing in aging: a systematic review. *Alzheimers Dement* 2008;4:428–437.
35. Lawrence AD, Sahakian BJ, Hodges JR, et al. Executive and mnemonic functions in early Huntington's disease. *Brain* 1996;119:1633–1645.
36. Owen AM, Downes JJ, Sahakian BJ, et al. Planning and spatial working memory following frontal lobe lesions in man. *Neuropsychologia* 1990;28:1021–1034.
37. Fried R, Hirshfeld-Becker D, Petty C, et al. How informative is the CANTAB to assess executive functioning in children with ADHD? A controlled study. J Atten Disord 2012, Aug 24 (Epub ahead of print).
38. Sahakian BJ, Downes JJ, Eagger S, et al. Sparing of attentional relative to mnemonic function in a subgroup of patients with dementia of the Alzheimer type. *Neuropsychologia* 1990;28:1197–1213.
39. Robbins TW, James M, Owen AM, et al. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia* 1994;5:266–281.
40. Sweeney JA, Kmiec JA, Kupfer DJ. Neuropsychologic impairments in bipolar and unipolar mood disorders on the CANTAB neurocognitive battery. *Biol Psychiatry* 2000; 48:674–684.
41. Cognition Limited. *CANTAB Eclipse Test Administration Guide*. Cambridge, UK: Cambridge Cognition Limited; 2012.
42. Charalampidou S, Nolan J, Loughman J, et al. Psychophysical impact and optical and morphological characteristics of symptomatic non-advanced cataract. *Eye (Lond)* 2011;25:1147–1154.
43. Hohberger B, Laemmer R, Adler W, et al. Measuring contrast sensitivity in normal subjects with OPTEC 6500: influence of age and glare. *Graefes Arch Clin Exp Ophthalmol* 2007;245:1805–1814.
44. Hitchcock EM, Dick RB, Krieg EF. Visual contrast sensitivity testing: a comparison of two F.A.C.T. test types. *Neurotoxicol Teratol* 2004;26:271–277.
45. Terzi E, Buhren J, Wesemann W, Kohnen T. [Frankfurt-Freiburg Contrast and Acuity Test System (FF-CATS). A new test to determine contrast sensitivity under variable

ambient and glare luminance levels]. *Ophthalmologe* 2005; 102:507–513.
46. Loughman J, Akkali MC, Beatty S, et al. The relationship between macular pigment and visual performance. *Vision Res* 2010;50:1249–1256.
47. van Gaalen KW, Jansonius NM, Koopmans SA, et al. Relationship between contrast sensitivity and spherical aberration: comparison of 7 contrast sensitivity tests with natural and artificial pupils in healthy eyes. *J Cataract Refract Surg* 2009;35:47–56.
48. Coppens JE, Franssen L, Van Rijn LJ, van den Berg TJ. Reliability of the compensation comparison stray-light measurement method. *J Biomed Opt* 2006;11:34027.
49. Franssen L, Coppens JE, van den Berg TJ. Compensation comparison method for assessment of retinal straylight. *Invest Ophthalmol Vis Sci* 2006;47:768–776.
50. van den Berg TJ, Van Rijn LJ, Michael R, et al. Straylight effects with aging and lens extraction. *Am J Ophthalmol* 2007;144:358–363.
51. Wooten BR, Hammond BR. Spectral absorbance and spatial distribution of macular pigment using heterochromatic flicker photometry. *Optometry Vis Sci* 2005;82:378–386.
52. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci* 1999;40:2481–2489.
53. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res* 2008;87:445–453.
54. Loane E, Stack J, Beatty S, Nolan JM. Measurement of macular pigment optical density using two different heterochromatic flicker photometers. *Curr Eye Res* 2007; 32:555–564.
55. Delori FC, Goger DG, Hammond BR, et al. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J Optical Soc Am A-Optics Image Sci Vis* 2001;18:1212–1230.
56. Wustemeyer H, Jahn C, Nestler A, et al. A new instrument for the quantification of macular pigment density: first results in patients with AMD and healthy subjects. *Graefes Arch Clin Exp Ophthalmol* 2002;240:666–671.
57. Trieschmann M, Heimes B, Hense H, Pauleikhoff D. Macular pigment optical density measurement in autofluorescence imaging: comparison of one- and two-wavelength methods. *Graefes Arch Clin Exp Ophthalmol* 2006;244:1565–1574.
58. Wolf-Schnurrbusch UE, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci* 2009;50:3432–3437.
59. Stifter E, Konig F, Lang T, et al. Reliability of a standardized reading chart system: variance component analysis, test-retest and inter-chart reliability. *Graefes Arch Clin Exp Ophthalmol* 2004;242:31–39.
60. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115–134.
61. Kassoff A, Kassoff J, Buehler J, et al. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss – AREDS Report No. *8. Arch Ophthalmol* 2001;119:1417–1436.
62. Chew EY, Clemons TE, SanGiovanni JP, et al. Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression: AREDS2 Report No. 3. *JAMA Ophthalmol* 2013.
63. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA. 2013;309: 2005–2015.
64. Neelam K, Hogg RE, Stevenson MR, et al. Carotenoids and co-antioxidants in age-related maculopathy: design and methods. *Ophthalmic Epidemiol* 2008;15:389–401.
65. Beatty S, Nolan JM, Muldrew KA, et al. Visual outcome after antioxidant supplementation. *Ophthalmology* 2013;120: 645.
66. Vishwanathan R, Neuringer M, Snodderly DM, et al. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutr Neurosci* 2013;16:21–29.
67. Rafnsson SB, Dilis V, Trichopoulou A. Antioxidant nutrients and age-related cognitive decline: a systematic review of population-based cohort studies. *Eur J Nutr* 2013;52: 1553–1567.
68. Gray SL, Hanlon JT, Landerman LR, et al. Is antioxidant use protective of cognitive function in the community-dwelling elderly? *Am J Geriatr Pharmacother* 2003;1:3–10.
69. Salerno-Kennedy R, Cashman KD. Relationship between dementia and nutrition-related factors and disorders: an overview. *Int J Vitam Nutr Res* 2005;75:83–95.

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Prevalence of age-related macular degeneration in the Republic of Ireland

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ABSTRACT

Background Age-related macular degeneration (AMD) remains the most common cause of visual loss among subjects over 50 years of age in the developed world. The Irish Longitudinal study on Ageing (TILDA) is a population-based study of subjects aged 50 years or older, designed to investigate factors that influence ageing, and has enabled this investigation of the prevalence of AMD in the Republic of Ireland (ROI).

Methods Data collected from a nationally representative sample of community-living older adults aged 50 years and over in ROI over the period November 2009 to July 2011. 5035 participants attended the TILDA health centre for assessment. Retinal photographs were obtained in 4859 of these participants. Retinal grading was performed in a masked fashion using a modified version of the International Classification and Grading System for AMD.

Results Adjusting for lower response rates among older subjects, the estimated overall prevalence of any AMD was 7.2% (95% CI 6.5% to 7.9%) in the population aged 50 years or older. The estimated prevalence of early AMD was 6.6% (95% CI 5.9% to 7.3%), and the estimated prevalence of late AMD was 0.6% (95% CI 0.4% to 0.8%). Statistically significant associations with AMD included increasing age and family history of the condition.

Conclusions This is the first study to provide prevalence estimates of AMD in ROI and will inform eye care professionals and policymakers involved in the delivery and planning of care for those afflicted with this condition.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blind registration in the developed world. In the Republic of Ireland (ROI), AMD is estimated to account for 25% of all blind registration (57.1 per 100 000 adults).[1] Early AMD is characterised by drusen and/or pigmentary abnormalities, whereas the late (advanced) form of AMD is visually consequential and can be classed as atrophic (geographic atrophy) or neovascular.[2]

Subjects with early AMD benefit from antioxidant supplementation, in terms of reduced risk of visual loss and disease progression.[3 4] Currently, there is no effective treatment for atrophic AMD, whereas neovascular AMD is treated by intravitreal injections of antivascular endothelial growth factor therapy.[5 6] The ongoing nature of treatment for neovascular AMD has profound cost implications to patients and to society, reflected in the recent retrospective observational study that demonstrated that new cases of neovascular AMD were associated with substantial discrepancies in total medical costs (41% higher compared with non-neovascular AMD controls).[7] The cost implications for neovascular AMD treatment are, however, balanced against savings associated with this treatment (improvement in visual acuity and reduction in cases of legal blindness).[8] Patients with untreated or untreatable advanced AMD invariably suffer from impairment of central vision, with consequential loss of social independence as a result of a concomitant inability to read, recognise faces, watch television or drive.[9]

The Irish Longitudinal Study on Ageing (TILDA, http://www.tilda.ie)[10] is a prospective cohort study aimed at providing representative and comprehensive data relating to older people and the ageing population in ROI, by collecting data on the social, economic and health status of participants aged 50 years and over. At baseline (wave 1), TILDA collected vision data, including retinal photographs for grading of AMD, as part of the health assessment.

Although the prevalence of AMD has been reported in population-based studies for many different countries,[11 12] the TILDA sample provides an unprecedented opportunity to investigate the prevalence of AMD from a population-based random sample selected from ROI.

MATERIALS AND METHODS

Study population

The design and methodology of TILDA has been described in detail elsewhere.[10] The TILDA sampling frame was based on a comprehensive record of all residential addresses in ROI compiled by the Irish Postal Service (An Post) and Ordnance Survey Ireland (RANSAM system, developed by the Economic and Social Research Institute of Ireland), and the sampling method was designed to achieve a population-representative sample of (community-resident) individuals aged 50 years or older. The sampling frame was made up of 3155 clusters (500–1180 residential addresses in each cluster). A total of 640 clusters were randomly selected using proportionate stratification by socioeconomic status (percentage in professional/managerial occupations), age structure (percentage of population aged 50 years or older) and geography. Forty residential addresses were randomly selected from each of the 640 clusters, resulting in a list of 25 600 addresses. A letter of invitation was sent to each of the sampled addresses, furnishing residents with information about the study and informing residents of the

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proposed visit by a member of the field staff. All sampled addresses were then visited by a member of the field staff, and residents that were deemed eligible were then invited to participate. All persons aged 50 years and over (primary respondents) and their spouses or partners of any age (secondary respondents) were eligible for inclusion in TILDA. Of note, secondary respondents are not included in this analysis.

In all, 8504 participants were sampled, with 8175 participants aged 50 years or older. Enrolled participants completed the computer-assisted personal interviewing questionnaire, self-completion questionnaires and were offered either a health centre assessment or a home-based assessment.[10 13] Of note, 5035 (62%) participants underwent a health centre assessment, which included retinal photographs for AMD grading. Figure 1 illustrates the TILDA baseline (wave 1) participants included in the current study. Data for this report were collected as part of the first wave of TILDA, which was initiated in October 2009, and completed in July 2011.

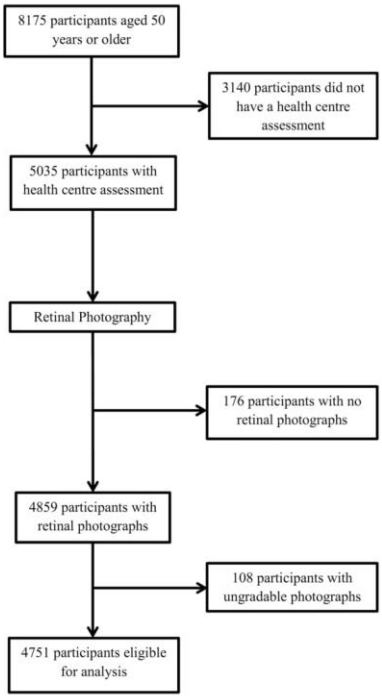

Figure 1 A total of 8175 participants aged 50 years or older completed the Irish Longitudinal study on Ageing (TILDA) baseline (wave 1) interview. Health assessments were conducted in clinical centres in Dublin and Cork, Republic of Ireland. Participants who refused or were unable to attend clinical centres were given the option of a home-based clinical assessment. Home-based clinical assessment did not include retinal photography. Retinal photographs were taken at the clinical centres using the NIDEK AFC-210 camera. Subjects with no photographs were either due to the following reasons: unable, unwilling and technical failure. Photographs were judged as ungradable based on photographic quality.

Retinal photography

Retinal photography was carried out using the NIDEK AFC-210 non-mydriatic auto-fundus camera, through a non-dilated pupil, by TILDA research nurses. TILDA nurses were trained and certified by experts from the Ocular Epidemiology Reading Centre at the University of Wisconsin, Madison, USA. One 45° monoscopic colour photograph, centred on the macula (Early Treatment Diabetic Retinopathy Study standard field 2), was obtained for each eye. The photographs were anonymised using a unique identifier and transferred to the Moorfields Eye Hospital (MEH) Reading Centre, London, UK (http://www.readingcentre.org) and the Macular Pigment Research Group (MPRG, http://www.mprg.ie), Vision Research Centre, Waterford, Ireland.

Retinal grading

Retinal photographs were graded at MPRG, Vision Research Centre, Waterford, Ireland, by a masked grader (KOA) who was trained and certified at the MEH Reading Centre. Grading was carried out under the supervision of the MEH Reading centre manager (TP) using a modified version of the International Classification and Grading System for AMD.[2]

The following AMD features were evaluated: the presence of >10 hard drusen (<63 μm), soft drusen (>125 μm), atrophic AMD and signs of neovascular AMD (choroidal neovascularisation, retinal pigment epithelium detachment, disciform scar). Early AMD was defined as the presence of >10 hard drusen (<63 μm) and/or the presence of soft drusen (>125 μm). Late AMD was defined as the presence of atrophic AMD and/or neovascular AMD. Mixed AMD was defined as the presence of atrophic AMD in one eye and neovascular AMD in the other eye.

AMD features graded as questionable were adjudicated by the MEH Reading Centre. To ensure that valid and reliable data with respect to AMD grading were secured, the following quality assurance measures were taken: first, 10% of images were regraded by the MEH Reading Centre for concordance. Second, intragrader reliability was assessed by the regrading of a 3% randomly selected sample of retinal photographs graded by the principal grader (KOA) with a minimum interval of 14 days between visualisation of the images in question.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, V.20.0. Armonk, New York, New York, USA; weighted kappa statistics, not available in SPSS, were obtained using the statistical programming language R.[14] For purposes of statistical analysis, the worst eye, in terms of AMD severity, was assigned to each participant.

Of 5035 TILDA participants who presented at health centres for clinical examination, 4859 had retinal photographs for at least one eye (right eye in 4808 and left eye in 4798). Intragrader reliability was assessed in 300 eyes using the kappa statistic. Demographic characteristics of participants with gradable photographs were compared with those with ungradable photographs using independent samples t test or χ^2 test of independence. After excluding subjects with ungradable fundus photographs, 4751 participants remained for estimating AMD prevalence.

Selection of households for inclusion in this study was random, but we identified two major sources of subsequent bias. In addition to the usual non-response bias, common to most social surveys, it was evident that non-attendance at health

centres was more common, for example, among older subjects, and this introduced additional bias. In order to identify and adjust for bias, study participants were initially classified by three variables—age (three categories, 50–64, 65–74 and ≥75), gender (male, female) and education (three categories, primary/none, secondary and tertiary/higher), resulting in a total of 18 (3×2×3) sample subgroups. Comparison of numbers in these subgroups, with what would be expected from the corresponding data for the population of ROI (available from the Central Statistics Office, Dublin),[15] revealed significant discrepancies. For instance, female, third-level educated and younger subjects were over-represented in the sample. However, before developing sample weights to adjust for these discrepancies, we first used logistic regression to investigate the relationship between AMD prevalence and these three variables jointly. As only the age variable was significantly related to AMD in the regression analysis, sample weights, adjusting for disproportionate representation, were calculated using just this (age) variable. These weights were then applied in all calculations of overall AMD prevalence.

The relationship between the prevalence of AMD, and established or putative risk factors for this condition, other than age, was investigated by logistic regression. Each such investigation controlled for age and included an age*risk factor interaction term. In reporting results, however, we elected to stratify by age and report prevalence with respect to potential risk factors within each age group. The 5% level of statistical significance was applied throughout all risk factor analyses, without adjustment for multiple testing.

RESULTS

Demographic characteristics of the TILDA participants studied as part of this investigation are reported in table 1. Participants with ungradable photographs were significantly older and had poorer visual acuity compared with participants with gradable photographs.

Intragrader reliability showed moderate agreement for all categories.[16] Kappa and weighted kappa scores varied from 0.51 to 0.61 and 0.60 to 0.61, respectively. Exact agreement for AMD features varied from 91% to 96%.

Prevalence of AMD

Increasing age was the only variable exhibiting a statistically significant association with AMD (defined as any AMD yes/no) in a logistic regression model including the variables age, gender and education. The development of sample weights based on this age variable is presented in table 2. The age group ≥75 constitutes over 18% of the over 50s in the Irish population, but only 8.5% of the sample reported herein. Therefore, ignoring this under-representation in the sample of the oldest age group would lead to an underestimate of prevalence of AMD. The weights (final column of table 2) adjust for this: every subject aged ≥75 in the sample is treated (in estimating overall prevalence) as representing 544 subjects in the population, whereas sample subjects in the other two age groups are treated as representative of about 225 subjects in the population.

Table 3 shows the prevalence of each category of AMD, as well as the estimated prevalence of AMD (all forms) for those aged 50 years or older in ROI. These estimates are based on the weights presented in table 2. Adjusting for age, the prevalence of AMD (any form) was 7.2% (95% CI 6.5% to 7.9%); the prevalence of early AMD was 6.6% (95% CI 5.9% to 7.3%); the prevalence of late AMD was 0.6% (95% CI 0.4% to 0.8%); the prevalence of atrophic AMD was 0.3% (95% CI 0.1% to 0.5%) and the prevalence of neovascular AMD was 0.3% (95% CI 0.1% to 0.5%).

Analysis of AMD by other demographic subgroups, stratifying by age, is shown in table 4. The p values displayed in table 4 were obtained from the χ^2 test for contingency tables. Some differences in prevalence of AMD are evident in table 4 with

Table 1 Demographic and other characteristics of TILDA baseline (wave 1) participants included in study analyses

Characteristic	Mean±SD
Age	61.61±8.10
BMI	28.42±4.51
VA	0.06±0.18
Characteristic	**n (%)**
Gender	
Male	2169 (45.7)
Female	2582 (54.3)
Total	4751 (100)
Education	
Primary/none	1013 (21.3)
Secondary	1986 (41.8)
Tertiary/higher	1750 (36.8)
Total	4749 (100)
Location	
Dublin	1383 (29.1)
Other urban	1259 (26.5)
Rural	2104 (44.3)
Total	4746 (100)
Smoking	
Never	2189 (46.1)
Past	1856 (39.1)
Current	706 (14.9)
Total	4751 (100)
Family history	
No/don't know	4496 (94.6)
Yes	255 (5.4)
Total	4751 (100)
Cardiovascular disease	
No	2987 (62.7)
Yes	1771 (37.3)
Total	4751 (100)
Stroke	
No	4690 (98.7)
Yes	61 (1.3)
Total	4751 (100)

Interval data presented as mean±SD. Categorical data presented as percentages. Cardiovascular disease refers to participants who reported no self-reported doctor's diagnosis of any of the following: angina, heart attack, heart failure, stroke, transient ischaemic attack and heart murmur. Stroke refers to participants who reported a doctor's diagnosis of stroke.
Age, age in years; BMI, body mass index (kg/m^2); Dublin, residence in Dublin city or county; Other urban, residence in other urban, another town or city in the Republic of Ireland; education, level of education; family history, subjects who reported a family history of age-related macular degeneration (AMD)—family history was defined as having a first-degree relative, that is, parent or sibling with AMD; location, location of residence in the Republic of Ireland; primary/none, subjects who did not have education and those with only primary education; rural, residence in rural area in the Republic of Ireland; secondary, subjects who completed a junior certificate or leaving certificate or equivalent; smoking, smoking status of subjects classified as never (no reported history of smoking), past (past smokers) and current (current smokers); tertiary, subjects who completed a diploma, first degree or higher degree; TILDA, the Irish Longitudinal study on Ageing; VA, visual acuity; visual acuity recorded in logarithm of the minimum angle of resolution (logMAR)—visual acuity was measured in both eyes using an Early Treatment Diabetic Retinopathy Study logMAR chart at a test distance of 4 m; only eye with best visual acuity is reported.

Table 2 Sample weights for analysis

Age group	Population (%)	Sample (%)	Weight
50–64	700 800 (58.4)	3093 (65.1)	226.6
65–74	280 900 (23.4)	1256 (26.4)	223.6
≥75	218 700 (18.2)	402 (8.5)	544.0

Weights developed from age variable. Population data were based on the Republic of Ireland population census 2011.

respect to gender, education and geographic location ('Dublin' vs 'other urban' vs 'rural'). However, a statistically significant difference was observed only for early AMD with respect to geographic location (with prevalence values of 10.8%, 18.4% and 6.3% of participants categorised as 'Dublin', 'other urban' and 'rural', respectively). Some other, statistically non-significant, findings in table 4 may be attributable to the small sample sizes of the respective subgroups; for example, prevalence of (any and early) AMD is clearly greater for women than for men in the ≥75 age group.

The prevalence of drusen within demographic subgroups, stratifying by age, is reported in table 5.

Risk factors for (any) AMD

Each risk factor for AMD (as listed in table 1) was investigated separately via logistic regression models containing that risk factor; each such model also included age, and the interaction of age with that risk factor. The dependent variable in these analyses was any AMD (yes/no); logistic analyses for smaller categories of AMD were deemed statistically infeasible. Subjects with ungradable photographs, and subjects unsure of family history for AMD, were omitted from all regression analyses.

Age was highly statistically significant in all logistic regression analyses ($p<0.005$ in all analyses). Family history was also statistically significant (OR=0.28, 95% CI for OR=0.11 to 0.69, $p=0.006$), but the age*family history interaction was not ($p=0.17$). None of the other risk factors analysed (gender, education, geographic location, body mass index (BMI), stroke, cardiovascular disease, smoking), nor their respective interactions with the age risk factor, were statistically significant ($p>0.05$ for all). For example, we obtained $p=0.10$ for BMI and $p=0.16$ for the interaction term, $p=0.44$ for cardiovascular disease and $p=0.76$ for the interaction, $p=0.32$ for stroke and $p=0.38$ for the interaction.

We considered that the other risk factors merited further exploration, beyond the basic regression findings, and that the best way to do this was to stratify by age and analyse each risk factor separately within each age group. Table 4 (first three age columns) contains this information for any AMD, and for each of the three demographic risk factors (gender, education, location). The p values displayed in table 4 were obtained from the χ^2 test for contingency tables; all p values exceed 0.05 and so support the earlier findings from the logistic regression analyses.

Positive family history was defined as having a first-degree relative, that is, parent or sibling, with AMD. The relationship of family history to (any) AMD, stratifying by age, is presented in table 6. The prevalence of AMD was significantly higher in those who reported a positive family history in the age group 65–74 (14.5% with AMD, $p=0.017$) and ≥75 (33.3% with AMD, $p=0.002$). These significant findings support the earlier findings from the logistic regression analysis.

The remaining risk factor (smoking) was not significantly associated with (any) AMD, after controlling for age ($p=0.59$ for smoking, $p=0.44$ for the interaction, in the logistic regression). Nevertheless, we have included some details of the smoking–AMD relationship in table 6. While not statistically significant, it is worth noting that in all three age groups, in table 6, prevalence of (any) AMD was higher for current smokers than for either of the other smoking groups. It is also worth reporting that, in the case of neovascular AMD (consistently associated with smoking in the literature), six of nine study subjects (67%) with this condition are past or current smokers, whereas just 54% of the TILDA sample are past or current smokers.

Other results

While logistic regression was not considered feasible for risk factor analysis for the rarer forms of AMD, table 4 has some interesting contingency table results for these. A statistically significant difference was observed for early AMD with respect to geographic location (with prevalence values of 10.8%, 18.4% and 6.3% of participants categorised as 'Dublin', 'other urban' and 'rural', respectively). Some other, statistically non-significant, findings in table 4 may be attributable to the small sample sizes of the respective subgroups; for example, prevalence of any AMD (and also early AMD) is clearly greater for women than for men in the ≥75 age group.

The prevalence of drusen within demographic subgroups, stratifying by age, is reported in table 5. There are three statistically significant results highlighted in table 5, but in general, definitive conclusions based on table 5 results (as in tables 4 and 6) are problematic because of the small numbers of subjects in certain subgroups.

DISCUSSION

This study was undertaken to investigate the prevalence of AMD in ROI using the TILDA wave 1 (baseline) sample. Subjects were randomly selected from the ROI population and therefore representative of the community-dwelling population

Table 3 Prevalence of age-related macular degeneration (AMD) by age category

Age groups (years)	Any AMD n (%)	Early AMD n (%)	Late AMD n (%)	Atrophic AMD n (%)	Neovascular AMD n (%)	Mixed AMD* n (%)
50–64	156 (5.0)	152 (4.9)	4 (0.1)	1 (0.0)	3 (0.1)	0 (0.0)
65–74	98 (7.8)	92 (7.3)	6 (0.5)	3 (0.2)	2 (0.2)	1 (0.1)
≥75	53 (13.2)	44 (11.0)	9 (2.2)	5 (1.3)	4 (1.0)	0 (0.0)
Overall unweighted	307 (6.5)	288 (6.1)	19 (0.4)	9 (0.2)	9 (0.2)	1 (0.0)
Overall weighted	86 095 (7.2)	78 950 (6.6)	7144 (0.6)	3618 (0.3)	3303 (0.3)	224 (0.0)
95% CI (overall weighted)	6.5 to 7.9	5.9 to 7.3	0.4 to 0.8	0.1 to 0.5	0.1 to 0.5	–

*Mixed AMD—subject has neovascular AMD in one eye and atrophic AMD in the other eye.

Table 4 Prevalence of age-related macular degeneration (AMD) by demographic subgroups, stratified by age group

	Any AMD Age groups			Early AMD Age groups			Late AMD Age groups			Atrophic AMD Age groups			Neovascular AMD Age groups		
Characteristic, n (%)	50–64	65–74	≥75	50–64	65–74	≥75	50–64	65–74	≥75	50–64	65–74	≥75	50–64	65–74	≥75
Gender															
Male	71 (5.2)	47 (7.8)	21 (11.1)	70 (5.1)	44 (7.3)	16 (8.4)	1 (0.1)	3 (0.5)	5 (2.6)	0 (0.0)	2 (0.3)	3 (1.6)	1 (0.1)	1 (0.2)	2 (1.1)
Female	85 (4.9)	51 (7.9)	32 (15.3)	82 (4.8)	48 (7.4)	28 (13.4)	3 (0.2)	3 (0.5)	4 (1.9)	1 (0.1)	1 (0.2)	2 (1.0)	2 (0.1)	1 (0.2)	2 (1.0)
p Value	0.771	0.947	0.211	0.672	0.927	0.113	0.435	0.933	0.630	0.372	0.524	0.577	0.700	0.961	0.924
Education															
Primary/none	28 (5.7)	34 (8.5)	15 (12.0)	27 (5.5)	33 (8.3)	9 (7.2)	1 (0.2)	1 (0.3)	6 (4.8)	0 (0.0)	0 (0.0)	3 (2.4)	1 (0.2)	1 (0.3)	3 (2.4)
Secondary	72 (5.2)	30 (6.9)	21 (13.8)	69 (4.9)	27 (6.2)	19 (12.5)	3 (0.2)	3 (0.7)	2 (1.3)	1 (0.1)	1 (0.2)	1 (0.7)	2 (0.1)	1 (0.2)	1 (0.7)
Tertiary	56 (4.6)	34 (8.1)	17 (14.2)	56 (4.6)	32 (7.6)	16 (13.3)	0 (0.0)	2 (0.5)	1 (0.8)	0 (0.0)	2 (0.5)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
p Value	0.628	0.658	0.863	0.744	0.501	0.242	0.277	0.656	0.069	0.545	0.377	0.382	0.356	0.603	0.147
Location															
Dublin	33 (4.0)	27 (7.0)	21 (13.3)	33 (4.0)	24 (6.2)	17 (10.8)	0 (0.0)	3 (0.8)	4 (2.5)	0 (0.0)	1 (0.3)	2 (1.3)	0 (0.0)	2 (0.5)	2 (1.3)
Other urban	48 (4.9)	27 (7.8)	18 (18.4)	46 (5.6)	26 (7.5)	18 (18.4)	2 (0.2)	1 (0.3)	0 (0.0)	1 (0.1)	1 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Rural	75 (5.2)	43 (8.3)	14 (9.8)	73 (5.1)	41 (7.9)	9 (6.3)	2 (0.1)	2 (0.4)	5 (3.5)	0 (0.0)	1 (0.2)	3 (2.1)	2 (0.1)	0 (0.0)	2 (1.4)
p Value	0.185	0.776	0.156	0.264	0.618	**0.013**	0.379	0.584	0.191	0.248	0.955	0.355	0.569	0.106	0.515

Level of significance set at p<0.05; statistical significance tested with χ^2 test for contingency tables.
Bold signifies statistically significant p value.
Dublin, residence in Dublin City or county; Other urban, residence in other urban, another town or city in the Republic of Ireland; education, level of education; location, location of residence in the Republic of Ireland; primary/none, subjects who did not have education and those with only primary education; rural, residence in rural area in the Republic of Ireland; secondary, subjects who completed a junior certificate or leaving certificate or equivalent; tertiary, subjects who completed a diploma, first degree or higher degree.

aged 50 years or older. The prevalence of AMD (any form) in ROI is estimated at 7.2%, after adjusting for different non-response rates (and different attendance rates at the health centres) in different age groups.

The prevalence estimates (and all other results presented in this paper) were obtained from subjects with gradable photographs only. Including the 108 ungradable subjects, and assuming these have the same prevalence rates within age groups as the gradable subjects, leads to some changes in AMD sample numbers within each age group, but also to changes in weights. The net effect is an overall age-adjusted estimate of 7.17% for any AMD, that is, practically identical to the estimate from the gradable subjects only.

Different population-based studies reporting prevalence estimates of AMD have adopted various photography/grading protocols and definitions for AMD. Table 7 provides AMD prevalence estimates from other studies for comparison with estimates from our TILDA study. Some large differences in reported AMD prevalence are evident in table 7 and could be either attributable to differences between the populations studied or to differences in study design (eg, grading techniques, photography protocols, sampling and recruitment strategies and age range of sample). A recent meta-analysis of the prevalence of AMD in populations of European ancestry found substantial variability in prevalence rates between studies, with differences in late AMD primarily due to differences in age profile and study design.[22] For the purpose of emphasising the important role of such variables on published findings, it is noteworthy that the prevalence of early AMD was as high as 52.3% and 58.6% in the Greenland Inuit Eye Study[23] and Prevalence of Age-related Macular Degeneration in Italy study,[24] respectively. However, in our study, we estimate the prevalence of early AMD in ROI to be 6.6%, consistent with many reports of ethnically comparable populations (eg, National Health and Nutrition Examination Survey (NHANES) 2005–2008 US population:[11] 5.7%).

The prevalence of late AMD in the current study was 0.6%, consistent with some previous reports (eg, NHANES 2005–2008 US population:[11] 0.8%; Visual Impairment Project:[25] 0.68%) but less than that reported by others (eg, Beaver Dam Eye Study:[18] 1.6%; Rotterdam Study:[12] 1.7%). However, the prevalence of neovascular AMD and atrophic AMD is known to vary between studies. In our study, we report prevalence for each of the two forms of late AMD (atrophic and neovascular) to be equal (at 0.3% each), whereas some previous studies have reported the atrophic form to be more common than the neovascular form (eg, Reykjavik Eye Study:[20] atrophic 3.2%, neovascular 0.7%). In contrast, however, the neovascular form of AMD has been reported to be more prevalent than the atrophic form of the condition in many other studies (eg, Beaver Dam Eye Study:[18] atrophic 0.6%, neovascular 1.2%; Blue Mountains Eye Study:[19] atrophic 0.7%, neovascular 1.3%; Rotterdam Study:[12] atrophic 0.6%, neovascular 1.1%; European Eye Study:[26] atrophic 1.2%, neovascular 2.3%).

In general, we found that differences in prevalence of AMD between demographic subgroups were not statistically significant, after controlling for age (tables 4 and 5). However, especially for the rarer forms of AMD, these findings are based on small cell frequencies and should be treated circumspectly.

For both men and women in this study, the impact of age on prevalence appears much stronger for the more severe forms of the disease. For example, in table 4, the prevalence of late AMD in the ≥75 age group (at 2.6%) is 5.2 times the prevalence observed for the 60–74 age group for men and 3.8 times the

Table 5 Prevalence of drusen by demographic subgroups, stratified by age group

	Hard drusen (<63 μm)* Age groups			Soft drusen (>125 μm) Age groups		
Characteristic, n (%)	**50–64**	**65–74**	**≥75**	**50–64**	**65–74**	**≥75**
Gender						
Male	36 (2.6)	15 (2.5)	2 (1.1)	34 (2.5)	29 (4.8)	14 (7.4)
Female	49 (2.9)	20 (3.1)	9 (4.3)	33 (1.9)	28 (4.3)	19 (9.1)
p Value	0.702	0.515	**0.047**	0.289	0.688	0.533
Education						
Primary/none	18 (3.7)	13 (3.3)	4 (3.2)	9 (1.8)	20 (5.0)	5 (4.0)
Secondary	40 (2.9)	11 (2.5)	4 (2.6)	29 (2.1)	16 (3.7)	15 (9.9)
Tertiary	27 (2.2)	11 (2.6)	3 (2.5)	29 (2.4)	21 (5.0)	13 (10.8)
p Value	0.240	0.789	0.938	0.735	0.559	0.104
Location						
Dublin	23 (2.8)	10 (2.6)	3 (1.9)	10 (1.2)	14 (3.6)	14 (8.9)
Other urban	21 (2.6)	11 (3.2)	5 (5.1)	25 (3.1)	15 (4.3)	13 (13.3)
Rural	41 (2.9)	13 (2.5)	3 (2.1)	32 (2.2)	28 (5.4)	6 (4.2)
p Value	0.929	0.816	0.262	**0.033**	0.445	**0.040**

Level of significance set at p<0.05; statistical significance tested with χ^2 test for contingency tables.
Bold signifies statistically significant p value.
*More than 10 hard drusen (<63 μm).
Dublin, residence in Dublin city or county; Other urban, residence in other urban, another town or city in the Republic of Ireland; education, level of education; location, location of residence in the Republic of Ireland; primary/none, subjects who did not have education and those with only primary education; rural, residence in rural area in the Republic of Ireland; secondary, subjects who completed a junior certificate or leaving certificate or equivalent; tertiary, subjects who completed a diploma, first degree or higher degree.

observed prevalence for women. In contrast, for early AMD, the corresponding risk ratios are 1.2 and 1.8 for men and women, respectively. Similarly, in table 5, prevalence of soft drusen in the ≥75 group is 1.5 times and 2.1 times the prevalence observed in the 60–74 group for men and women, respectively, whereas the corresponding risk ratios for hard drusen are just 0.4 and 1.4 for men and women, respectively.

While primarily concerned with the prevalence of AMD, we also investigated possible associations with this condition, especially for variables that have been previously identified as risk factors for AMD. In this regard, we report that the prevalence of AMD increases with increasing age, consistent with all other studies.[18 19] Also, family history for AMD was strongly associated with prevalence of this condition, consistent with other studies.[27 28] In fact, in the 65–74 and ≥75 age groups, the prevalence of AMD is strikingly greater for subjects who reported a family history of this condition. Self-reported data with respect to family history for AMD are problematic for the following reasons: reporting of AMD among siblings is subject to influence by the number of siblings; reporting of AMD among parents is subject to influence by the longevity of those parents; reporting of AMD among participants who were adopted will be irrelevant with respect to a genetic predisposition for AMD; and finally, reporting of early AMD is likely to be under-represented because it is typically asymptomatic. Nevertheless, and with full appreciation of these limitations, and given that we excluded subjects who replied that they did not know whether or not a first-degree relative suffered from AMD, we believe that our findings that self-reported family history of AMD is a risk factor for the condition are important.

Table 6 Risk factors for prevalence of age-related macular degeneration (AMD), stratified by age group

	Any AMD Age groups		
Characteristic, n (%)	**50–64**	**65–74**	**≥75**
Smoking			
Never	77 (5.4)	39 (6.7)	24 (13.0)
Past	50 (4.4)	44 (8.5)	25 (13.1)
Current	29 (5.5)	15 (10.1)	4 (16.7)
p Value	0.420	0.290	0.881
Family history			
No	134 (5.0)	75 (7.2)	40 (11.7)
Yes	9 (6.1)	12 (14.5)	8 (33.3)
p Value	0.564	**0.017**	**0.002**

Level of significance set at p<0.05; statistical significance tested with χ^2 test for contingency tables.
Bold signifies statistically significant p value.
Family history, subjects who reported a family history of AMD—family history was defined as having a first-degree relative, that is, parent or sibling with AMD; smoking, smoking status of subjects classified as never (no reported history of smoking), past (past smokers) and current (current smokers).

However, with respect to other potential risk factors for which no statistically significant associations with AMD were observed in the current study, it should be appreciated that controlling for age in the logistic regression analyses, and stratifying AMD prevalence by age group, may have contributed to the non-identification of some potentially significant associations with AMD.

The strengths of our study include (1) the use of a population-representative cohort of subjects aged 50 years and older in ROI; (2) the study population is racially homogeneous, over 99% being white; and (3) retinal photographs were graded in a masked fashion using standard protocols by the same person and therefore reducing intergrader variability. The large sample size (nearly 5000) could also be considered a strength, but the need to stratify by age group meant that, for some analyses, subgroup sizes were small.

The limitations of this study include the use of monoscopic retinal photographs through undilated pupils, rendering it difficult to obtain quality photographs in the presence of significant media opacities. The TILDA investigators elected to use monoscopic retinal photographs in the study because other health

Table 7 Prevalence of age-related macular degeneration (AMD) in comparable population-based studies

Study name	Country	Age group (year)	Early AMD (%)	Late AMD (%)	Atrophic AMD (%)	Neovascular AMD (%)
Baltimore Eye Survey*	USA	40–49		0.0	0.0	0.0
1985–1988[17]		50–59		0.5	0.2	0.4
		60–69		0.7	0.7	0.0
		70–79		2.9	1.8	1.6
		80+		7.0	4.0	5.6
Beaver Dam Eye Study	USA	43–54	8.4	0.1		
1988–1990[18]		55–64	13.8	0.6		
		65–74	18.0	1.4		
		75+	29.7	7.1		
Blue Mountains Eye Study	Australia	49–54	1.3	0.0		
1992–1993[19]		55–64	2.6	0.2		
		65–74	8.5	0.7		
		75–84	15.5	5.4		
		85+	28.0	18.5		
Reykjavik Eye Study	Iceland	50–59	8.9	0.3	0.3	0.0
1996[20]		60–69	16.4	1.2	1.2	0.0
		70–79	27.5	5.8	5.3	0.5
		>80	37.1	30.8	25.0	9.8
MESA*	USA	45–54	1.8	0.0		
2000–2002[21]		55–64	2.8	0.1		
		65–74	5.5	0.3		
		75–84	13.3	2.9		
TILDA Study	ROI	50–64	4.9	0.1	0.1	0.1
2009–2011		65–74	7.3	0.5	0.2	0.2
		≥75	11.0	2.2	1.3	1.0

*Data on only white participants.
MESA, Multi-ethnic Study of Atherosclerosis; ROI, Republic of Ireland; TILDA, the Irish Longitudinal Study on Ageing.

assessment measures (eg, gait) were to be conducted immediately following retinal photography, and the results of such tests would have been influenced and confounded by pharmacological pupillary dilation. Also, subjects with ungradable images were more likely to be older and have poor vision, although (upon investigation) this did not appear to have much effect on our prevalence estimates.

The response rate in the TILDA study (62% of eligible households participated) is in line with other national household surveys of older people, for example, in the Survey of Health, Ageing and Retirement in Europe, the average response rate across all countries was 55%.[29] Moreover, a non-response rate of this magnitude had been anticipated (from pilot surveys prior to the main survey) and built into the sample size calculations for the TILDA study. However, non-attendance at health centres reduced the effective participation rate further, so that just 5035 of 8175 participants (61.6%), who were successfully enrolled in the broader TILDA study, actually took part in this AMD study; this represents just 38% of the individuals originally selected. This has to be acknowledged as a weakness of our study, although we were able to adjust our prevalence calculations, to take account of the distorted sample age structure that arose from this non-participation. Of note, while many of the studies listed in table 7 reported much higher response rates than our AMD study (eg, 83.1% for the Beaver Dam Study), most of these were not nationally representative population-based studies and are not directly comparable.

In conclusion, this study reports the prevalence of AMD in ROI for the first time and will inform healthcare providers and planners involved in the delivery of care to those suffering with this condition.

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Competing interests SB and JN do consultancy work for Nutrasight Consultancy Limited. All other authors report no potential conflict of interest.

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REFERENCES

1 Kelliher C, Kenny D, O'Brien C. Trends in blind registration in the adult population of the Republic of Ireland 1996–2003. *Br J Ophthalmol* 2006;90:367–71.
2 Bird AC, Bressler NM, Bressler SB, *et al*. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 1995;39:367–74.
3 Age-Related Eye disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* 2001;119:1417–36.
4 Age Related Eye Disease Study 2 Research Group. Lutein+zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 2013;309:2005–15.
5 Chakravarthy U, Harding SP, Rogers CA, *et al*. Ranibizumab versus bevacizumab to treat neovascular age-related macular degeneration: one-year findings from the IVAN randomized trial. *Ophthalmology* 2012;119:1399–411.
6 Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, *et al*. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. *Ophthalmology* 2014;121:193–201.
7 Qualls LG, Hammill BG, Wang F, *et al*. Costs of newly diagnosed neovascular age-related macular degeneration among medicare beneficiaries, 2004–2008. *Retina* 2013;33:854–61.
8 Bloch SB, Larsen M, Munch IC. Incidence of legal blindness from age-related macular degeneration in denmark: year 2000 to 2010. *Am J Ophthalmol* 2012;153:209–13.
9 Slakter JS, Stur M. Quality of life in patients with age-related macular degeneration: impact of the condition and benefits of treatment. *Surv Ophthalmol* 2005;50:263–73.
10 Kearney PM, Cronin H, O'Regan C, *et al*. Cohort profile: the Irish longitudinal study on ageing. *Int J Epidemiol* 2011;40:877–84.
11 Klein R, Chou CF, Klein BE, *et al*. Prevalence of age-related macular degeneration in the US population. *Arch Ophthalmol* 2011;129:75–80.
12 Vingerling JR, Dielemans I, Hofman A, *et al*. The prevalence of age-related maculopathy in the rotterdam study. *Ophthalmology* 1995;102:205–10.
13 Cronin H, O'Regan C, Finucane C, *et al*. Health and aging: development of the Irish longitudinal study on ageing health assessment. *J Am Geriatr Soc* 2013;61 (Suppl 2):S269–78.
14 R Core team. *R: a language and environment for statistical computing [computer program]*. Vienna, Austria: R Foundation for Statistical Computing, 2008.
15 Central Statistics Office. Census 2011. 2012. Ref Type: Online Source.
16 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
17 Friedman DS, Katz J, Bressler NM, *et al*. Racial differences in the prevalence of age-related macular degeneration—The Baltimore eye survey. *Ophthalmology* 1999;106:1049–55.
18 Klein R, Klein BEK, Linton KLP. Prevalence of Age-Related Maculopathy—the Beaver Dam Eye Study. *Ophthalmology* 1992;99:933–43.
19 Mitchell P, Smith W, Attebo K, *et al*. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology* 1995;102: 1450–60.
20 Jonasson F, Arnarsson A, Sasaki H, *et al*. The prevalence of age-related maculopathy in iceland: Reykjavik eye study. *Arch Ophthalmol* 2003;121:379–85.
21 Klein R, Klein BE, Knudtson MD, *et al*. Prevalence of age-related macular degeneration in 4 racial/ethnic groups in the multi-ethnic study of atherosclerosis. *Ophthalmology* 2006;113:373–80.
22 Rudnicka AR, Jarrar Z, Wormald R, *et al*. Age and gender variations in age-related macular degeneration prevalence in populations of European ancestry: a meta-analysis. *Ophthalmology* 2012;119:571–80.
23 Andersen MV, Rosenberg T, la CM, *et al*. Prevalence of age-related maculopathy and age-related macular degeneration among the inuit in Greenland. The Greenland Inuit Eye Study. *Ophthalmology* 2008;115:700–7.
24 Piermarocchi S, Segato T, Scopa P, *et al*. The prevalence of age-related macular degeneration in Italy (PAMDI) study: report 1. *Ophthalmic Epidemiol* 2011;18:129–36.
25 VanNewkirk MR, Nanjan MB, Wang JJ, *et al*. The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology* 2000;107:1593–600.
26 Augood CA, Vingerling JR, de Jong PT, *et al*. Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE). *Arch Ophthalmol* 2006;124:529–35.
27 Smith W, Mitchell P. Family history and age-related maculopathy: the Blue Mountains Eye Study. *Aust N Z J Ophthalmol* 1998;26:203–6.
28 Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol* 1997;123:199–206.
29 Brugiavini A, Hendrik J, Johan M, *et al*., eds. *Health, ageing and retirement in Europe: first results from the Survey of Health, Ageing and Retirement in Europe*. Mannheim: Mannheim Research Institute for the Economics of Aging (MEA), 2005.

Prevalence of age-related macular degeneration in the Republic of Ireland

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CLINICAL STUDY

Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration

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Abstract

Purpose **To compare the impact of sustained supplementation using different macular carotenoid formulations on macular pigment (MP) and visual function in early age-related macular degeneration (AMD).**

Patients and methods **Sixty-seven subjects with early AMD were randomly assigned to: Group 1 (20 mg per day lutein (L), 0.86 mg per day zeaxanthin (Z); Ultra Lutein), Group 2 (10 mg per day *meso*-zeaxanthin (MZ), 10 mg per day L, 2 mg per day Z; Macushield; Macuhealth), Group 3 (17 mg per day MZ, 3 mg per day L, 2 mg per day Z). MP was measured using customised heterochromatic flicker photometry and visual function was assessed by measuring contrast sensitivity (CS) and best-corrected visual acuity (BCVA). AMD was graded using the Wisconsin Age-Related Maculopathy Grading System (AREDS 11-step severity scale).**

Results **At 3 years, a significant increase in MP from baseline was observed in all groups at each eccentricity ($P<0.05$), except at 1.75° in Group 1 ($P=0.160$). Between 24 and 36 months, significant increases in MP at each eccentricity were seen in Group 3 ($P<0.05$ for all), and at 0.50° in Group 2 ($P<0.05$), whereas no significant increases were seen in Group 1 ($P>0.05$ for all). At 36 months, compared with baseline, the following significant improvements ($P<0.05$) in CS were observed: Group 2—1.2, 6, and 9.6 cycles per degree (c.p.d.); Group 1—15.15 c.p.d.; and Group 3—6, 9.6, and 15.15 c.p.d. No significant changes in BCVA, or progression to advanced AMD, were observed.**

Conclusion **In early AMD, MP can be augmented with a variety of supplements, although the inclusion of MZ may confer benefits in terms of panprofile augmentation and in terms of CS enhancement.**

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Introduction

Age-related macular degeneration (AMD) is characterised by a spectrum of degenerative changes at the macula, which include drusen and/or hyper-/hypopigmentary changes (known as early AMD), atrophic changes (geographic atrophy, GA, a form of advanced AMD), and choroidal neovascularisation (neovascular or 'wet AMD', another form of advanced AMD).[1]

Macular pigment (MP) is a yellow pigment located in the macular region of the human retina, and is composed of lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (MZ).[2] MP filters short-wavelength blue light (and therefore limits photooxidative damage passively) and its constituent carotenoids act as antioxidants by neutralizing free radicals.[3,4]

In the current study, known as the Meso-zeaxanthin Ocular Supplementation Trial (MOST) AMD study, we compared the effect of sustained supplementation with some or all of MP's constituent carotenoids on visual function, and evaluated the impact of such supplementation on vision and disease progression. Observations that MZ, the dominant carotenoid in the epicentre of the MP's spatial profile, may offer advantages in terms of MP augmentation across its spatial profile[5] and

in terms of enhancement of visual function[6] prompted this investigation. The 8-week[7] and 12-month[8] reports of the MOST AMD study have been published. In the current study, we present new data on a 3-year follow-up of subjects in the MOST AMD study. Of note, this is the first study to monitor MP, visual function, and AMD status in response to supplementation with all three macular carotenoids in patients with early AMD, over a 36-month period.

Materials and methods

The design and methodology of the MOST AMD study has been reported previously.[8] In brief, MOST AMD is a single-blind, randomised controlled clinical trial. Clinical assessments were carried out at the Institute of Eye Surgery (http://www.ioes.ie/), Waterford, Ireland. Before study enrolment, an eligibility screening visit was conducted by an ophthalmologist with a special interest in retinal disease (SB). The eligibility criteria included early AMD (one to eight on AREDS 11-step severity scale[9] in at least one eye (the study eye), confirmed by the Ocular Epidemiology Reading Center at the University of Wisconsin, Madison, WI, USA); best-corrected visual acuity (BCVA) $\geq$6/12 in the study eye; and no other ocular pathology.

Subjects were randomly assigned to one of three parallel groups: Group 1—20 mg L, 0.86 mg Z (Ultra Lutein supplied by Natural Organics, Inc., Melville, NY, USA); Group 2—10 mg MZ, 10 mg L, 2 mg Z (Macushield (Macuvision Europe Limited, Solihull, UK)/Macuhealth LMZ3 (MacuHealth LLC, Birmingham, MI, USA)); Group 3—17 mg MZ, 3 mg L, 2 mg Z (supplied by Industrial Organica, Monterrey, Mexico (not commercially available)). The above treatment groups (formulations) were selected to be comparable total concentrations of macular carotenoids (ie 22 mg). Of note, however, discrepancies between label claim and measured values of the supplements used in this trial have been reported previously, and in particular, the finding that the Group 1 supplement contained small amounts of MZ (0.30 mg).[10,11] This has implications for the findings presented below.

The supplements were prepared in a soft gel capsule. Subjects were instructed to take one capsule daily with a meal. All study supplements were indistinguishable in terms of external appearance, and were packaged in identical containers. Study visits were conducted at baseline, 12 months, 24 months, and 36 months.

Ethics

Ethics approval was granted by the Waterford Regional Hospital Ethics Committee. Written and informed consent was obtained from each subject before study enrolment. The tenets of the Declaration of Helsinki were adhered to in all study procedures.

Outcome measures

The primary outcome measure was change in MP as measured by customized heterochromatic flicker photometry (cHFP) at 36 months. Secondary outcome measures included BCVA, letter contrast sensitivity (CS), serum concentrations of macular carotenoids, and grade of AMD.

Study procedures

MP measurement MP was measured using the Macular Densitometer (Macular Metrics, Corp., Providence, RI, USA) at 0.25°, 0.5°, 1.0°, and 1.75° retinal eccentricity, with a reference point at 7°.[12]

Serum L, Z, and MZ analysis Serum L, Z, and MZ were quantified by high-performance liquid chromatography using methodology described previously.[7,13]

Visual acuity BCVA was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (LogMAR) chart (Test Chart 2000 PRO; Thomson Software Solutions, Hatfield, Hertfordshire, UK) viewed at 4 m.

Letter CS Letter CS was assessed using the LogMAR ETDRS (Test Chart 2000 PRO; Thomson Software Solutions) chart at five different spatial frequencies (1.2, 2.4, 6.0, 9.6, and 15.15 c.p.d., respectively) viewed at 4 m.

Retinal photography and AMD grading

Following prior pupillary dilation (0.5% proxymetacaine hydrochloride, 2.5% phenylephrine hydrochloride, and 1% tropicamide), 45° stereoscopic color fundus photographs were taken in three retinal photographic fields (optic disc, macula, temporal to macula) using a Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany). Photographs were transferred to the Ocular Epidemiology Reading Center at the University of Wisconsin via an encrypted system. Photographs were graded in a masked manner using a modified version of the Wisconsin Age-Related Maculopathy Grading System[14,15] and adhered to the AREDS 11-step severity scale.[9]

Statistical analysis

One eye (the study eye) of each subject comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM, Armonk, NY, USA). To compare the effects of the three supplements (on each outcome measure, over time), we used repeated-measures analysis of variance, and contingency table analysis, as appropriate. Cognisant that this exploratory study would likely have insufficient power for such analyses, however, we did some additional analyses. In fact, and beyond the previously reported 12-month data,[8] we decided upon two strands of analysis: (a) between supplement group analysis over time: despite the small sample sizes, supplement groups were compared with each other, for changes in each outcome variable over the 3 years of the study. For interval outcome variables (MP, serum carotenoids, BCVA, CS), the method of analysis was repeated-measures analysis of variance, with time as a within-subjects factor and supplement as a between-subjects factor; we used the Greenhouse–Geisser correction for lack of sphericity. *Post hoc* analysis, with Bonferroni adjustment for multiple testing, was used where appropriate. For categorical outcome variables (AMD grade), we used contingency table analysis to compare supplements; (b) within-supplement group changes in each outcome variable, over the 3 years of the study. We used paired *t*-tests analysis here.

Tests of significance, for all *t*-test analyses, were two-tailed, and the 5% level of significance was used throughout. With the exception of *post hoc* analyses for the repeated-measures analysis of variance, we did not correct for multiple tests.

Results

Sixty-seven subjects were enrolled at baseline, with 47 subjects completing the final study visit at 36 months. Only those subjects who completed each study visit were included in analysis. Therefore, if a subject attended his/her 12- or 24-month visit, but did not complete the 36-month visit, he/she was not included in the analysis. Where a subject did complete a study visit, but where a variable was not measured or recorded, that subject was also excluded from all analyses relating to that variable. Exclusions occurred only in the MP and CS analysis because data were not available at all study visits (MP analysis: 5 subjects; CS analysis: 6 subjects). We have also included the sample size in all tables for clarity.

Baseline characteristics (eg age, gender, smoking status, education) of participants in intervention groups have been described previously, and the intervention groups were statistically comparable in terms of these variables.[8]

MP and its constituent carotenoids in serum

Macular pigment

(a) Comparing supplement groups In the repeated-measures analysis of change in MP (at 0.25°, 0.5°, 1.0°, and 1.75°), the within-subjects Time × Supplement interaction effect was not significant ($P = 0.759$, 0.726, 0.703, 0.110, respectively, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect (on MP levels) over time, at any eccentricity, does not differ significantly between supplement groups. The boxplots in Figure 1 graphically illustrate these findings.

(b) Within-supplement group analyses of MP are given in Table 1.

Serum concentrations of lutein

(a) Comparing supplement groups In the repeated-measures analysis of change in serum L, the within-subjects Time × Supplement interaction effect was significant ($P = 0.029$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect (on serum L levels) over time differs significantly between the supplements used. *Post hoc* analysis indicates that increases in serum L over time in groups 1 and 2 are comparable ($P = 1$, after Bonferroni adjustment for multiple testing), and each of these groups exhibit significantly greater increases than group 3 ($P = 0.029$ and $P = 0.004$, respectively, after Bonferroni adjustment for multiple testing). The boxplots in Figure 2a graphically illustrate these findings.

(b) Within-supplement group analyses of serum L are given in Table 2.

Serum concentrations of MZ

(a) Comparing supplement groups In the repeated-measures analysis of change in serum MZ, the within-subjects Time × Supplement interaction effect was significant ($P = 0.011$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect over time (on serum levels of MZ) differs significantly between the supplement groups. *Post hoc* analysis indicates that increases in MZ over time in Groups 2 and 3 are comparable ($P = 1$, after Bonferroni adjustment for multiple testing), and each of these groups exhibits significantly greater increases than Group 1 ($P = 0.001$ for both, after Bonferroni adjustment for multiple testing). The boxplots in Figure 2b graphically illustrate these findings.

(b) Within-supplement group analyses of serum MZ are given in Table 2.

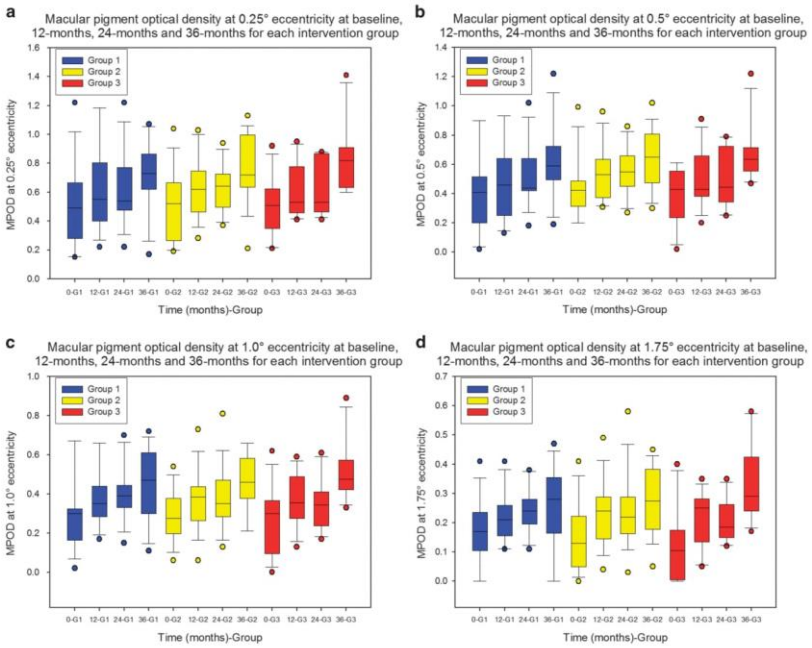

Figure 1 Macular pigment response at different retinal eccentricities over the course of the MOST AMD study. Boxplots representing macular pigment optical density at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group: Group 1—20 mg L and 0.86 mg Z; Group 2—10 mg MZ, 10 mg L, and 2 mg Z; Group 3—17 mg MZ, 3 mg L, and 2 mg Z Macular pigment was measured at 0.25° (a), 0.5° (b), 1.0° (c), and 1.75° (d) eccentricity using cHFP. 0-G1, Baseline Group 1; 12-G1, 12 months Group 1; 24-G1, 24 months Group 1; 36-G1, 36 months Group 1; 0-G2, Baseline Group 2; 12-G2, 12 months Group 2; 24-G2, 24 months Group 2; 36-G2, 36 months Group 2; 0-G3, Baseline Group 3; 12-G3, 12 months Group 3; 24-G3, 24 months Group 3; 36-G3, 36 months Group 3. MPOD, macular pigment optical density.

Serum concentrations of zeaxanthin

(a) Comparing supplement groups In the repeated-measures analysis of change in serum Z, the within-subjects Time × Supplement interaction effect was not significant ($P = 0.081$, using the Greenhouse–Geisser adjustment for lack of sphericity). Thus, the effect over time does not differ significantly between the supplements. The boxplots in Figure 2c graphically illustrate these findings.

(b) Within-supplement group analyses of serum Z are given in Table 2.

Changes in visual function

(a) Comparing supplement groups There were no significant Time × Supplement interaction effects for any vision-related outcome measures (BCVA, letter CS at any spatial frequency), indicating that the observed effects over time in terms of these variables (see below) did not differ between intervention groups.

Best-corrected visual acuity

Within-supplement group analysis There were no significant within-supplement changes in BCVA ($P > 0.05$, for all), with the exception of a statistically significant improvement in Group 3 between 12 and 24 months.

Contrast sensitivity

Within-supplement group analysis of CS are given in Table 3. At 36 months, compared with baseline, the following significant improvements ($P < 0.05$) in CS were observed: Group 2—1.2, 6, and 9.6 c.p.d.; Group 1—15.15 c.p.d.; Group 3—6, 9.6, and 15.15 c.p.d.

Table 1 Within-supplement group analysis of macular pigment by intervention groups

Intervention	*N*	*Baseline, mean ± SD*	*36 Months, mean ± SD*	*%Δ*	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	*%Δ*	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	*%Δ*	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*
MP at 0.25																	
Group 1	13	0.51 ± 0.29	0.72 ± 0.24	41	0.004	0.51 ± 0.29	0.61 ± 0.30	20	0.039	0.61 ± 0.30	0.61 ± 0.25	0	0.896	0.61 ± 0.25	0.72 ± 0.24	18	0.134
Group 2	16	0.50 ± 0.24	0.76 ± 0.23	52	0.001	0.50 ± 0.24	0.63 ± 0.21	26	0.001	0.63 ± 0.21	0.64 ± 0.17	2	0.802	0.64 ± 0.17	0.76 ± 0.23	19	0.095
Group 3	12	0.51 ± 0.20	0.85 ± 0.25	67	0.000	0.51 ± 0.20	0.62 ± 0.19	22	0.021	0.62 ± 0.19	0.62 ± 0.19	0	0.924	0.62 ± 0.19	0.85 ± 0.25	37	0.003
MP at 0.5																	
Group 1	13	0.41 ± 0.28	0.62 ± 0.26	51	0.000	0.41 ± 0.28	0.47 ± 0.27	15	0.194	0.47 ± 0.26	0.53 ± 0.21	13	0.092	0.53 ± 0.21	0.62 ± 0.26	16	0.087
Group 2	16	0.45 ± 0.21	0.64 ± 0.20	42	0.000	0.45 ± 0.21	0.54 ± 0.18	20	0.011	0.54 ± 0.18	0.55 ± 0.16	2	0.343	0.55 ± 0.16	0.64 ± 0.20	16	0.034
Group 3	12	0.39 ± 0.19	0.68 ± 0.20	74	0.000	0.39 ± 0.19	0.50 ± 0.20	22	0.016	0.50 ± 0.20	0.50 ± 0.20	0	0.879	0.50 ± 0.20	0.68 ± 0.20	36	0.011
MP at 1.0																	
Group 1	13	0.30 ± 0.19	0.45 ± 0.19	50	0.006	0.30 ± 0.19	0.38 ± 0.15	27	0.053	0.38 ± 0.15	0.40 ± 0.14	5	0.339	0.40 ± 0.14	0.45 ± 0.18	13	0.298
Group 2	16	0.29 ± 0.13	0.46 ± 0.15	59	0.000	0.29 ± 0.13	0.37 ± 0.16	28	0.010	0.37 ± 0.16	0.38 ± 0.16	3	0.730	0.38 ± 0.16	0.46 ± 0.15	21	0.071
Group 3	12	0.26 ± 0.17	0.52 ± 0.16	100	0.000	0.26 ± 0.17	0.37 ± 0.14	42	0.010	0.37 ± 0.14	0.35 ± 0.13	−6	0.473	0.35 ± 0.13	0.52 ± 0.16	49	0.011
MP at 1.75																	
Group 1	13	0.17 ± 0.11	0.23 ± 0.19	35	0.160	0.17 ± 0.11	0.22 ± 0.09	29	0.055	0.22 ± 0.09	0.24 ± 0.08	9	0.256	0.24 ± 0.08	0.23 ± 0.19	−4	0.870
Group 2	16	0.15 ± 0.12	0.28 ± 0.11	87	0.000	0.15 ± 0.12	0.24 ± 0.11	60	0.007	0.24 ± 0.11	0.24 ± 0.13	0	0.793	0.24 ± 0.13	0.28 ± 0.11	17	0.383
Group 3	12	0.12 ± 0.13	0.34 ± 0.14	183	0.000	0.12 ± 0.13	0.21 ± 0.09	75	0.006	0.21 ± 0.09	0.21 ± 0.07	0	0.899	0.21 ± 0.07	0.34 ± 0.14	62	0.003

Abbreviations: MP, macular pigment; N, number of subjects; SD, standard deviation; Sig., significance; %Δ, percentage change.
Macular pigment was measured at 0.25°, 0.5°, 1.0°, and 1.75° eccentricity using customized heterochromatic flicker photometry. Statistical significance was tested using paired *t*-test. Level of significance set at $P < 0.05$. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).
Group 1, 20 mg lutein and 0.86 mg zeaxanthin; Group 2, 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3, 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

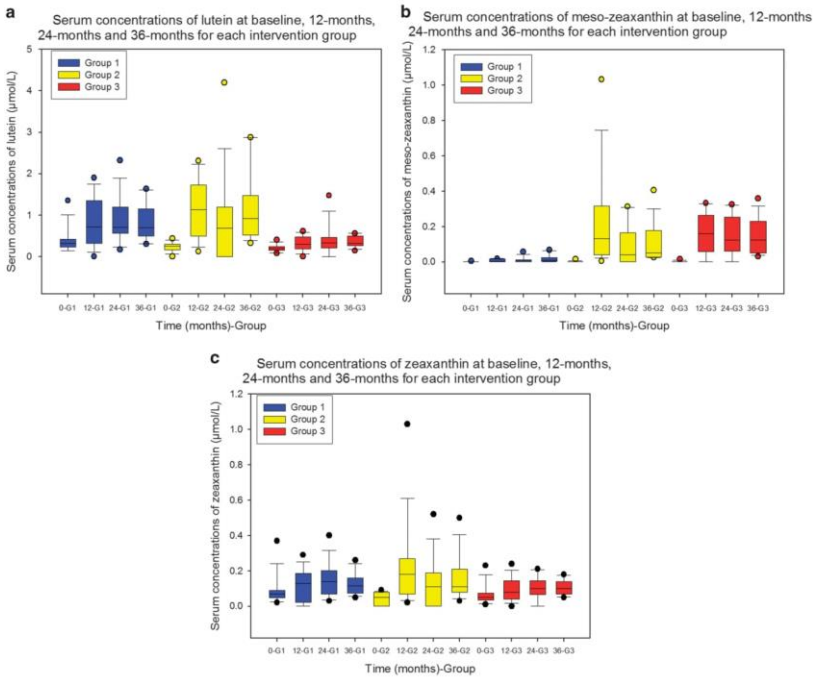

Figure 2 Serum response of L, MZ, and Z over the course of the MOST AMD study. Boxplots representing serum concentrations of L (a), MZ (b), and zeaxanthin (c) at four time points (baseline, 12 months, 24 months, and 36 months) for each intervention group: Group 1—20 mg L and 0.86 mg Z; Group 2—10 mg MZ, 10 mg L, and 2 mg Z; Group 3—17 mg MZ, 3 mg L, and 2 mg Z. Serum macular carotenoids were analysed by HPLC and expressed as µmol/L; 0-G1, Baseline Group 1; 12-G1, 12 months Group 1; 24-G1, 24 months Group 1; 36-G1, 36 months Group 1; 0-G2, Baseline Group 2; 12-G2, 12 months Group 2; 24-G2, 24 months Group 2; 36-G2, 36 months Group 2; 0-G3, Baseline Group 3; 12-G3, 12 months Group 3; 24-G3, 24 months Group 3; 36-G3, 36 months Group 3.

Changes in grade of AMD

Because of the limited number of subjects in this study, we collapsed adjacent grades of AMD, as follows: AREDS grades 1–3 (representing eyes at low risk of progression to advanced AMD), and AREDS grades 4–8 (representing eyes at high risk of progression to advanced AMD). In terms of this collapsed and simplified classification, intervention groups were statistically similar in terms of baseline findings ($P = 0.44$, χ^2 test). Using this simplified and modified system, no study eye in any intervention group progressed from low risk to high risk of progression to advanced AMD over the course of the study period, and no study eye regressed from high risk to low risk of progression to advanced AMD in any intervention group, and finally, no subject progressed to advanced AMD (AREDS grades 9–11) over the study period. Given that findings were identical for all three intervention groups, there was no need for statistical investigation of differences between intervention groups in terms of changes in risk for progression to advanced AMD.

We also investigated clinically meaningful change in AMD grade along the AREDS 11-step scale, defined as a change of at least two steps along this scale. Thus, an increase of two steps between baseline and final visit at 36 months was considered clinically meaningful disease progression and a decrease of two steps was considered a clinically meaningful disease regression. On this basis, there was no clinically meaningful change in AMD grade

Table 2 Within-supplement group analysis of serum macular carotenoids by intervention groups

Intervention	N	*Baseline, mean ± SD*	*36 Months, mean ± SD*	%Δ	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	%Δ	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	%Δ	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	% Δ	*Sig.*
Lutein																	
Group 1	14	0.39 ± 0.31	0.81 ± 0.44	108	0.006	0.39 ± 0.31	0.81 ± 0.58	108	0.014	0.81 ± 0.58	0.90 ± 0.57	11	0.616	0.90 ± 0.57	0.81 ± 0.44	−10	0.412
Group 2	15	0.24 ± 0.11	1.14 ± 0.83	375	0.001	0.24 ± 0.11	1.11 ± 0.67	363	0.000	1.11 ± 0.67	0.85 ± 1.05	−23	0.336	0.85 ± 1.05	1.14 ± 0.83	34	0.250
Group 3	13	0.20 ± 0.08	0.36 ± 0.13	80	0.001	0.20 ± 0.08	0.31 ± 0.17	55	0.021	0.31 ± 0.17	0.39 ± 0.36	26	0.367	0.39 ± 0.36	0.36 ± 0.13	−8	0.694
Zeaxanthin																	
Group 1	14	0.09 ± 0.09	0.13 ± 0.06	44	0.124	0.09 ± 0.09	0.12 ± 0.09	33	0.314	0.12 ± 0.09	0.15 ± 0.09	25	0.251	0.15 ± 0.09	0.13 ± 0.06	−13	0.202
Group 2	15	0.04 ± 0.03	0.16 ± 0.12	300	0.001	0.04 ± 0.03	0.22 ± 0.24	450	0.012	0.22 ± 0.24	0.13 ± 0.14	−41	0.221	0.13 ± 0.14	0.16 ± 0.12	23	0.298
Group 3	13	0.06 ± 0.05	0.11 ± 0.04	83	0.005	0.06 ± 0.05	0.09 ± 0.06	50	0.031	0.09 ± 0.06	0.10 ± 0.07	11	0.653	0.10 ± 0.07	0.11 ± 0.04	10	0.799
Meso-zeaxanthin																	
Group 1	14	0.00 ± 0.00	0.02 ± 0.02		0.010	0.00 ± 0.00	0.01 ± 0.01		0.008	0.01 ± 0.01	0.01 ± 0.02	0	0.393	0.01 ± 0.02	0.02 ± 0.02	100	0.371
Group 2	15	0.00 ± 0.00	0.11 ± 0.11		0.001	0.00 ± 0.00	0.22 ± 0.27		0.007	0.22 ± 0.27	0.09 ± 0.11	−59	0.083	0.09 ± 0.11	0.11 ± 0.11	22	0.314
Group 3	13	0.00 ± 0.00	0.14 ± 0.10		0.000	0.00 ± 0.00	0.16 ± 0.11		0.000	0.16 ± 0.11	0.15 ± 0.11	−6	0.911	0.15 ± 0.11	0.14 ± 0.10	−7	0.743

Abbreviations: N, number of subjects; SD, standard deviation; Sig., significance; %Δ, percentage change.

Serum Lutein, Zeaxanthin and Meso-zeaxanthin were analysed by HPLC and expressed as μmol/L. Statistical significance tested using paired *t*-test. Level of significance set at $P < 0.05$. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).

Group 1, 20 mg lutein and 0.86 mg zeaxanthin; Group 2, 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3, 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

Table 3 Within-supplement group analysis of letter contrast sensitivity by intervention groups

Intervention	N	*Baseline, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*	*Baseline, mean ± SD*	*12 Months, mean ± SD*	*% Δ*	*Sig.*	*12 Months, mean ± SD*	*24 Months, mean ± SD*	*%Δ*	*Sig.*	*24 Months, mean ± SD*	*36 Months, mean ± SD*	*% Δ*	*Sig.*
Letter CS 1.2cpd																	
Group 1	12	1.87 ±0.25	1.89 ± 0.16	1	0.817	1.87 ± 0.25	1.96 ± 0.23	5	0.222	1.96 ± 0.23	1.76 ± 0.22	−10	0.000	1.76 ± 0.22	1.89 ± 0.16	7	0.059
Group 2	15	1.71 ± 0.24	1.86 ± 0.18	9	0.012	1.71 ± 0.24	1.93 ± 0.29	13	0.004	1.93 ± 0.29	1.85 ± 0.25	−4	0.207	1.85 ± 0.25	1.86 ± 0.18	1	0.861
Group 3	13	1.75 ± 0.31	1.82 ± 0.20	4	0.432	1.75 ± 0.31	1.89 ± 0.27	8	0.069	1.89 ± 0.27	1.86 ± 0.24	−2	0.602	1.86 ± 0.24	1.82 ± 0.20	−2	0.494
Letter CS 2.4cpd																	
Group 1	12	1.76 ± 0.30	1.87 ± 0.17	6	0.227	1.76 ± 0.30	1.89 ± 0.33	7	0.077	1.89 ± 0.33	1.70 ± 0.25	−10	0.011	1.70 ± 0.25	1.87 ± 0.17	10	0.065
Group 2	15	1.68 ± 0.31	1.81 ± 0.21	8	0.087	1.68 ± 0.31	1.86 ± 0.31	11	0.000	1.86 ± 0.31	1.78 ± 0.26	−4	0.221	1.78 ± 0.26	1.81 ± 0.21	2	0.657
Group 3	13	1.63 ± 0.31	1.78 ± 0.21	9	0.083	1.63 ± 0.31	1.85 ± 0.29	13	0.005	1.85 ± 0.29	1.77 ± 0.22	−4	0.225	1.77 ± 0.22	1.78 ± 0.21	1	0.947
Letter CS 6cpd																	
Group 1	12	1.42 ± 0.30	1.60 ± 0.15	13	0.112	1.42 ± 0.30	1.49 ± 0.41	5	0.224	1.49 ± 0.41	1.39 ± 0.26	−7	0.200	1.39 ± 0.26	1.60 ± 0.15	15	0.037
Group 2	15	1.37 ± 0.24	1.52 ± 0.25	11	0.040	1.37 ± 0.24	1.44 ± 0.30	5	0.079	1.44 ± 0.30	1.39 ± 0.34	−3	0.357	1.39 ± 0.34	1.52 ± 0.25	9	0.164
Group 3	13	1.23 ± 0.44	1.52 ± 0.27	24	0.034	1.23 ± 0.44	1.55 ± 0.29	26	0.005	1.55 ± 0.29	1.48 ± 0.20	−5	0.357	1.48 ± 0.20	1.52 ± 0.27	3	0.547
Letter CS 9.6cpd																	
Group 1	12	1.14 ± 0.31	1.35 ± 0.16	18	0.043	1.14 ± 0.31	1.14 ± 0.32	0	0.959	1.14 ± 0.32	1.14 ± 0.28	0	1.000	1.14 ± 0.28	1.35 ± 0.16	18	0.031
Group 2	15	1.06 ± 0.27	1.27 ± 0.34	20	0.024	1.06 ± 0.27	1.17 ± 0.39	10	0.072	1.17 ± 0.39	1.06 ± 0.37	−9	0.115	1.06 ± 0.37	1.27 ± 0.34	20	0.025
Group 3	13	0.94 ± 0.48	1.30 ± 0.22	38	0.020	0.94 ± 0.48	1.17 ± 0.44	24	0.031	1.17 ± 0.44	1.23 ± 0.27	5	0.503	1.23 ± 0.27	1.30 ± 0.22	6	0.201
Letter CS 15.15cpd																	
Group 1	12	0.75 ± 0.32	1.02 ± 0.23	36	0.033	0.75 ± 0.32	0.83 ± 0.31	11	0.055	0.83 ± 0.31	0.79 ± 0.29	−5	0.509	0.79 ± 0.29	1.02 ± 0.23	29	0.011
Group 2	15	0.70 ± 0.37	0.91 ± 0.38	30	0.083	0.70 ± 0.37	0.78 ± 0.44	11	0.278	0.78 ± 0.44	0.60 ± 0.47	−23	0.013	0.60 ± 0.47	0.91 ± 0.38	52	0.029
Group 3	13	0.61 ± 0.48	0.97 ± 0.25	59	0.019	0.61 ± 0.48	0.81 ± 0.38	33	0.028	0.81 ± 0.38	0.93 ± 0.35	15	0.169	0.93 ± 0.35	0.97 ± 0.25	4	0.555

Abbreviations: CS, contrast sensitivity; cpd, cycles per degree; N, number of subjects; SD, standard deviation; Sig., level of significance set at $P < 0.05$; %Δ, percentage change.
Letter contrast sensitivity was assessed using Thompson Test Chart PRO and recorded in the logarithm of contrast sensitivity (Log CS) units. Statistical significance was tested using paired *t*-test. The calculated percentage change from baseline to 36 months, calculated as the 36-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from baseline to 12 months, calculated as the 12-month value minus baseline value divided by baseline value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 12 to 24 months, calculated as the 24-month value minus the 12-month value divided by the 12-month value, multiplied by 100 (−, negative change; +, positive change); the calculated percentage change from 24 to 36 months, calculated as the 36-month value minus the 24-month value divided by the 24-month value, multiplied by 100 (−, negative change; +, positive change).
Group 1: 20 mg lutein and 0.86 mg zeaxanthin; Group 2: 10 mg *meso*-zeaxanthin, 10 mg lutein, and 2 mg zeaxanthin; Group 3: 17 mg *meso*-zeaxanthin, 3 mg lutein, and 2 mg zeaxanthin.

in 43 (93%) study eyes, whereas 3 (7%) study eyes (one subject in Group 1 and two subjects in Group 3) exhibited a clinically meaningful progression along the AREDS 11-step scale, and these observed changes were not statistically different between intervention groups ($P = 0.29$, Fisher's exact test).

Discussion

The present study reports on the impact of sustained supplementation with different carotenoid formulations on serum concentrations of MP's constituent carotenoids, MP, visual function (BCVA and letter CS), and disease progression in subjects with early AMD.

The strengths of this study include: (1) it is a randomized clinical trial comparing three different formulations containing some or all of MP's constituent carotenoids, with a follow-up of 3 years; (2) MP was measured using a validated technique at regular intervals throughout the study period; (3) assessment of visual function was not restricted to BCVA, and included CS; (4) assessment of AMD morphology was performed by an accredited reading centre in a masked manner.

Serum response to supplementation reflected the carotenoid content of the supplement used. For example, serum L exhibited an increase in all three supplementation groups, but to a greater extent in Groups 1 and 2, where intake of L was at least three times the typical dietary intake of this carotenoid.[16,17] Similarly, a significant rise in serum Z was noted following supplementation, but that was comparable across supplement groups, reflecting similar concentrations of this carotenoid in each of the three formulations tested. Finally, serum MZ response is noteworthy for several reasons. First, MZ was detected in the serum of patients supplemented with a formulation with no declared MZ content. However, we have shown that MZ is indeed present in commercially available formulations containing L, including Ultra Lutein, the Group 1 supplement used in this study.[10] Finally, it is also worth noting that serum L and serum Z responses were unaffected by the presence of substantial concentrations of MZ (10 mg or more) in the formulation used, thereby allaying previously expressed concerns that the inclusion of MZ in a supplement may adversely impact upon the circulating bioavailability of the other two macular carotenoids.

MP increased significantly in all groups at each eccentricity (with the exception of Group 1 at 1.75°) at 3 years. It is surprising to see that MP did not increase at 1.75° in Group 1, given that L is the dominant carotenoid at this locus, and this seemingly counterintuitive observation might be because subjects in Group 1 were bioconverting L to MZ at the macula.[18,19] Consistent with this hypothesis, only groups that received supplemental MZ exhibited significant augmentation of MP across the spatial profile of this pigment.

In terms of MP increase over the course of the study, it was observed that MP continues to increase further and significantly in the third year of supplementation (but only in groups supplemented with meaningful concentrations of MZ) following a relative plateau in the second year of supplementation. Indeed, MP did not increase significantly between 12 and 24 months in any intervention group, at any eccentricity. Although the exact mechanism of macular carotenoid uptake has not been fully elucidated, it is plausible that there are several mediators (eg binding proteins, enzymes) that influence the capture, accumulation, and stabilisation of these carotenoids at the macula,[20] but further research is needed to understand these mechanisms.

There was no significant change in BCVA over the course of the present study, other than a transient improvement between 12 and 24 months in Group 3. Murray *et al*[21] reported the impact of supplemental L on MP and visual acuity in patients with early AMD in a randomised, double-blind, placebo-controlled, multicentre 12-month trial. At the end of their study, there was no change in BCVA in the L group, whereas BCVA in the placebo group had deteriorated significantly.[21] In the present study, there was a nonsignificant increase in BCVA in all intervention groups, consistent with the view that BCVA stabilised over the 3-year period of the study in this cohort of patients with early AMD. The CARMA trial, a randomised controlled trial of L, Z, and coantioxidants *vs* placebo, reported no significant change in BCVA at 1 year, although there was a demonstrable benefit in terms of differential BCVA between intervention and placebo groups at 3 years.[22,23] Of note, visual acuity, which is a measure of the spatial resolving power of the visual system and remains the most commonly used measure of vision in clinical practice,[24] is probably not sensitive enough to detect subtle but important changes in visual function experienced when monitoring subjects with early AMD.[25]

CS measures the threshold between visible and invisible at a given spatial frequency, and could be loosely described as 'faintness appreciation'[26] and is a better tool than BCVA for assessing visual function in early AMD.[25] In Group 2 (a supplement with a formulation containing all three of MP's constituent carotenoids), there was a statistically significant improvement in CS at the lowest spatial frequency (2.4 c.p.d.), whereas this was not observed for Groups 1 and 3. At the highest spatial frequency (15.15 c.p.d.), letter CS improved in Groups 1 and 3 at 36 months, but not in Group 2. At intermediate spatial frequencies (6 and 9.6 c.p.d.), however, only supplementation with formulations containing

appreciable amounts of MZ (Groups 2 and 3) resulted in a significant improvement in letter CS. Although some, but not all, previous studies have reported improvements in CS following supplementation with macular carotenoids in subjects with early AMD, our results suggest that those studies that failed to report an improvement in CS may be explained, at least in part, by a lack of MZ in the supplement formulation used.[23,27] Finally, an important and novel finding of the current study rests on the observation that further and significant improvements in CS are experienced beyond 24 months of supplementation with MP's constituent carotenoids, suggesting that sustained supplementation is indeed necessary to exert a beneficial effect on visual function.

With respect to AMD, only three study eyes exhibited clinically meaningful disease progression (1 subject from Group 1 and 2 subjects from Group 3), and no study eye progressed to advanced AMD over the 3-year study period. This study is not adequately powered or designed to make meaningful comment on AMD progression.

The current study compared the impact of supplementation with different carotenoid formulations on visual function, and our findings suggest that a formulation containing MZ yields benefits in terms of MP augmentation and in terms of CS enhancement. Further, sustained supplementation appears necessary, for at least 3 years, if MP is to be augmented maximally and CS is to be optimised over that period of time. Of note, modest visual benefits were observed in the current study. Future clinical trials should examine the impact of supplementation with formulations containing MZ and Z at similar doses. The Central Retinal Enrichment Supplementation Trial (CREST), currently underway, will also add to our understanding of the role of the macular carotenoids, including MZ, on vision in healthy and diseased eyes.[28]

Limitations of the MOST AMD study include its small numbers and the fact that it is a single blind clinical trial with no placebo arm. With respect to the use of placebo in the current study, we believe that the findings arising from the secondary analysis of the AREDS2 may render the use of placebo in patients with early (including intermediate) AMD ethically questionable.[29,30] Of note, the term early AMD in this study includes patients with intermediate AMD (as defined by AREDS). However, the absence of placebo may render it difficult to demonstrate clinical efficacy of the different carotenoid formulations used in this study and our results should be interpreted with full appreciation of this limitation. We used the single-blind design because the current study was the first clinical trial to compare the impact of supplementation with three different carotenoid formulations (including MZ) on visual function in subjects with early AMD and therefore we wanted to monitor more closely the effects of the three carotenoid formulations in terms of response among these subjects. Statistically, this exploratory study was underpowered for a direct comparison of the three supplements. Differences in effects between supplements were, in general, likely to be small, meaning that impractically large numbers of subjects would have been required to obtain statistically significant results.

In conclusion, we report that the inclusion of MZ in a supplement formulation seems to confer benefits in terms of MP augmentation and in terms of enhanced CS in subjects with early AMD. An important and novel finding rests on the observation that sustained supplementation with the macular carotenoids seems necessary to maximally augment MP and to optimise CS over a 3-year period in patients with early AMD.

Summary

What was known before

- MP augmentation can be achieved with a variety of supplements.
- The inclusion of MZ in a formulation appears to confer greater benefits in terms of visual function and augmentation of MP in subjects with early AMD at 12 months.

What this study adds

- Sustained supplementation in subjects with early AMD results in further augmentation of MP following 2 years of continuous supplementation, and confers visual benefit in these patients in terms of CS.
- The inclusion of MZ in a formulation appears to be important if increases in MP, and consequential improvements in vision, are to be maximised in subjects with early AMD receiving supplements.

Conflict of interest

JMN and SB do consultancy work for nutraceutrical companies in a personal capacity and as directors of Nutrasight Consultancy Limited. ANH is a 'honorary director' of Howard Foundation Holdings Limited and Nutriproducts Limited, which licence and supply nutraceutical ingredients. DIT is a consultant of Howard Foundation Holdings Limited. All other authors declare no conflict of interest.

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References

1 Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, Davis MD *et al.* An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 1995; **39**: 367–374.

2 Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 2001; **385**: 28–40.

3 Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 1997; **38**: 1802–1811.

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

5 Nolan JM, Akkali MC, Loughman J, Howard AN, Beatty S. Macular carotenoid supplementation in subjects with atypical spatial profiles of macular pigment. *Exp Eye Res* 2012; **101**: 9–15.

6 Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci* 2012; **53**(12): 7871–7880.

7 Nolan JM, Loskutova E, Howard AN, Moran R, Mulcahy R, Stack J *et al.* Macular pigment, visual function, and macular disease among subjects with Alzheimer's disease: an exploratory study. *J Alzheimers Dis* 2014; **42**(4): 1191–1202.

8 Sabour-Pickett S, Beatty S, Connolly E, Loughman J, Stack J, Howard A *et al.* Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina* 2014; **34**(9): 1757–1766.

9 Davis MD, Gangnon RE, Lee LY, Hubbard LD, Klein BE, Klein R *et al.* The age-related eye disease study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol* 2005; **123**: 1484–1498.

10 Meagher KA, Thurnham DI, Beatty S, Howard AN, Connolly E, Cummins W *et al.* Serum response to supplemental macular carotenoids in subjects with and without age-related macular degeneration. *Br J Nutr* 2013; **110**: 289–300.

11 Nolan JM, Meagher K, Kashani S, Beatty S. What is meso-zeaxanthin, and where does it come from? *Eye (Lond)* 2013; **27**: 899–905.

12 Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci* 1999; **40**: 2481–2489.

13 Thurnham DI, Tremel A, Howard AN. A supplementation study in human subjects with a combination of meso-zeaxanthin, (3*R*,3′*R*)-zeaxanthin and (3*R*,3′*R*,6′*R*)-lutein. *Br J Nutr* 2008; **100**: 1307–1314.

14 Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology* 1991; **98**: 1128–1134.

15 Sparrow JM, Dickinson AJ, Duke AM. The Wisconsin Age-related Macular Degeneration grading system: performance in an independent centre. *Ophthalmic Epidemiol* 1997; **4**: 49–55.

16 O'Neill ME, Carroll Y, Corridan B, Olmedilla B, Granado F, Blanco I *et al.* A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *Br J Nutr* 2001; **85**: 499–507.

17 O'Connell ED, Nolan JM, Stack J, Greenberg D, Kyle J, Maddock L *et al.* Diet and risk factors for age-related maculopathy. *Am J Clin Nutr* 2008; **87**: 712–722.

18 Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate retinas, III: effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. *Invest Ophthalmol Vis Sci* 2005; **46**: 692–702.

19 Bhosale P, Zhao dY, Serban B, Bernstein PS. Identification of 3-methoxyzeaxanthin as a novel age-related carotenoid metabolite in the human macula. *Invest Ophthalmol Vis Sci* 2007; **48**: 1435–1440.

20 Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Beatty S. Transport and retinal capture of lutein and zeaxanthin with reference to age-related macular degeneration. *Surv Ophthalmol* 2008; **53**: 68–81.

21 Murray IJ, Makridaki M, van der Veen RL, Carden D, Parry NR, Berendschot TT. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. *Invest Ophthalmol Vis Sci* 2013; **54**: 1781–1788.

22 Beatty S, Nolan JM, Muldrew KA, Woodside J, Stevenson MR, Chakravarthy U. Visual outcome after antioxidant supplementation. *Ophthalmology* 2013; **120**: 645.

23 Beatty S, Chakravarthy U, Nolan JM, Muldrew KA, Woodside JV, Denny F *et al.* Secondary outcomes in a clinical trial of carotenoids with coantioxidants versus placebo in early age-related macular degeneration. *Ophthalmology* 2013; **120**: 600–606.

24 Charalampidou S, Loughman J, Nolan J, Stack J, Cassidy L, Pesudovs K *et al.* Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol* 2011; **129**(9): 1155–1161.

25 Kleiner RC, Enger C, Alexander MF, Fine SL. Contrast sensitivity in age-related macular degeneration. *Arch Ophthalmol* 1988; **106**: 55–57.

26 Pelli DG, Bex P. Measuring contrast sensitivity. *Vision Res* 2013; **90**: 10–14.

27 Garcia-Layana A, Recalde S, Alaman AS, Robredo PF. Effects of lutein and docosahexaenoic acid supplementation on macular pigment optical density in a randomized controlled trial. *Nutrients* 2013; **5**: 543–551.

28 Akuffo KO, Beatty S, Stack J, Dennison J, O'Regan S, Meagher KA *et al.* Central Retinal Enrichment Supplementation Trials (CREST): Design and Methodology of the CREST Randomized Controlled Trials. *Ophthalmic Epidemiol* 2014; **21**: 111–123.

29 Age Related Eye Disease Study 2 Research Group. Lutein +zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 2013; **309**: 2005–2015.

30 Chew EY, Clemons TE, SanGiovanni JP, Danis RP, Ferris FL III, Elman MJ *et al.* Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 Report No. 3. *JAMA Ophthalmol* 2014; **132**(2): 142–149.

Concordance of Macular Pigment Measurement Using Customized Heterochromatic Flicker Photometry and Fundus Autofluorescence in Age-Related Macular Degeneration

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Purpose. We compared macular pigment (MP) measurements using customized heterochromatic flicker photometry (Macular Metrics Densitometer) and dual-wavelength fundus autofluorescence (Heidelberg Spectralis HRA + OCT MultiColor) in subjects with early age-related macular degeneration (AMD).

Methods. Macular pigment was measured in 117 subjects with early AMD (age, 44–88 years) using the Densitometer and Spectralis, as part of the Central Retinal Enrichment Supplementation Trial (CREST; ISRCTN13894787). Baseline and 6-month study visits data were used for the analyses. Agreement was investigated at four different retinal eccentricities, graphically and using indices of agreement, including Pearson correlation coefficient (precision), accuracy coefficient, and concordance correlation coefficient (ccc).

Results. Agreement was poor between the Densitometer and Spectralis at all eccentricities, at baseline (e.g., at 0.25° eccentricity, accuracy = 0.63, precision = 0.35, ccc = 0.22) and at 6 months (e.g., at 0.25° eccentricity, accuracy = 0.52, precision = 0.43, ccc = 0.22). Agreement between the two devices was significantly greater for males at 0.5° and 1.0° of eccentricity. At all eccentricities, agreement was unaffected by cataract grade.

Conclusions. In subjects with early AMD, MP measurements obtained using the Densitometer and Spectralis are not statistically comparable and should not be used interchangeably in either the clinical or research setting. Despite this lack of agreement, statistically significant increases in MP, following 6 months of supplementation with macular carotenoids, were detected with each device, confirming that these devices are capable of measuring change in MP within subjects over time. (http://www.controlled-trials.com number, ISRCTN13894787.)

Keywords: macular pigment, customized heterochromatic flicker photometry, fundus autofluorescence, age-related macular degeneration, concordance, agreement

Macular pigment (MP) is composed of the carotenoids, lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ).[1] Macular pigment is found at the macula (the specialized part of the retina that mediates central vision) between the receptor axon and inner plexiform layers.[2,3] Macular pigment filters short-wavelength (blue) light and its constituent carotenoids have antioxidant[4,5] and anti-inflammatory properties.[6–9] Macular pigment's unique anatomic location, blue light filtering properties, and antioxidant and anti-inflammatory properties make this pigment important for visual function.[10] Macular pigment (and its constituent carotenoids) may have an important role in age-related macular degeneration (AMD) by reducing oxidative stress via its antioxidant properties as well as limiting the effect of inflammatory mediators in the pathogenesis of this condition.[11] Some studies also have reported that MP may be lower compared to controls among persons with glaucoma,[12] Alzheimer's disease (AD),[13] and diabetes,[14,15] suggesting that MP could be a useful biomarker for these conditions, providing a biologically plausible rationale to investigate whether MP has a role in these pathologies.

Given the importance of MP for visual function in diseased[16–18] and nondiseased retinas,[19,20] and the emerging evidence that MP may be a useful biomarker for AD,[21,22] researchers and clinicians must measure MP levels accurately across different populations. A variety of techniques are available for measuring MP (and its constituent carotenoids) and the measurement techniques can be classified broadly as ex vivo (i.e., outside cell/tissue) and in vivo techniques (i.e., inside cell/tissue). Ex vivo techniques include high performance liquid chromatography (HPLC) and microdensitometry. However, these ex vivo techniques can be performed only in postmortem eyes. In vivo techniques include physical techniques (e.g., dual-wavelength fundus autofluorescence (2WAF), fundus reflectometry, and raman spectroscopy) and psychophysical techniques (e.g., heterochromatic flicker photometry [HFP], customized heterochromatic flicker photometry [cHFP], color matching, and motion photometry), and these methods are desirable because they can be performed noninvasively in the living subject. The 2WAF technique also can be used ex vivo. Of note, however, there remains debate as to which

device, if any, should be deemed as "gold standard" for measuring MP. A review of the literature shows that HFP and cHFP are the most commonly used, but it is important to note that each device has its own advantages and limitations.

The Heidelberg Spectralis HRA+OCT MultiColor (Heidelberg Engineering GmbH, Heidelberg, Germany) is a new, commercially available device which uses the 2WAF technique to measure MP, whereas the Macular Densitometer (Macular Metrics, Corp., Providence, RI, USA) has been available for over a decade, with over 100 peer-reviewed scientific publications which have used this device. A previous study from our group compared MP measurements using the Spectralis to measurements obtained using the Densitometer (which uses cHFP), and reported good concordance between the results obtained by the two devices at four different retinal eccentricities.[23] However, that previous study was performed in subjects free of retinal disease with a mean age of 49 ± 13 years.[23] Therefore, to our knowledge until now, concordance between the Spectralis and the Densitometer has not been evaluated appropriately in the AMD population. The current study was designed to investigate concordance between these two devices, and was done as part of the Central Retinal Enrichment Supplementation Trials (CREST),[24] a randomized double-blind clinical trial designed to investigate the impact of supplementation with the macular carotenoids (L, Z, and MZ) on visual function in healthy subjects with low MP and in subjects with early AMD (the study population used in the current investigation; CREST AMD [ISRCTN13894787]). We see this MP measurement concordance study as an important experiment, as it will inform researchers and clinicians on the agreement between the devices and emphasize the importance of not changing the measurement technique when assessing subjects/patients over time.

Methods

The design and methodology of the CREST study, including intervention assignment, has been described in detail previously.[24] For CREST AMD, the population of interest for the current investigation, the eligibility criteria included early AMD (one to eight on AREDS 11-step severity scale[25] in at least one eye [the study eye], confirmed by the Moorfields Eye Hospital Reading Centre [MEHRC], London, UK), best-corrected visual acuity (BCVA) of 6/12 (20/40) or better, no more than five diopters spherical equivalence of refraction, no previous consumption of supplements containing the macular carotenoids (L, Z, and/or MZ), no other retinal pathology beyond AMD, and no diabetes mellitus (by self-report). Ethical approval was granted by Research Ethics Committee of the Waterford Institute of Technology (WIT), Waterford, Ireland, and the Ethics Committee of the European Research Council (ERC). Written informed consent was obtained from each subject before study enrollment. The tenets of the Declaration of Helsinki were followed in the experimental procedures. A comprehensive clinical assessment, which included MP measurement using cHFP and 2WAF (see below), was conducted at the Macular Pigment Research Group (available in the public domain at www.mprg.ie), Vision Research Centre, Waterford Institute of Technology, Ireland by the study investigator (KOA), who was trained in all aspects of the CREST protocol. All subjects for this investigation were naïve to the MP measurement protocols and had no previous experience with any of the tests.

Macular Pigment Measurement

MP Measurement by cHFP. Macular pigment was measured by cHFP using the Macular Densitometer (Macular Metrics, Corp.) at 0.25°, 0.5°, 1.0°, and 1.75° of retinal eccentricity, with a reference point at 7°.[26] This protocol has been described in detail previously and has been validated in AMD subjects by comparing the in vivo spectral absorption curves from this device to the ex vivo spectral absorption curves of the macular carotenoids.[27] Two wavelengths of light, one at 458 nm (blue light; wavelength that is well absorbed by MP) and the other at 550 nm (green light; wavelength that is not absorbed by MP) are arranged in a stimulus-surround configuration where the stimulus consists of a target presented in counterphase flicker (alternating blue to green). The blue and green alternating lights are inverse-yoked so that when the blue light is adjusted to be more intense, the green light is decreased commensurately and vice versa. The radiance of the blue and green lights are adjusted by turning a dial until the flicker of the disk stops (null flicker) or it is at a point of minimal flicker. Thus, null flicker occurs when there is isoluminance of the blue and green lights.

Before MP measurements, the testing procedure was explained and the subject's critical flicker frequency (CFF) was estimated using a prediction table based on age. Setting the flicker rate according to an expected optimal for a narrow null zone helps to minimize the variance in radiance values obtained during MP measurements at a given retinal eccentricity. If the subject could not reach the null flicker, the CFF was increased by 1 Hz in a stepwise fashion until null flicker was perceived. Also, if the subject exhibited a wide variation in null flicker reading, the CFF was decreased in steps of 1 Hz until an acceptable null range was achieved. During the test, subjects were instructed to turn the radiance knob clockwise or counterclockwise until the flickering stops or it is at a point of minimal flicker. The starting radiance is alternated, so that the knob is not always turned in the same direction. Throughout the testing, subjects were reminded to blink, and instructions were repeated where necessary. Six measurements at each of the targets (0.25°, 0.5°, 1°, 1.75°, and a reference point at 7°) were taken for each subject. The MP measurement at a specified retinal eccentricity was computed from the radiance values obtained where the subject reported null flicker and these radiance values were deemed reliable and acceptable only when the standard deviation of the MP value was below 0.1 optical density units (ODU). The log ratio of the difference in radiance values between the measurement at a particular retinal eccentricity (0.25°, 0.5°, 1°, 1.75°) and the measurement at 7° yields the MP optical density at the specified test locus. Data were not obtained from three (2.6%) subjects because they could not complete the test reliably.

Pupillary Dilation. Pupils were dilated using a drop each of 0.5% proxymetacaine hydrochloride, 2.5% phenylephrine hydrochloride, and 1% tropicamide before performing MP measurement by 2WAF, retinal photography, and cataract grading.

MP Measurement by 2WAF. Macular pigment was measured by 2WAF[28–30] using the Spectralis HRA+OCT MultiColor (Heidelberg Engineering GmbH). This new technology uses confocal scanning laser ophthalmoscopy imaging with diode lasers to measure MP.[23,31] The 2WAF technique works on the principle of excitation of fluorophores (primarily lipofuscin) in the retina and provides a single-pass MP measurement. Fluorescence is an internal property of certain molecules (known as fluorophores) which makes them emit light when excited at certain wavelengths. Lipofuscin is excited by light between 400 and 590 nm and emits light between 520 and 800 nm.[32,33] The excitation spectrum of lipofuscin overlaps with the absorption spectrum of MP (400–550 nm with maximum absorption at 460 nm)[2] and this property is used in the 2WAF technique.

Table 1. Demographic, Lifestyle, Cataract, and AMD Characteristics of Subjects Included in This Report

Variable	
Age, y, mean ± SD	64.68 ± 9.08
Sex, *n* (%)	
Male	39 (33.7)
Female	78 (66.7)
Cataract, mean ± SD*	
Nuclear opalescence	1.65 ± 0.83
Nuclear color	2.40 ± 0.95
Cortical	0.84 ± 1.03
Posterior subcapsular	0.35 ± 0.56
Pseudophakia, *n* (%)*	6 (5.1)
AMD grades, *n* (%)	
1–3	30 (25.6)
4–5	57 (48.7)
6–8	30 (25.6)
SP_MP, mean ± SD*	
0.23	0.52 ± 0.19
0.47	0.44 ± 0.17
0.98	0.33 ± 0.14
1.76	0.14 ± 0.08
DM_MP, mean ± SD*	
0.25	0.75 ± 0.26
0.5	0.63 ± 0.22
1	0.45 ± 0.17
1.75	0.32 ± 0.14

Data displayed are mean ± SD for interval data and percentages for categorical data. SP_MP, macular pigment at 0.23°, 0.47°, 0.98°, and 1.76° eccentricity using dual-wavelength fundus autofluorescence (Heidelberg Spectralis HRA + OCT MultiColor); DM_MP, macular pigment at 0.25°, 0.5°, 1.0° and 1.75° eccentricity using customized heterochromatic flicker photometry (cHFP; Macular Metrics Densitometer). Age-related macular degeneration grades are based on the AREDS 11-step severity scale.

* $n = 117$ as certain tests/measures could not be obtained.

Before MP measurement, alignment, focus and illumination are first adjusted in infrared mode. Once the image is illuminated evenly, the laser mode is switched from infrared to blue plus green laser light autofluorescence. Focus and illumination are readjusted for optimal acquisition. The retina is illuminated simultaneously with two wavelengths of light (486 nm, which is absorbed by MP, and 518 nm, which is not well absorbed by MP) to obtain a series of autofluorescence images within 30 seconds. These images are digitally subtracted to generate MP spatial distribution maps with the reference set at 7°. Macular pigment at 0.23°, 0.47°, 0.98°, and 1.76° retinal eccentricities was recorded. Data were not obtained in two (1.7%) subjects because one subject found bright lights unbearable and refused to continue the test, and the other subject was not able to open the eyes sufficiently for a reliable measurement to be obtained.

Retinal Photography and AMD Grading

Stereoscopic color fundus photographs (45°) were taken in three retinal photographic fields (optic disc, macula, temporal to macula) using a Zeiss Visucam 200 (Carl Zeiss Meditec AG, Jena, Germany). In addition, a monoscopic photograph of the anterior segment was taken to document any media opacities. Photographs were transferred to the MEHRC, London, UK via an encrypted system and were graded in a masked fashion adhering to the Age-Related Eye Disease Study (AREDS) 11-step severity scale.[25]

Cataract Grading

Cataract grading was performed using the Haag-Streit BM 900 slit-lamp biomicroscope (Haag-Streit AG, Koeniz, Switzerland) adhering to the Lens Opacities Classification System III (LOCS III)[34] within the first year of this study by a trained and certified grader (KOA).

The degree of nuclear opalescence (NO) and color (NC) was graded on a scale ranging from 0.1 to 6.9 while cortical (C) and posterior subcapscular (PSC) opacities were graded on a scale ranging from 0.1 to 5.9. In addition, pseudophakia versus cataract in the study eye also was recorded during grading.

Statistical Analysis

One eye (the study eye) of each subject comprised the unit of analysis. The study eye was chosen by adhering to the eligibility criteria with particular emphasis on the presence of early AMD, BCVA of 6/12 (20/40), no more than five diopters spherical equivalence of refraction and no other retinal pathology beyond AMD. The study eye could be either the right or left eye. If both eyes had early AMD, the eye with the best BCVA was chosen as the study eye. However, if both eyes had the same BCVA, the right eye was selected. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (IBM, Armonk, NY). Data analyzed included baseline and 6-month study visits. We decided to use 6-month data, in addition to baseline data, for the following reasons: (1) to investigate whether agreement between the two devices was the same at two different time points (in other words, is there better agreement [as determined by agreement indices] at 6 months in comparison to baseline?); and (2) to investigate whether the two devices are able to consistently detect changes in MP following 6 months of supplementation with the macular carotenoids. Of note, 6-month data should represent increased MP levels in all subjects, given that all subjects in CREST AMD were consuming a formulation containing either 10 mg/day L, 10 mg/day MZ, and 2 mg/day Z or 10 mg/day L and 2 mg/day Z.[24] Uniquely, this allows us to assess concordance at baseline and at 6 months, and also the capacity of each device to detect change in MP over time. Agreement indices, and confidence limits for these indices, were obtained using the statistical programming language R code[35] supplied with Lin et al.[36] Agreement was investigated graphically using ordinary scatterplots of MP from the two devices being compared (with line $y = x$ superimposed). In addition, agreement was assessed using three indices of agreement (see Appendix): (1) precision, the Pearson correlation coefficient, measures the degree of scatter, with values close to 1 indicating closeness to the ordinary least squares regression line (and, hence, little scatter); (2) accuracy, constructed from the means and standard deviations of the two variables being compared, with values close to 1 indicating that the two means are close to each other and that the two standard deviations are close to each other; and (3) concordance correlation coefficient (ccc), obtained as the product of the other two coefficients.

The possible effect on agreement of age, sex, AMD, and cataract, was investigated using a general linear model. Level of significance was set at $P < 0.05$ without adjusting for multiple comparisons.

Results

Table 1 presents the demographic, MP, cataract, and AMD grades of all subjects included in this report. Agreement indices between the Densitometer and Spectralis at baseline study visit are shown in Table 2. The Figure shows the scatterplots of the

Table 2. Agreement Indices for MP Measurements Using the Macular Densitometer and the Heidelberg Spectralis HRA+OCT Multicolor at Baseline (V1)

Measure	ccc	Precision	Accuracy
0.25	0.22 (0.12)	0.35 (0.21)	0.63 (0.54)
0.5	0.27 (0.17)	0.41 (0.27)	0.67 (0.58)
1.0	0.20 (0.08)	0.26 (0.11)	0.76 (0.66)
1.75	0.08 (0.01)	0.19 (0.04)	0.41 (0.34)

For each coefficient, the 95% lower confidence limit is shown in parentheses.

MP values at 0.25°, 0.50°, 1.00°, and 1.75° eccentricity, with the line $y = x$ superimposed. Table 2 and the Figure exhibit poor agreement between the two devices at all eccentricites.

We investigated the effect on agreement of other study variables, using general linear models with difference in measured MP (Densitometer–Spectralis) as dependent variable.

Effect of Age and Sex on Agreement Between the Two Devices

The effect of age and sex on agreement at the four retinal eccentricities was investigated using a general linear model. Agreement was unaffected by age ($P > 0.05$, for all eccentricities). However, disagreement between the two

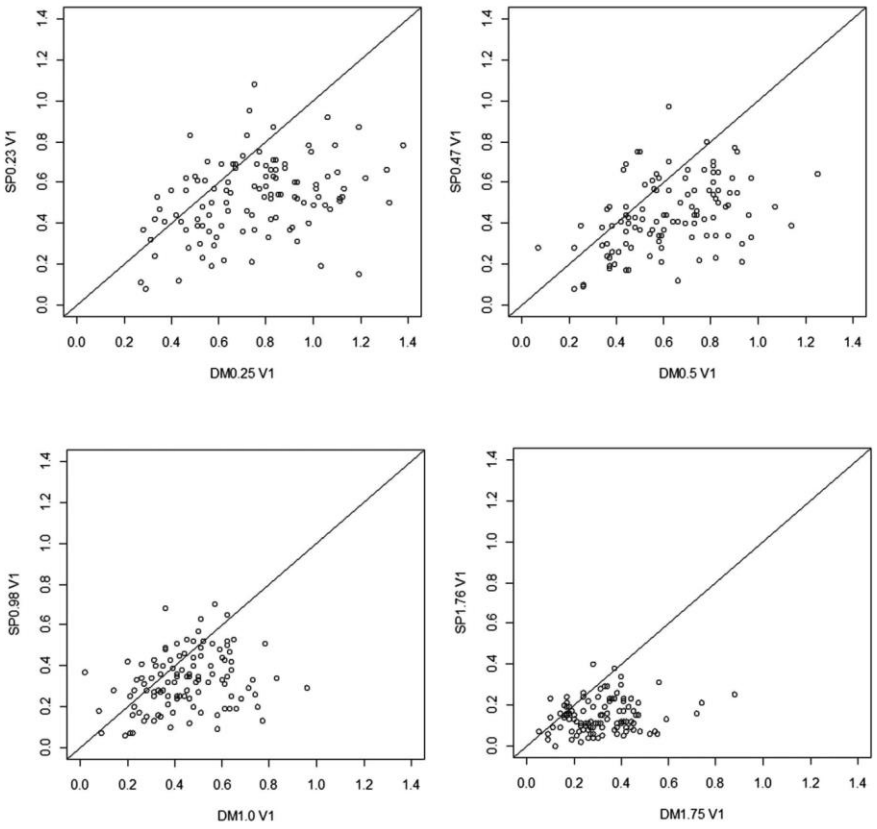

Figure. Scatterplots with the line $y = x$ superimposed comparing macular pigment measurements using the Macular Densitometer (DM) to the Heidelberg Spectralis HRA+OCT Multicolor (SP) at baseline (V1).

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TABLE 3. Subgroup Analyses of the Average Differences in MP Measurements Using the Macular Densitometer and the Heidelberg Spectralis HRA+OCT Multicolor at Baseline (V1)

		MP at 0.25		MP at 0.5		MP at 1.0		MP at 1.75	
Subgroups	*n*	Av. Diff., DM-SP	Sig.	Av. Diff., DM-SP	Sig.	Av. Diff., DM-SP	Sig.	Av. Diff., DM-SP	Sig.
Age, y									
<65	61	0.25	0.302	0.22	0.093	0.16	0.029	0.19	0.161
≥65	56	0.20		0.15		0.08		0.15	
Sex									
Male	36	0.27	0.164	0.25	0.025	0.19	0.007	0.20	0.112
Female	76	0.20		0.16		0.09		0.15	
AMD grades									
1–3	28	0.26	0.356	0.23	0.248	0.19	0.019	0.21	0.111
4–8	84	0.21		0.17		0.10		0.16	
Cataract grades*									
Nuclear opalescence									
<2.5	83	0.24	0.805	0.20	0.844	0.13	0.834	0.18	0.529
≥2.5	7	0.27		0.22		0.11		0.21	
Nuclear Color									
<2.5	55	0.23	0.621	0.18	0.329	0.12	0.829	0.16	0.177
≥2.5	35	0.26		0.23		0.13		0.21	
Cortical									
<1.0	60	0.25	0.678	0.22	0.360	0.13	0.727	0.18	0.830
≥1.0	30	0.23		0.17		0.12		0.18	
Posterior subcapsular									
<0.5	74	0.25	0.688	0.21	0.557	0.14	0.135	0.19	0.053
≥0.5	16	0.22		0.17		0.06		0.11	

Age-related macular degeneration grades are based on the AREDS 11-step severity scale. *n*, number; Av. Diff., DM-SP, average differences in macular pigment between the Densitometer (Macular Metrics Densitometer) and the Spectralis (Heidelberg Spectralis HRA + OCT MultiColor); Sig., level of significance set at standard $P < 0.05$ and obtained using the Independent samples *t*-test.

* $n \neq 117$ as certain tests/measures could not be obtained.

devices was significantly greater for males at 0.5° ($P = 0.025$) and 1.0° ($P = 0.007$) of eccentricity.

Effect of Cataract Grade and Pseudophakia on Agreement Between the Two Devices

The effect of cataract grades (NO, NC, C, PSC) on agreement between the two devices was investigated using a general linear model, independently and after controlling for age and sex. Agreement was unaffected by grade of cataract in all our analyses ($P > 0.05$). The effect of pseudophakia on agreement also was investigated using a general linear model, but the nonsignificant result in this case may be due to lack of statistical power, as there were few cases of pseudophakia in this study (see Table 1).

Effect of AMD Grade on Agreement

The effect of AMD grades (as defined on the AREDS 11-step severity scale) on agreement between the two devices was investigated using a general linear model, independently and after controlling for age and sex. We combined AMD grades 1 to 3 and 4 to 8 for this analysis. Agreement was unaffected by AMD grade in all general linear model analyses controlling for age and sex ($P > 0.05$, for all). Not controlling for age and sex, there is a relationship between AMD grade and disagreement at 1.0° eccentricity ($P = 0.019$), but greater disagreement occurs in the low-risk (1–3) AMD category (Table 3).

Agreement Within Study Subgroups

These findings (on the relationship between agreement and other study variables) are summarized in Table 3, using simple binary classifications of age and cataract grades. The *P* values displayed in Table 3 are from the independent samples *t*-test, and there is one more statistically significant result than from the earlier general linear model analysis; however, greater disagreement in Table 3 is found among the under-65s, not the older subjects.

Is Agreement Different Between Study Visits, That Is, Baseline (V1) and 6 Months (V2)?

The agreement indices at baseline and 6 months were similar; that is, there still is, at 6 months, poor agreement between the Densitometer and Spectralis at all retinal eccentricities (e.g., baseline at 0.25° eccentricity, accuracy = 0.63, precision = 0.35, ccc = 0.22; 6 months at 0.25° eccentricity, accuracy = 0.52, precision = 0.43, ccc = 0.22).

Measuring Change in MP Over Time

Despite these differences between MP measured by the two devices, when we look at change in MP over time (baseline versus 6-month study visit), using a paired *t*-test, the conclusion is the same; that is, on average, MP increases significantly after 6 months of supplementation, whichever

device is used. For example, mean MP at 0.25° eccentricity measured on the Densitometer increases from 0.76 to 0.85 (12%) after 6 months ($P < 0.001$), and mean MP at 0.23° eccentricity measured on Spectralis increases from 0.52 to 0.60 (15%; $P < 0.001$).

Discussion

In the present study, we evaluated the concordance of two MP measuring devices (Densitometer and Spectralis) in subjects with early AMD, and assessed the ability of these devices to detect change in MP over time. In brief, the results of this experiment suggested that MP measurement on both devices are not statistically comparable. Assessing the data (see Fig.), it appears that the Spectralis tends to undervalue MP measurements when compared to the Densitometer. Moreover, this poor agreement was not only present at baseline (before supplementation with the macular carotenoids), but it also was seen at 6 months following supplementation with the macular carotenoids. However, it is important to point out that both devices were capable of detecting changes within subjects following supplementation with formulations containing the macular carotenoids.

Of note, the Spectralis device used in this study has been compared previously (by our group) with the Densitometer in normal healthy subjects.[23] In contrast to the current study, concordance in subjects free of retinal disease was good.[23]

Possible reasons for the lack of agreement in the current study are discussed. Firstly, we studied factors that we believe may contribute to the poor concordance we identified between the two devices in the early AMD population. The variables examined included age, sex, cataract, and AMD grade, and the analysis was done with general linear models (i.e., controlling for other variables) and based on binary versions of each variable (older and younger, lower and higher risk of AMD, and so forth). Of note, we found that only sex was associated consistently with disagreement, at 0.5° and 1.0° eccentricities, with greater disagreement between devices for male subjects. Age and AMD grades were related to disagreement at 1.0° eccentricity, but only in the simplified binary analysis (Table 3), and in both cases the finding was counterintuitive, with younger and lower-risk subjects exhibiting greater disagreement. Given that we did not adjust for multiple testing in this study, these reported significant differences in agreement (for age, sex, and AMD status) should be treated circumspectly: they may be the result of Type I errors.

Of note, MP measurement by cHFP has been shown to be unaffected by cataracts.[37,38] However, it may be surprising that cataract grade did not explain, at least in part, the lack of agreement we observed between the two devices, given that MP measurements by fundus autofluorescence have been reported previously to be affected by cataracts.[39,40] It is important to note, however, that these previous studies, testing the impact of cataract on MP measurement using fundus autofluorescence, used different hardware and software to that used by the Spectralis. Also, in our study, cataract grading was conducted within the first year of the study, rather than at baseline, and it is possible, but unlikely, that this may have influenced our results. Also, it is important to point out that the level of cataract in our study population was not severe (e.g., mean NO 1.65; see Table 1), and it is likely that if cataract is to impact on MP measurement when using fundus autofluorescence, that this would be directly related to severity grade of cataract.[39,40] Finally, and most importantly, this study was not designed to investigate the impact of cataract on Spectralis 2WAF MP measurement. Indeed, a study sufficiently powered with appropriate design to investigate the influence of cataract on Spectralis 2WAF MP measurement would involve MP measurements before and after cataract surgery, and this precise experiment currently is underway at our research center.

Fundamentally, the two devices work on different principles to obtain measurements of MP, with many different assumptions inherent in each device. For example, cHFP requires subjects to follow instructions and to make decisions to obtain MP readings, whereas the 2WAF method does not require subjects' responses or decision-making to obtain MP readings. The subject simply is required to fixate on an internal target within the system for circa 30 seconds. Therefore, MP is measured quickly using the Spectralis, and subject fatigue is not an issue, whereas with the Densitometer, MP measurement takes circa 30 minutes to obtain MP data at four different retinal eccentricities. During cHFP, Troxler's fading may be induced when viewing the target at the peripheral reference locus (7° eccentricity), which is a limitation of this technique. The Spectralis, however, does require pupil dilation, and some subjects do find the bright lights used in the Spectralis uncomfortable. Nevertheless, the Spectralis is a class 1 laser device, which complies with all applicable international standards with respect to safety. Interestingly, the poor concordance between the devices also was seen at 6 months following supplementation with the macular carotenoids, ruling out any possible learning effect with MP measurement using either device. The lack of concordance may be explained, at least in part, by the following assumptions of the 2WAF method: (1) no fluorophores anterior to MP, which cannot be compensated for by digital subtraction of the two autofluorescence images, and (2) the type and composition of fluorophores is assumed to be constant.[28,31,41] The assumption that lipofuscin is equally distributed has been suggested to be compensated by the use of two wavelengths.[28,31]

Despite the lack of agreement between the two devices, statistically significant increases in MP, following 6 months of supplementation with macular carotenoids, were recorded using each device. This finding suggests that, for longitudinal analyses, if the requirement is to detect change over time, both devices are capable of detecting such change (i.e., following supplementation with the macular carotenoids). An important finding from our study is that it is not appropriate to switch between devices when measuring MP in the same subjects over time. For example, mean MP at 0.25° eccentricity, in our study, was 0.76 ODU on the Densitometer at baseline, whereas at 6 months mean MP at 0.23° eccentricity was 0.60 ODU on the Spectralis. Comparison of these two results (baseline for Densitometer and 6 months for Spectralis) would suggest a decrease of 21% in average MP after 6 months of macular carotenoid supplementation in AMD subjects, which clearly is not the case, as a significant and comparable increase was seen with each instrument in subjects over time (12% at 0.25° for Densitometer and 15% at 0.23° for Spectralis).

Each device, therefore, may be suited to a given population, and perhaps more appropriate for a particular research setting. It is important that each device is underpinned by its normative database, given the current diversity of MP research, and the need to measure MP accurately in the research and clinic setting. In summary, for clinical or research purposes, it is advisable that the same device be used for baseline and follow-up visits within a given study.

The strengths of this study include the following: (1) MP measurements were conducted in a relatively large sample ($n = 117$) of subjects with early AMD at two time points (baseline and 6 months); (2) assessment of AMD morphology was performed by an accredited reading center in a masked fashion; (3) cataract grading was conducted by a trained and

certified LOCS III grader; (4) subjects were naïve to both tests, thereby limiting potential confounding attributable to subject bias; and (5) one trained examiner conducted MP measurements on both devices; therefore, eliminating interexaminer bias and variability. The main limitation of this study is that cataract grades were obtained within the first year of the study rather than at baseline.

In conclusion, specific MP values obtained using the Spectralis are not comparable to MP values obtained using the Densitometer in subjects with early AMD. These two devices should not, for AMD subjects, be used interchangeably in the clinical or research settings, but each device is capable of reliably detecting and quantifying change in MP following supplementation with MP's constituent carotenoids. Accordingly, it is advisable that the same device be used within a given study. Furthermore, each of these two devices should be underpinned by its respective and separate normative database.

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References

1. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys*. 2001;385:28–40.
2. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. 1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
3. Trieschmann M, van Kuijk FJ, Alexander R, et al. Macular pigment in the human retina: histological evaluation of localization and distribution. *Eye (Lond)*. 2008;22:132–137.
4. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802–1811.
5. Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY, Katz NB. Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Exp Eye Res*. 2001;72:215–223.
6. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. Lutein supplementation leads to decreased soluble complement membrane attack complex sC5b-9 plasma levels. *Acta Ophthalmol*. 2015;93:141–145.
7. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a and C3d. *PLoS One*. 2013;8:e73387.
8. Li SY, Fung FK, Fu ZJ, Wong D, Chan HH, Lo AC. Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Invest Ophthalmol Vis Sci*. 2012;53:5976–5984.
9. Firdous AP, Kuttan G, Kuttan R. Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. *Pharm Biol*. 2015;53:961–967.
10. Loughman J, Davison PA, Nolan JM, Akkali MC, Beatty S. Macular pigment and its contribution to visual performance and experience. *J Optom*. 2010;3:74–90.
11. Sabour-Pickett S, Nolan JM, Loughman J, Beatty S. A review of the evidence germane to the putative protective role of the macular carotenoids for age-related macular degeneration. *Mol Nutr Food Res*. 2011;56:270–286.
12. Igras E, Loughman J, Ratzlaff M, O'Caoimh R, O'Brien C. Evidence of lower macular pigment optical density in chronic open angle glaucoma. *Br J Ophthalmol*. 2013;97:994–998.
13. Nolan JM, Loskutova E, Howard AN, et al. Macular pigment, visual function, and macular disease among subjects with alzheimer's disease: an exploratory study. *J Alzheimer's Dis*. 2014;42:1191–1202.
14. Scanlon G, Connell P, Ratzlaff M, et al. Macular pigment optical density is lower in type 2 diabetes, compared with type 1 diabetes and normal controls. *Retina*. 2015;35:1808–1816.
15. Lima VC, Rosen RB, Maia M, et al. Macular pigment optical density measured by dual-wavelength autofluorescence imaging in diabetic and nondiabetic patients: a comparative study. *Invest Ophthalmol Vis Sci*. 2010;51:5840–5845.
16. Liu R, Wang T, Zhang B, et al. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:252–258.
17. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902–912.
18. Ma L, Yan SF, Huang YM, et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology*. 2012;119:2290–2297.
19. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871–7880.
20. Hammond BR, Fletcher LM, Roos F, Wittwer J, Schalch W. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Invest Ophthalmol Vis Sci*. 2014;55:8583–8589.
21. Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly. *Am J Clin Nutr*. 2012;96:1161S–1165S.
22. Johnson EJ. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. *Nutr Rev*. 2014;72:605–612.
23. Dennison JL, Stack J, Beatty S, Nolan JM. Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-wavelength autofluorescence, and single-wavelength reflectance. *Exp Eye Res*. 2013;116:190–198.
24. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111–123.
25. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol*. 2005;123:1484–1498.
26. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Invest Ophthalmol Vis Sci*. 1999;40:2481–2489.
27. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445–453.

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Relationship between macular pigment and visual function in subjects with early age-related macular degeneration

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ABSTRACT

Purpose To investigate the relationship between macular pigment (MP) and visual function in subjects with early age-related macular degeneration (AMD).

Methods 121 subjects with early AMD enrolled as part of the Central Retinal Enrichment Supplementation Trial (CREST; ISRCTN13894787) were assessed using a range of psychophysical measures of visual function, including best corrected visual acuity (BCVA), letter contrast sensitivity (CS), mesopic and photopic CS, mesopic and photopic glare disability (GD), photostress recovery time (PRT), reading performance and subjective visual function, using the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25). MP was measured using customised heterochromatic flicker photometry.

Results Letter CS, mesopic and photopic CS, photopic GD and mean reading speed were each significantly ($p<0.05$) associated with MP across a range of retinal eccentricities, and these statistically significant relationships persisted after controlling for age, sex and cataract grade. BCVA, NEI VFQ-25 score, PRT and mesopic GD were unrelated to MP after controlling for age, sex and cataract grade ($p>0.05$, for all).

Conclusions MP relates positively to many measures of visual function in unsupplemented subjects with early AMD. The CREST trial will investigate whether enrichment of MP influences visual function among those afflicted with this condition.

Trial registration number ISRCTN13894787.

INTRODUCTION

The carotenoids (lutein (L), zeaxanthin (Z) and *meso*-zeaxanthin (MZ)) are found in the human macula, where they are collectively known as macular pigment (MP). MP gives the macula its eponymous yellow appearance (*macula lutea*). Interestingly, the pigment is captured and normally (typically) distributed at the macula in such a way that it peaks centrally at the foveola (the part of the retina responsible for high acuity and colour vision) and declines with increasing retinal eccentricity.[1]

MP filters short-wavelength light (thereby passively limiting photo-oxidative damage), and its constituent carotenoids have antioxidant properties (and therefore actively neutralise reactive oxygen species).[2]

Several hypotheses (eg, the acuity hypothesis,[3] glare hypothesis,[4] visibility hypothesis[5]) have been put forward to explain how MP may influence visual function. Indeed, studies have been performed to examine the role of MP for visual function across diverse populations of subjects (eg, healthy subjects free of retinal disease and subjects with age-related macular degeneration (AMD)). In general, cross-sectional studies have shown positive and statistically significant relationships between MP and visual function.[4 6–10] Furthermore, most interventional trials have shown that supplementation with the macular carotenoids impacts positively on visual function in subjects with and without retinal disease,[11–16] and it appears that supplementation with all three macular carotenoids offers advantages over formulations containing only two of these three nutrients.[15 16] However, we await the outcome of a double-blind placebo-controlled clinical trial to confirm this hypothesis.[17]

The Central Retinal Enrichment Supplementation Trials (CREST), which commenced in 2012 and will be completed in 2016, was designed to investigate the impact of macular carotenoid supplementation on vision in normal healthy subjects with low MP (Trial 1: CREST Normal (ISRCTN68270512)) and subjects with early AMD (the latter representing the study population in the current investigation; Trial 2: CREST AMD (ISRCTN13894787)).[17] In this report, we present findings on the relationship between MP and psychophysical (and subjective) measures of visual function in unsupplemented subjects with early AMD, using baseline data from CREST AMD.[17]

METHODS

Study population

The design and methodology of CREST AMD have been described in detail elsewhere.[17] Inclusion criteria included: early AMD (one to eight on AREDS 11-step severity scale[18] in at least one eye (the study eye), confirmed by the Reading Centre at the Moorfields Eye Hospital Reading Centre, London, UK); best corrected visual acuity (BCVA) of 6/12 or better; spherical equivalence of refraction no more than 5 dioptres; no previous consumption of supplements containing the macular carotenoids (L, Z and/or MZ); no retinal pathology beyond AMD; no diabetes mellitus (by self-report). All clinical assessments were conducted by the study investigator (KOA) who was trained in all aspects of the CREST protocol.[17] Clinical assessments pertaining to this report are briefly described below.

MP measurement

MP was measured using the Macular Densitometer (Macular Metrics, Providence, Rhode Island, USA)

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at 0.25°, 0.5°, 1.0° and 1.75° retinal eccentricity, with a reference point at 7°. This protocol has been validated for subjects with early AMD.[19]

Visual function assessment

Best corrected visual acuity

BCVA was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (LogMAR) chart (Test Chart 2000 Xpert; Thomson Software Solutions, UK) viewed at 4 m.

Letter contrast sensitivity

Letter contrast sensitivity (CS) was assessed using the LogMAR ETDRS (Test Chart 2000 PRO; Thomson Software Solutions, UK) chart viewed at 4 m.

CS with the Functional Vision Analyzer

CS was also assessed using the Functional Vision Analyzer (Stereo Optical Co., Chicago, Illinois, USA). The test was conducted under four simulated conditions: (1) mesopic (3.0 candela per metre square (cd/m^2)), (2) photopic (85 cd/m^2), (3) mesopic with glare (28 Lux, mesopic glare disability (GD)), (4) photopic with glare (135 Lux, photopic GD).

Photostress recovery time

Photostress recovery time (PRT) (in seconds) was assessed by measuring the time of recovery (time taken to see the 6/24 letterset ETDRS (Test Chart 2000 PRO) at a specified contrast threshold) after exposure to a 300 W tungsten spotlight (ARRI 300 Plus lamp, ARRI Lighting Solutions, GmbH, Germany) with a low-pass glass dichroic filter.

Reading performance

Reading performance was assessed using the English version of the Radner reading chart at 40 cm.

Subjective visual function

Subjective visual function was assessed using the National Eye Institute Visual Function questionnaire-25 (NEI VFQ-25).

Cataract grading

Cataract grading was performed using the Haag-Streit BM 900 Slit lamp biomicroscope (Haag-Streit AG, Switzerland) adhering to the Lens Opacities Classification System III (LOCS III)[20] within the first year of this study by a trained and certified grader (KOA).

Statistical analysis

One eye (the study eye) of each subject comprised the unit of analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows, V.21.0 (Armonk, New York, USA). Pearson's correlation coefficients were used to investigate bivariate relationships between MP and visual function parameters. General linear models were used to control for variables such as age, sex and cataract grade (while excluding subjects with pseudophakia). We used the 5% level of significance throughout, without adjusting for multiple comparisons.

RESULTS

Table 1 presents the demographic and lifestyle characteristics of all subjects included in this report. Table 2 presents the relationship between MP and visual function, as assessed by the bivariate Pearson correlation coefficients. General linear models were used to assess which visual function variables are statistically related to MP, controlling for age, sex and cataract grade; these results are reported in the text, separately for each visual function variable.

Table 1 Demographic, lifestyle, visual function and MP of subjects included in this report

Variables	n (%)
Sex	
Male	40 (33.1)
Female	81 (66.9)
Education	
Primary	18 (14.9)
Secondary	57 (47.1)
Tertiary	46 (38.0)
	Mean±SD
Age (years)	64.77±9.03
Cataract*	
NO	1.64±0.81
NC	2.41±0.95
C	0.88±1.08
PSC	0.37±0.6
BCVA	
Study eye	99.97±5.88
Fellow eye	95.34±11.45
Letter CS (cpd)	
1.2	1.81±0.17
2.4	1.80±0.20
6	1.52±0.23
9.6	1.27±0.28
15.15*	0.90±0.32
CSmesopic (cpd)	
1.5	1.57±0.22
3	1.65±0.21
6	1.27±0.35
12	0.81±0.27
18	0.32±0.11
CSphotopic (cpd)	
1.5	1.49±0.17
3	1.76±0.21
6	1.63±0.31
12	1.22±0.37
18	0.57±0.34
GDmesopic (cpd)	
1.5	0.95±0.31
3	1.15±0.35
6	0.93±0.24
12	0.64±0.13
18	0.30±0.03
GDphotopic (cpd)	
1.5	1.43±0.19
3	1.70±0.20
6	1.54±0.32
12	1.15±0.37
18	0.54±0.33
PRT	15.98±8.23
Reading	
RAcuity	0.10±0.12
Mean RS	154.68±27.36
Max RS	199.79±33.02
SVF	
VFQ_Tscore	88.95±9.84

Continued

Table 1 Continued

Variables	n (%)
MP*	
0.25	0.75±0.25
0.5	0.63±0.21
1	0.44±0.17
1.75	0.31±0.14

Cataracts graded using the Lens Opacities Classification System (LOCS) III. Cataract grades - NO, nuclear opalescence; NC, nuclear colour; C, cortical; PSC, posterior subcapsular cataract.
Data displayed are mean±SD for interval data and percentages for categorical data.
*n≠121 for all variables as certain tests/measures could not be obtained.
Age, age in years; BCVA, best corrected visual acuity measured with Thompson Test Chart 2000 Xpert and recorded in visual acuity rating (VAR); cpd, cycles per degrees; CS, contrast sensitivity; CSmesopic, contrast sensitivity measured under nighttime conditions (3.0 candela per metre square (cd/m^2)) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; CSphotopic, contrast sensitivity measured under daytime conditions (85 cd/m^2) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; education, highest level of education; GD, glare disability; GDmesopic, glare disability measured under nighttime conditions (28 Lux) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; GDphotopic, glare disability measured under daytime conditions (135 Lux) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; LetterCS, letter contrast sensitivity (assessed using Thompson Test Chart 2000 PRO and recorded in logarithm of contrast sensitivity (LogCS) units); maxRS, maximum reading speed; meanRS, mean reading speed (calculated as the average of the reading speed scores recorded for each of the standardised sentences); MP, macular pigment measured at 0.25°, 0.5°, 1.0° and 1.75° eccentricities using customised heterochromatic flicker photometry; n, number of subjects; PRT, photostress recovery time recorded in seconds (reading assessed using the English version of the standardised Radner reading chart); RAcuity, reading acuity (Reading acuity is recorded in logarithm of the reading acuity determination (LogRAD). The formula (logRAD+total number of incorrectly read syllables ×0.005) is used to calculate the LogRAD-score.); RS, reading speed (the time taken to read the number of words in a sentence) measured in words per minute (w/min) with a stop watch for each standardised sentence (14 words ×60 s divided by reading time in seconds); SVF, subjective visual function assessed using the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25; score range from 0 (worst) to 100 (best)); VFQ_Tscore, NEI VFQ-25 overall vision score.

MP and BCVA

There is a positive and significant relationship between central MP (0.25° up to 1°) and BCVA (table 2). In a general linear model controlling for age, sex and cataract grade, there is no statistically significant relationship between MP (at any eccentricity) and BCVA (p>0.05, for all).

MP and letter CS

MP is not significantly related to letter CS, except for MP at 1° and letter CS at 1.2 cpd (table 2). In a general linear model controlling for age, sex and cataract grade, letter CS at 1.2 cpd remains positively and significantly related to MP at 1° (p=0.023; figure 1A).

MP and measures of CS using the Functional Vision Analyzer

A range of mesopic CS variables are positively and significantly related to MP (table 2). In a general linear model controlling for age, sex and cataract grade, the following relationships remained statistically significant: (a) the relationship between MP at 0.5° and mesopic CS at 3 cpd (p=0.047) and 6 cpd (p=0.033) (figure 1A); (b) the relationship between MP at 1° and mesopic CS at 1.5 cpd (p=0.033; figure 1A). However, MP at 1.75° is now positively and significantly related to mesopic CS at 1.5 cpd (p=0.041) (figure 1A).

For photopic CS and MP, correlations similar to those between MP and mesopic CS are observed (table 2). In a general linear model controlling for age, sex and cataract grade: (a) there is a significant relationship between MP at 0.25° and photopic CS at 12 cpd (p=0.013; figure 1A); (b) there is a significant relationship between MP at 0.5° and photopic CS at 6 cpd (p=0.006) and 12 cpd (p=0.003) (figure 1A); (c) there is a significant relationship between MP at 1° and photopic CS at 6 cpd (p=0.039; figure 1A) and 12 cpd (p=0.025; figure 1B). However, MP at 1.75° is now positively and significantly related to photopic CS at 12 cpd (p=0.049; figure 1B).

MP and GD

No significant correlations between MP and mesopic GD are observed (table 2), even after controlling for age, sex and cataract grade (p>0.05, for all).

In a general linear model for photopic GD controlling for age, sex and cataract grade, the following relationships remain statistically significant: (a) the relationship between MP at 0.25° and photopic GD at 6 cpd (p=0.006; figure 1B); (b) the relationship between MP at 0.5° and photopic GD at 3 cpd (p=0.025) and 6 cpd (p=0.004) (figure 1B); (c) the relationship between MP at 1° and photopic GD at 3 cpd (p=0.030) (figure 1B). However, MP at 1.75° is now positively and significantly related to photopic GD at 3 cpd (p=0.037) (figure 1B).

MP and reading performance

Correlations between MP and reading performance are shown in table 2. In a general linear model controlling for age, sex and cataract grade, there is a significant relationship between mean reading speed and MP at eccentricities 1.0° (p=0.046) and 1.75° (p=0.034) (figure 1B).

Of note, these reading performance variables are also significantly related to education (p<0.05, for all). Therefore, we repeated the analyses controlling for age, sex, cataract grade and education. In a general linear model, and after controlling for age, sex, cataract grade and education, only the significant and positive relationship between MP at 1.75° and mean reading speed persisted (p=0.048).

MP and subjective visual function

Correlations between MP and subjective visual function are shown in table 2. In a general linear model controlling for age, sex and cataract grade, MP (at any eccentricity) is not related to subjective visual function (p>0.05, for all).

MP and PRT

MP (at any eccentricity) is not related to PRT, either in the correlation analyses (table 2) or in the general linear model analyses controlling for age, sex and cataract grade (p>0.05, for all).

DISCUSSION

This study presents findings on the relationship between MP and visual function in subjects with early AMD who are not yet using supplements (CREST AMD baseline data).

Our main findings show that MP relates to several measures of visual function, even after controlling for age, sex and cataract grade. Indeed, we report that MP is positively associated with a range of CS measures in both mesopic (nighttime) and photopic (daytime) conditions. A possible explanation for the role that MP plays in optimising CS may rest on the visibility hypothesis of MP. This hypothesis posits that MP can enhance detail of a target by the absorption of blue haze.[5] Blue haze is caused by scattered short-wavelength dominant air light (blue light) that produces a veiling luminance when we view objects at a distance.[5] MP accentuates the luminance of an object relative to its background by attenuating this scattered (veiling) short-wavelength visible blue light and, by consequence, extends the

Table 2 Relationship between MP and visual function using baseline data in the Central Retinal Enrichment Supplementation Trial (CREST) age-related macular degeneration study

Variables	MP at 0.25	MP at 0.5	MP at 1.0	MP at 1.75
BCVA	r=0.241, p=**0.009**	r=0.243, p=**0.008**	r=0.210, p=**0.022**	r=0.107, p=0.248
Letter CS (cpd)				
1.2	r=0.101, p=0.278	r=0.113, p=0.225	r=0.219, p=**0.017**	r=0.102, p=0.272
2.4	r=0.094, p=0.310	r=0.116, p=0.210	r=0.171, p=0.064	r=0.020, p=0.829
6	r=0.166, p=0.073	r=0.157, p=0.089	r=0.153, p=0.098	r=0.016, p=0.859
9.6	r=0.113, p=0.221	r=0.096, p=0.302	r=0.082, p=0.375	r=−0.014, p=0.883
15.15	r=0.106, p=0.257	r=0.106, p=0.256	r=0.080, p=0.393	r=0.037, p=0.690
CSmesopic (cpd)				
1.5	r=0.103, p=0.267	r=0.123, p=0.185	r=0.194, p=**0.035**	r=0.131, p=0.157
3	r=0.139, p=0.133	r=0.217, p=**0.018**	r=0.165, p=0.074	r=0.038, p=0.680
6	r=0.169, p=0.067	r=0.203, p=**0.028**	r=0.148, p=0.111	r=0.111, p=0.230
12	r=0.209, p=**0.023**	r=0.220, p=**0.017**	r=0.209, p=**0.023**	r=0.159, p=0.086
18	r=0.175, p=0.058	r=0.170, p=0.066	r=0.071, p=0.447	r=0.228, p=**0.013**
CSphotopic (cpd)				
1.5	r=−0.002, p=0.987	r=0.050, p=0.589	r=0.097, p=0.295	r=−0.025, p=0.787
3	r=0.091, p=0.326	r=0.162, p=0.079	r=0.137, p=0.138	r=0.125, p=0.178
6	r=0.168, p=0.069	r=0.243, p=**0.008**	r=0.194, p=**0.036**	r=0.075, p=0.422
12	r=0.259, p=**0.005**	r=0.290, p=**0.001**	r=0.238, p=**0.009**	r=0.169, p=0.068
18	r=0.123, p=0.186	r=0.144, p=0.119	r=0.067, p=0.468	r=0.062, p=0.502
GDmesopic (cpd)				
1.5	r=0.051, p=0.580	r=0.068, p=0.463	r=0.069, p=0.461	r=0.002, p=0.983
3	r=0.077, p=0.407	r=0.113, p=0.225	r=0.070, p=0.454	r=−0.010, p=0.916
6	r=0.041, p=0.656	r=0.098, p=0.293	r=0.032, p=0.733	r=−0.096, p=0.303
12	r=0.029, p=0.758	r=0.060, p=0.520	r=0.067, p=0.474	r=−0.034, p=0.713
18	r=0.047, p=0.611	r=0.080, p=0.390	r=0.042, p=0.654	r=0.031, p=0.740
GDphotopic (cpd)				
1.5	r=0.051, p=0.580	r=0.083, p=0.374	r=0.081, p=0.382	r=0.061, p=0.515
3	r=0.164, p=0.076	r=0.256, p=**0.005**	r=0.231, p=**0.012**	r=0.153, p=0.098
6	r=0.233, p=**0.011**	r=0.242, p=**0.008**	r=0.186, p=**0.043**	r=0.116, p=0.211
12	r=0.222, p=**0.016**	r=0.225, p=**0.014**	r=0.164, p=0.077	r=0.085, p=0.359
18	r=0.166, p=0.073	r=0.180, p=0.051	r=0.070, p=0.450	r=0.069, p=0.458
PRT	r=0.073, p=0.433	r=0.153, p=0.097	r=0.068, p=0.464	r=0.001, p=0.990
Reading				
RAcuity	r=−0.183, p=**0.047**	r=−0.187, p=**0.042**	r=−0.113, p=0.152	r=−0.056, p=0.547
Mean RS	r=0.177, p=0.056	r=0.170, p=0.065	r=0.222, p=**0.016**	r=0.132, p=0.153
Max RS	r=0.135, p=0.146	r=0.119, p=0.201	r=0.164, p=0.076	r=0.040, p=0.664
SVF				
VFQ_Tscore	r=0.188, p=**0.042**	r=0.132, p=0.155	r=0.124, p=0.181	r=0.025, p=0.787

Bold typeface signifies statistically significant p value (p<0.05).
BCVA, best corrected visual acuity measured with Thompson Test Chart 2000 Xpert and recorded in visual acuity rating (VAR); cpd, cycles per degrees; CS, contrast sensitivity; CSmesopic, contrast sensitivity measured under nighttime conditions (3.0 candela per metre square (cd/m^2)) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; CSphotopic, contrast sensitivity measured under daytime conditions (85 cd/m^2) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; GD, glare disability; GDmesopic, glare disability measured under nighttime conditions (28 Lux) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; GDphotopic, glare disability measured under daytime conditions (135 Lux) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; LetterCS, letter contrast sensitivity assessed using Thompson Test Chart 2000 PRO and recorded in logarithm of contrast sensitivity (LogCS) units; maxRS, maximum reading speed; meanRS, mean reading speed calculated as the average of the reading speed scores recorded for each of the standardised sentences; MP, macular pigment measured at 0.25°, 0.5°, 1.0° and 1.75° eccentricities using customised heterochromatic flicker photometry; p, level of statistical significance set at p<0.05; PRT, photostress recovery time recorded in seconds (reading assessed using the English version of the standardised Radner reading chart); r, Pearson's correlation coefficient; RAcuity, reading acuity (Reading acuity is recorded in logarithm of the reading acuity determination (LogRAD). The formula (logRAD+total number of incorrectly read syllables ×0.005) is used to calculate the LogRAD-score.); RS, reading speed (the time taken to read the number of words in a sentence) measured in words per minute (w/min) with a stop watch for each standardised sentence (14 words×60 s divided by reading time in seconds); SVF, subjective visual function assessed using the National Eye Institute Visual Function Questionnaire-25 (NEI VFQ-25; scores range from 0 (worst) to 100 (best)); VFQ_Tscore, NEI VFQ-25 overall vision score.

visual range. The visibility hypothesis has been tested empirically and is supported by two studies which demonstrate the beneficial effect of MP on simulated blue haze conditions.[8 9] Furthermore, Hammond and Renzi propose that MP may improve CS by the differential absorption of chromatic edges (ie, the ability of MP to absorb the short-wavelength (blue) component of an isoluminant edge).[7 10 11] Of note, we found significant associations between MP and CS at 6 cpd, which is the primary outcome measure of the prospective arm of the current study.[17] The importance of our findings with respect to the relationship between MP and CS rests on the observation that CS has been shown to be an important determinant of quality of life,[21] which has important implications for the population studied here.

In our study, we also found that reading performance is related to MP levels (table 2). For example, we found that

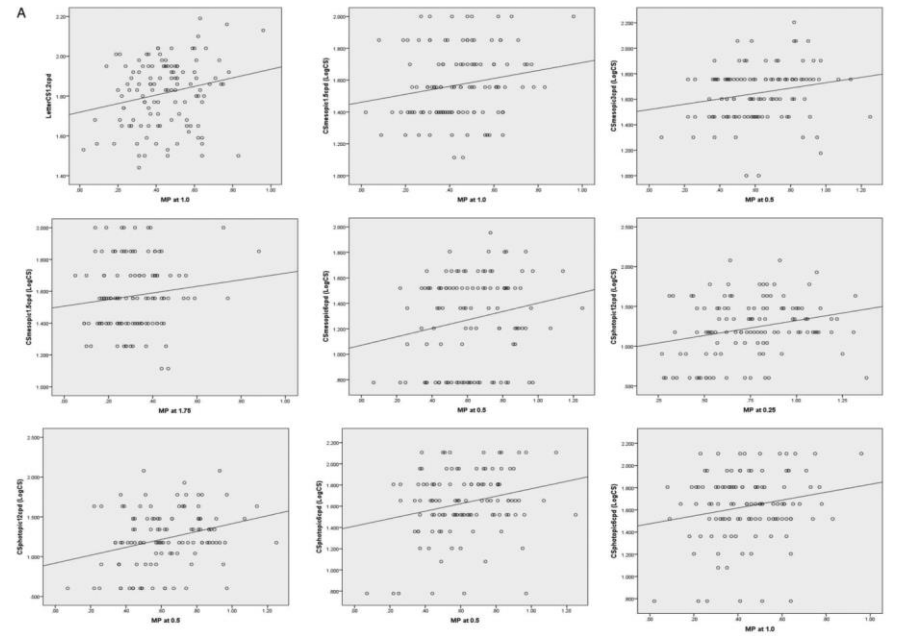

Figure 1 Relationship between measures of macular pigment and visual function. CSmesopic, contrast sensitivity measured under nighttime conditions (3.0 candela per metre square (cd/m^2)) using the Functional Vision Analyzer and recorded in logarithm of contrast sensitivity (LogCS) units; CSphotopic, contrast sensitivity measured under daytime conditions (85 cd/m^2) using the Functional Vision Analyzer and recorded in LogCS units; GD, glare disability; GDmesopic, GD measured under nighttime conditions (28 Lux) using the Functional Vision Analyzer and recorded in LogCS units; GDphotopic, GD measured under daytime conditions (135 Lux) using the Functional Vision Analyzer and recorded in LogCS units; LetterCS, letter contrast sensitivity.

higher MP at 1° is associated with better reading speed, even after controlling for age, sex and cataract grade. However, we believe that this finding is not solely attributable to the optical filtration properties of MP at the macula, as it is possible that the role of carotenoids in brain health[22] and cognition[23] may have contributed to our finding (and therefore that MP simply represents a biomarker for concentrations of these nutrients in brain). Indeed, this notion is consistent with our finding that education is also related to reading performance in the current study, although it is noteworthy that MP at 1.75° eccentricity was positively related to mean reading speed even after correction for education. Indeed, and for instance, we know that L and Z concentrations at the macula correlate with their respective concentrations in the frontal cortex and cerebellum (brain areas engaged in reading performance) in primates.[24] It has also been reported that reading speed is positively related to visual processing in persons with AMD.[25] These carotenoids, and their putative contribution to optimal neural processing and efficiency,[26 27] may contribute positively to reading performance. Reading performance is also a measure of subjects' ability to perform tasks related to near-vision, with important and positive implications for quality of life.[28]

Although the current study did not find any relationship between MP and PRT or mesopic GD, we found a significant and positive relationship between MP and photopic GD at some spatial frequencies (even after controlling for age, sex and cataract grade). Some previous cross-sectional studies have shown that MP is inversely related to GD and PRT,[29 30] and that augmentation of MP results in improvements in GD and PRT,[11 13] whereas others have found no association with GD (or PRT).[6] These inconsistencies may be attributable to the differences in study design, including stimulus conditions. Of note, glare is an important clinical symptom, and reducing GD would be beneficial for patients with early AMD, especially for driving.[31]

We found that higher central MP (ie, at 0.25°, 0.5°, 1°) is associated with better BCVA in our study group (table 2). However, after controlling for age, sex and cataract grade, there was no significant relationship between MP (at any eccentricity) and BCVA. The acuity hypothesis posits that MP is necessary for optimal visual acuity by reducing the effects of chromatic aberration,[3] by attenuating the penumbra/blur circle formed as a result of this phenomenon.[32] However, studies have tested this hypothesis, and the results have been mixed, perhaps, explained at least in part, by differences in study design and outcome

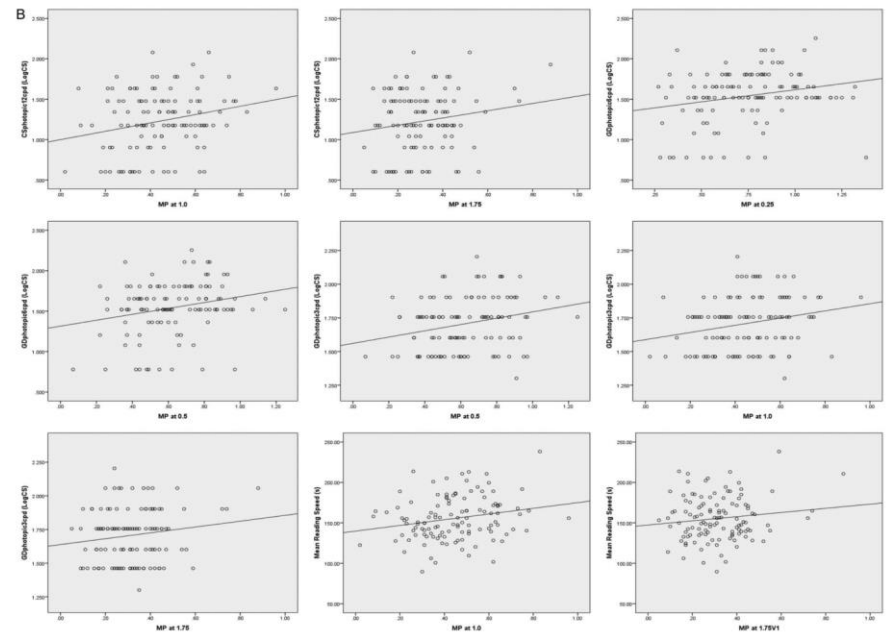

Figure 1 Continued.

measures.[6 33 34] For example, our results are consistent with those of a study by Engles *et al*,[34] who assessed resolution acuity and hyperacuity using a customised experimental setup (ie, two solid black lines on either white or yellow background), whereas they are not consistent with those reported by Loughman *et al*,[6] who used a methodology similar to the current study, although in normal subjects free of retinal disease.

In this study we performed multiple statistical tests (eg, table 2 presents p values for more than 120 different tests of bivariate correlations). For any one such test, the level of significance is, conventionally, set at 5%. For correlation analysis, this means that we start with the null hypothesis ('the correlation between the two variables is zero in the population'), then calculate the sample correlation and then reject the null hypothesis if the probability is less than 5% of getting this sample correlation when the null hypothesis is actually true. For multiple tests, however, the recommendation is sometimes made that the level of significance should be reduced well below 5%, so as to reduce the probability of a type I error—rejecting null hypotheses which we should accept—arising from the multiplicity of tests. In particular, Bonferroni adjustment is often advocated for multiple tests. This would entail, for the tests reported in table 2, reducing the significance level from 0.05 to about 0.0004 (which is 0.05 divided by 120). Not one of the 27 correlations in table 2 (significant at the 5% level) would still be reported as significant at this reduced level of significance. We regard this approach as extreme and unwise, however, because it greatly increases the risk of a type II error—accepting null hypotheses which we should reject. This, to us, is the more serious error, failing to report a relationship that does, in fact, exist. In the current study, therefore, we followed our usual practice and used the 5% level of significance in all statistical analyses, without adjusting for multiple comparisons. It must be conceded, however, that the reported significance for some of our 27 correlations may well, therefore, be spurious.

It must also be conceded that the significant correlations in table 2 are, in general, weak. However, there is remarkable consistency in the direction and location of many of the significant correlations in table 2. In particular, many of the correlations between central MP and CS, when significant at 6 cpd, are significant, or nearly so, at 3 cpd and 12 cpd also. Furthermore, all significant correlations between MP and visual function are, without exception, positive—higher MP is associated with better vision. While the importance of a single correlation of this magnitude should not be exaggerated (and might well be explained as a consequence of multiple tests), we believe that the frequency and consistent directionality of these significant correlations, in our study, do constitute a substantial body of evidence that MP and visual function are positively correlated.

Average central MP (see table 1) may be considered higher than expected in this study, given that subjects were recruited only if they had not previously taken supplements containing the macular carotenoids and, accordingly, all subjects were supplement-naive. The eligibility criteria did not include a threshold for MP at a specific retinal eccentricity. In other words, subjects with a range of MP values were enrolled into

the study. Of note, MP is augmented either through the diet or by taking nutritional supplements containing the macular carotenoids. Given that subjects in the current study were supplement-naive patients with early AMD, it is more likely that the high average central MP may reflect healthy dietary habits in our study population. Indeed, results from the dietary carotenoid (L/Z) screener used in the current study (see detailed description in Akuffo *et al*[17]) show that subjects had, on average, medium dietary intake of L and Z (which reflects 3–13 mg/day (data previously reported by Kelly *et al*[35])). Furthermore, there was a positive and statistically significant correlation ($r=0.343$, $p<0.0005$) between this diet score and MP at 0.25°—evidence that high MP is indeed associated with better diet in this study.

AMD is a multifactorial disease, which causes degenerative changes at the macula and consequential central vision impairment, thereby adversely affecting normal daily activities (eg, reading, driving, watching television and recognising faces) and, ultimately, leading to an overall loss of social independence and reduced quality of life among sufferers of this condition.[36] Overall, our results highlight the role of MP in vision-related quality of life, and, by extension, the potential benefits of supplementation with the macular carotenoids for improved quality of life through optimised visual function.

Strengths of this study include: (1) MP was measured using a validated technique at four different retinal eccentricities; (2) the outcome measures for the current study are known and important determinants of quality of life in patients afflicted with AMD. Limitations of this study include its cross-sectional design, and therefore its findings are associative. However, the impact of supplementation with the macular carotenoids on visual function in non-advanced AMD is currently under investigation as part of the prospective arm of the CREST trial, which is designed to investigate whether enrichment of MP impacts on visual function in these subjects.[17]

In conclusion, we report that MP relates positively to visual function in AMD-afflicted eyes of unsupplemented subjects, suggesting that augmentation of MP may enhance vision in patients with this condition.

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Competing interests JMN and SB do consultancy work for nutraceutical companies in a personal capacity and as directors of Nutrasight Consultancy Limited.

Patient consent Obtained.

Ethics approval Ethical approval was granted by the Research Ethics Committee of the Waterford Institute of Technology (WIT), Waterford, Ireland, and the Ethics Committee of the European Research Council (ERC).

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REFERENCES

1 Hammond BR, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A Opt Image Sci Vis* 1997;14:1187–96.
2 Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 1997;38:1802–11.
3 Schultze M. *Ueber den gelben Fleck der Retina, seinen Einfluss auf normales Sehen und auf Farbenblindheit*. Cohen & Sohn, 1866.
4 Stringham JM, Hammond BR Jr. The glare hypothesis of macular pigment function. *Optom Vis Sci* 2007;84:859–64.
5 Wooten BR, Hammond BR. Macular pigment: influences on visual acuity and visibility. *Prog Retin Eye Res* 2002;21:225–40.
6 Loughman J, Akkali MC, Beatty S, *et al*. The relationship between macular pigment and visual performance. *Vision Res* 2010;50:1249–56.
7 Hammond BR Jr, Fletcher LM, Elliott JG. Glare disability, photostress recovery, and chromatic contrast: relation to macular pigment and serum lutein and zeaxanthin. *Invest Ophthalmol Vis Sci* 2013;54:476–81.
8 Fletcher LM, Engles M, Hammond BR Jr. Visibility through atmospheric haze and its relation to macular pigment. *Optom Vis Sci* 2014;91:1089–96.
9 Hammond BR Jr, Wooten BR, Engles M, *et al*. The influence of filtering by the macular carotenoids on contrast sensitivity measured under simulated blue haze conditions. *Vision Res* 2012;63:58–62.
10 Renzi LM, Hammond BR. The effect of macular pigment on heterochromatic luminance contrast. *Exp Eye Res* 2010;91:896–900.
11 Hammond BR, Fletcher LM, Roos F, *et al*. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Invest Ophthalmol Vis Sci* 2014;55:8583–9.
12 Sabour-Pickett S, Beatty S, Connolly E, *et al*. Supplementation with three different macular carotenoid formulations in patients with early age-related macular degeneration. *Retina* 2014;34:1757–66.
13 Stringham JM, Hammond BR. Macular pigment and visual performance under glare conditions. *Optom Vis Sci* 2008;85:82–8.
14 Ma L, Yan SF, Huang YM, *et al*. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology* 2012;119:2290–7.
15 Loughman J, Nolan JM, Howard AN, *et al*. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci* 2012;53:7871–80.
16 Akuffo KO, Nolan JM, Howard AN, *et al*. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)* 2015;29:902–12.
17 Akuffo KO, Beatty S, Stack J, *et al*. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol* 2014;21:111–23.
18 Davis MD, Gangnon RE, Lee LY, *et al*. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol* 2005;123:1484–98.
19 Stringham JM, Hammond BR, Nolan JM, *et al*. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res* 2008;87:445–53.
20 Chylack LT Jr, Wolfe JK, Singer DM, *et al*. The lens opacities classification system III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol* 1993;111:831–6.
21 Bansback N, Czoski-Murray C, Carlton J, *et al*. Determinants of health related quality of life and health state utility in patients with age related macular degeneration: the association of contrast sensitivity and visual acuity. *Qual Life Res* 2007;16:533–43.
22 Craft NE, Haitema TB, Garnett KM, *et al*. Carotenoid, tocopherol, and retinol concentrations in elderly human brain. *J Nutr Health Aging* 2004;8:156–62.
23 Johnson EJ. A possible role for lutein and zeaxanthin in cognitive function in the elderly. *Am J Clin Nutr* 2012;96:1161S–5S.
24 Vishwanathan R, Neuringer M, Snodderly DM, *et al*. Macular lutein and zeaxanthin are related to brain lutein and zeaxanthin in primates. *Nutr Neurosci* 2013;16:21–9.
25 Cheong AM, Legge GE, Lawrence MG, *et al*. Relationship between slow visual processing and reading speed in people with macular degeneration. *Vision Res* 2007;47:2943–55.
26 Renzi LM, Bovier ER, Hammond BR Jr. A role for the macular carotenoids in visual motor response. *Nutr Neurosci* 2013;16:262–8.
27 Bovier ER, Renzi LM, Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on neural processing speed and efficiency. *PLoS ONE* 2014;9:e108178.
28 Hassell JB, Lamoureux EL, Keeffe JE. Impact of age related macular degeneration on quality of life. *Br J Ophthalmol* 2006;90:593–6.
29 Stringham JM, Garcia PV, Smith PA, *et al*. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. *Invest Ophthalmol Vis Sci* 2011;52:7406–15.

30 Putnam CM, Bassi CJ. Macular pigment spatial distribution effects on glare disability. *J Optom* 2015;8:258–65.
31 Mainster MA, Turner PL. Glare's causes, consequences, and clinical challenges after a century of ophthalmic study. *Am J Ophthalmol* 2012;153:587–93.
32 Reading VM, Weale RA. Macular pigment and chromatic aberration. *J Opt Soc Am A* 1974;64:231–4.
33 Puell MC, Palomo-Alvarez C, Barrio AR, *et al*. Relationship between macular pigment and visual acuity in eyes with early age-related macular degeneration. *Acta Ophthalmol* 2013;91:e298–303.
34 Engles M, Wooten B, Hammond B. Macular pigment: a test of the acuity hypothesis. *Invest Ophthalmol Vis Sci* 2007;48:2922–31.
35 Kelly D, Coen RF, Akuffo KO, *et al*. Cognitive function and its relationship with macular pigment optical density and serum concentrations of its constituent carotenoids. *J Alzheimers Dis* 2015;48:261–77.
36 Slakter JS, Stur M. Quality of life in patients with age-related macular degeneration: impact of the condition and benefits of treatment. *Surv Ophthalmol* 2005;50:263–73.

The Impact of Cataract, and Its Surgical Removal, on Measures of Macular Pigment Using the Heidelberg Spectralis HRA+OCT MultiColor Device

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Purpose. To investigate the effect of cataract (and cataract surgery) on macular pigment (MP) measurements using the Heidelberg Spectralis HRA | OCT MultiColor device.

Methods. Thirty-six patients (age, 54–87 years) scheduled for cataract surgery at the Institute of Eye Surgery, Ireland, were enrolled in this study. Cataracts were graded using the Lens Opacities Classification System (LOCS) III, and surgery was performed using standard phacoemulsification technique with implantation of a Tecnis ZCB00 or Tecnis ZCT intraocular lens. Macular pigment was measured before and after cataract surgery in the operated (study) eye and in the fellow (control) eye.

Results. In the study eye, there was statistically significant disagreement in measures of MP taken before and after surgery. At all eccentricities, and also for MP volume, the postsurgery measurements were significantly ($P < 0.05$) greater, ranging from an average 16% greater at 1.72° to an average 35% greater at 0.23° eccentricity. Eyes exhibiting large disagreement between pre- and postsurgery measurements at a given eccentricity also generally exhibited substantial disagreement at other eccentricities. Overall severity of cataract contributed to greater disagreement between pre- and postoperative measures of MP, as did grade of nuclear opalescence, nuclear color, and posterior subcapsular cataract. In control eyes, there was no statistically significant disagreement in terms of measures of MP taken before and after cataract surgery ($P > 0.05$ for all; 1-sample *t*-test).

Conclusions. Macular pigment measurements using the Spectralis are affected by cataract. Accordingly, we recommend that cataract be graded when measuring MP with a device that utilizes dual-wavelength fundus autofluorescence and propose the employment of a correction factor to compensate for cataract when measuring MP.

Keywords: macular pigment, macular pigment optical density, fundus autofluorescence, concordance correlation coefficient, agreement, spectralis, cataract, cataract surgery, phacoemulsification, Tecnis IOL, lutein, zeaxanthin, meso-zeaxanthin, visual acuity, optical coherence tomography

Macular pigment (MP) is composed of the carotenoids lutein (L), zeaxanthin (Z), and meso-zeaxanthin (MZ). Macular pigment is found at the macula, the specialized part of the retina that mediates fine central and color vision.[1] Macular pigment's anatomic location,[2] short-wavelength (blue) light filtering properties,[3] and antioxidant[4–6] and anti-inflammatory properties[7–10] make this pigment important for vision in diseased[11–13] and nondiseased retinas.[14,15]

Given the importance of MP for vision and its role in reducing risk of age-related macular degeneration (AMD) progression,[16] there is clearly a need to measure this pigment accurately in vivo in the clinical and the research setting. Moreover, it is important to be able to measure changes in MP over time.

There are several techniques for measuring MP in vivo, and the most common include heterochromatic flicker photometry (HFP)[17] and fundus autofluorescence (AF).[18] Measurement of MP using either of these techniques rests on assumptions, and each has its own advantages and limitations. While HFP is most widely used, it requires the patient to fixate on the targets presented and follow operator instructions, rendering this method unsuitable for persons with advanced retinal disease (e.g., advanced AMD), dementia, learning difficulties, or memory problems. In addition, when measuring the MP spatial profile, this technique can take up to 30 minutes per eye and provides data only at specific points (retinal eccentricities) across the retina, and therefore does not yield a continuous profile of the pigment.

The Heidelberg Spectralis HRA+OCT MultiColor device (a new commercially available device) utilizes the dual-wavelength AF technique. The Spectralis does not require responses from the patient in order to measure MP. Limitations of this device include the need to pharmacologically dilate the pupil and the relatively bright lights required for photopigment bleaching.

TABLE 1. Study Procedures Conducted Before Cataract Surgery (V1) and After Cataract Surgery (V2)

Study Procedures	V1	V2
Informed consent	•	
Demographic and lifestyle questionnaire	•	
Dietary carotenoid assessment	•	•
Visual acuity	•	•
Pupillary dilation	•	•
Cataract grading	•	
MP measurement using the Heidelberg Spectralis	•	•
Optical coherence tomography	•	•
Blood sample collection	•	•
Serum carotenoid assessment	•	•

Concordance of MP measurements using the Spectralis and the Densitometer (an established and validated device[19,20] that utilizes customized HFP [cHFP]) has been examined in healthy eyes (i.e., free of retinal disease)[21] as well as in patients with early AMD.[22] In persons with no retinal disease, Dennison et al.[21] reported good concordance between MP readings using the Densitometer and the Spectralis. However, in patients with early AMD, Akuffo et al.[22] recently reported poor concordance between these two devices; they recommended that readings on these devices not be considered interchangeable in a given study in the clinical and research setting, but also concluded that each device yielded reliable measures of MP (and changes in MP) within subjects over time.

One important question with respect to MP measurements using the Spectralis relates to the impact of lens opacification (cataract) on the measurement. A cataract is any opacity of the crystalline lens and causes visual disturbance. Cataracts absorb blue light, and this blue light–absorbing property may affect measures of MP using AF devices. Of note, a previous study conducted by Sasamoto et al.[23] reported that cataracts (especially the nuclear component) affect AF-derived measures of MP. Although the Spectralis also utilizes dual-wavelength AF, it is mechanistically different from the device employed by Sasamoto et al.,[23] and therefore the effect of cataract on MP measurement using the Spectralis merits investigation.

This study was designed to investigate the impact, if any, of cataract on MP measurements obtained using the Spectralis, by measuring MP before and after cataract surgery in each eye of patients scheduled for cataract surgery in one eye.

METHODS

Study Design and Population

Thirty-six patients scheduled for cataract surgery at the Institute of Eye Surgery (IOES; www.ioes.ie), Whitfield Clinic, Waterford, Ireland, were enrolled in this study. Figure 1 shows the flow diagram summarizing the study design, patient enrollment, and follow-up. Ethical approval was granted by the Research Ethics Committee, University Hospital Waterford (UHW), Ireland. Written informed consent was obtained from each patient, and the experimental procedures adhered to the tenets of the Declaration of Helsinki. Patients were eligible for this study if they were scheduled for cataract surgery and if they had no evidence of coexisting ocular disease (e.g., AMD, glaucoma) other than mild ocular surface disease (i.e., dry eye and/or blepharitis).

Study clinical assessments (Table 1) were conducted before (baseline; V1) and after cataract surgery (final visit; V2) at the IOES. Study visits began in February 2015 (i.e., first patient visit) and were completed in November 2015 (last patient visit). Each study visit lasted approximately 30 minutes; the interval between visits ranged from 19 to 107 days, with a mean interval per patient of 37.6 days.

Demographic and Lifestyle Questionnaire

The following details were obtained from each patient before cataract surgery: contact information, age, sex, body mass index (BMI in kg/m^2), and spectacle lens prescription.

Visual Acuity

Visual acuity (VA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) logarithm of the minimum angle of resolution (logMAR) chart (Test Chart 2000 Pro; Thomson Software Solutions, Hatfield, UK), viewed at 4 meters.

Dietary Carotenoid Assessment

Dietary intake of L and Z was estimated using a crude carotenoid screener known as the "L/Z screener," which was developed by Elizabeth Johnson (Tufts University, Boston, Massachusetts). This screener gives a dietary score (from 0 to 75), which can be categorized as follows: low intake, category 1, 0 to 15: $\leq$2 mg/day; medium intake, category 2, 16 to 30: 3 to 13 mg/day; high intake, category 3, 31 to 75: >13 mg/day. This tool has been described in detail elsewhere.[24,25]

Blood Sample Collection and Serum Carotenoid Assessment

Nonfasting blood samples were collected in 9-mL VACUETTE tubes containing the Z Serum Sep Clot Activator (BD Vacutainer SST Serum Separation Tubes; Becton, Dickinson and Company, Plymouth, UK), adhering to standard venipuncture protocols. The blood samples were allowed to clot at room temperature for approximately 30 minutes and then centrifuged at 725*g* for 10 minutes in a Gruppe GC 12 centrifuge (Desaga Sarstedt, Hampshire, UK) to separate the serum from the whole blood. The resulting serum samples were stored in light-resistant microtubes at −80°C until the time of batch analysis using high-performance liquid chromatography (HPLC). Serum carotenoid analysis was conducted using a method previously described.[26]

Pupillary Dilation

Pupils were dilated using a drop each of 0.5% proxymetacaine hydrochloride and 1% tropicamide prior to performing MP measurement using the Spectralis, optical coherence tomography (OCT), and cataract grading.

Cataract Grading

Cataract grading was performed by a trained and certified grader (CK), using the Haag-Streit 900 Slit Lamp biomicroscope (Haag-Streit AG, Koeniz, Switzerland), adhering to the Lens Opacities Classification System III (LOCS III).[27] The degree of nuclear opalescence (NO) and color (NC) was graded on a scale ranging from 0.1 to 6.9 while cortical (C) and posterior subcapscular (P) opacities were graded on a scale ranging from 0.1 to 5.9.

Optical Coherence Tomography

Optical coherence tomography was performed using the Spectralis HRA+OCT MultiColor (Heidelberg Engineering

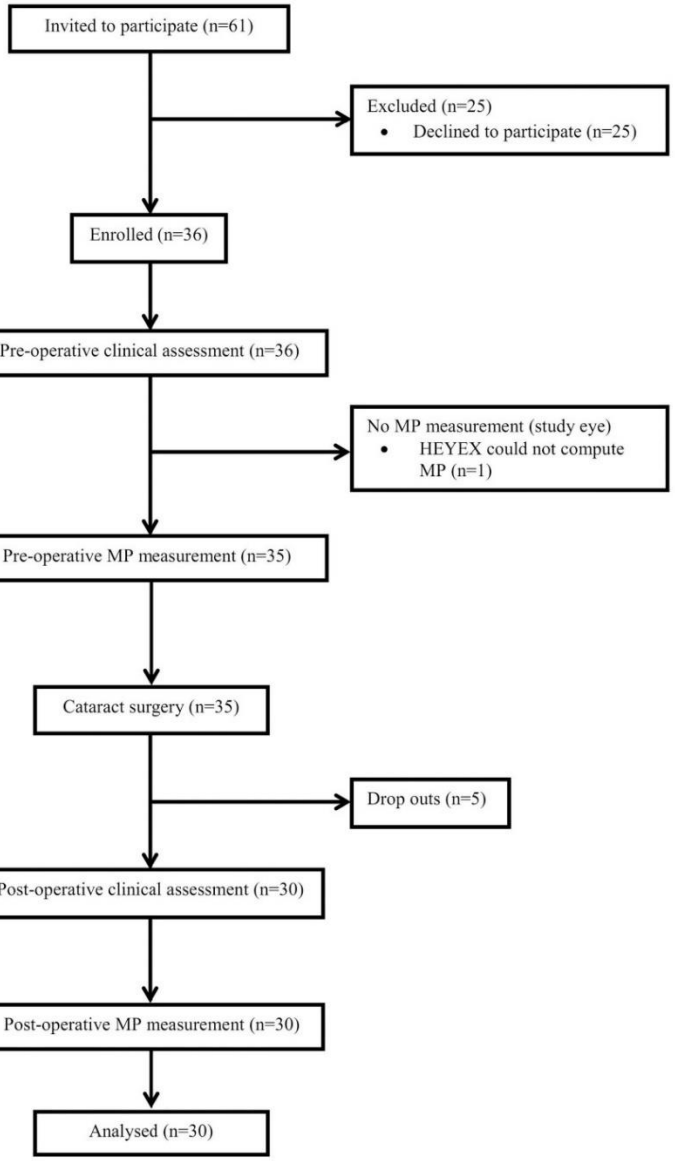

FIGURE 1. Flow chart showing study design, enrollment, clinical procedures, follow-up, and participants included in study analyses. MP, macular pigment; HEYEX: Heidelberg Eye Explorer software. The study eye was the patient's eye that was designated for cataract surgery and that fulfilled the inclusion criteria.

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GmbH, Heidelberg, Germany).[28] The following acquisition protocol was used: 20×15 volume scan, 19 scans each 239 μm apart at high speed, automatic real-time mean (ART) of 8 frames per B-scan. Foveal thicknesses (minimum and mean) were recorded following analysis using Heidelberg Eye Explorer software (HEYEX, version 1.9.10.0).

Cataract Surgery

Cataract surgery was performed at the IOES by a single surgeon (SB) using standard phacoemulsification technique, with the implantation of either a Tecnis ZCB00 (Advanced Medical Optics, Inc., Santa Ana, CA, USA) or the toric version of the same intraocular lens (Tecnis ZCT, Advanced Medical Optics, Inc.), as described elsewhere.[29] The two intraocular lenses have the same lens design and absorbance properties and do not block blue light, but may differ only in refractive power. Only one study eye was implanted with a toric Tecnis ZCT, with all other eyes being implanted with a monofocal Tecnis ZCB00. Pre-, intra-, and postoperative procedures adhered to standard protocols at the IOES, which have recently been published.[30] No intra- or postoperative complications were encountered.

Measurement of Macular Pigment Using Dual-Wavelength Fundus Autofluorescence

Macular pigment was measured using the Spectralis HRA+OCT MultiColor. The Spectralis has a confocal scanning laser ophthalmoscope (cSLO) with diode lasers and uses dual-wavelength AF technique (two excitation wavelengths, one that is well absorbed by MP [486 nm, blue] and one that is not well absorbed by MP [518 nm, green]) for measuring MP.

During the measurement, the patient's head was positioned with the help of the canthus alignment mark, and forehead and chin rest. The patient was then instructed to fixate on an internal fixation target. Initial camera alignment, illumination, and focus were done in infrared (IR) mode. Once the image was evenly illuminated, the camera mode was switched to simultaneous blue AF and green AF imaging (BAF+GAF) mode for MP measurement acquisition. After additional adjustments to illumination and focus in order to ensure optimal image quality, a 30-second video was recorded.

The AF images in the video were aligned and digitally subtracted using the Heidelberg Eye Explorer software (HEYEX, version 1.9.10.0), generating the MP spatial distribution profile. Macular pigment at 0.23°, 0.47°, 0.98°, and 1.72° and MP volume were recorded, with the parafoveal reference set at 7°.

When tear film was so poor as to interfere with MP measurement, a drop of Hyloforte (an intensive ocular lubricant; Scope Ophthalmics, London, UK/Dublin, Ireland) was applied. Approximately 1 minute following instillation of the lubricant, a further attempt was made to measure MP. In these cases, the application of the lubricant facilitated acquisition of MP measurements.

Statistical Analysis

One eye (the study eye) of each patient composed the unit of our primary analyses. The study eye was the patient's eye that was designated for cataract surgery and fulfilled the inclusion criteria. We also analyzed MP data from the nonstudy eye as control (secondary analyses). In our analyses of study eyes, we excluded one patient because the MP measurement had a high level of "noise," indicating questionable reliability. In the nonstudy eyes, we excluded two eyes because of macular hole; some nonstudy eyes (14, 38.9%) were pseudophakic, precluding the need to grade cataract, and such eyes were necessarily excluded from some analyses. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, USA); the statistical programming language R[31] was used to generate agreement statistics such as the concordance correlation coefficient.[32] Disagreement in pre- and postoperative measures of MP was expressed in terms of the ratio of postsurgery (visit 2, V2) measurements to presurgery (visit 1, V1) measurements. The 1-sample *t*-test was used to test if this ratio was significantly different from 1, a ratio of 1 being ideal.

The relationship between the MP V2/V1 ratio and grade of cataract (and other study variables, such as age, sex, BMI) was investigated in the study eye using correlation analyses and general linear models.

Results

Of the 61 patients invited to participate, 36 (56.3%) were assessed before cataract surgery with 30 (46.9% of those invited to participate, but 83.3% of those deemed eligible to participate at the preoperative assessment) completing final study visits after cataract surgery (Fig. 1). Table 2 presents the demographic, lifestyle, cataract, dietary, and serum carotenoid characteristics of all patients who completed clinical assessment before cataract surgery.

Before cataract surgery in the study eye, MP measurements were not obtained in one patient because the HEYEX software failed computation of MP spatial density profile. In the nonstudy eye, MP measurements were not obtained in two patients before cataract surgery and in three patients after cataract surgery, mainly because HEYEX software failed computation of MP spatial density profile.

Serum concentrations of L and Z did not change significantly ($P > 0.05$ for all), on average, in the course of the study.

Comparing Study and Nonstudy Eyes for Disagreement in MP Measurements

Figure 2 shows the scatter plots of the pre- and postsurgery MP values (in study eyes) at 0.23°, 0.47°, 0.98°, and 1.72° and MP volume, with the line $y = x$ superimposed. Figure 3 shows the corresponding scatter plots for nonstudy eyes.

In Figure 2 (study eyes), nearly all points lie to the left of the line $y = x$, indicating that postsurgery MP measurements are consistently higher than presurgery measurements. In Figure 3, there is no such pattern evident for nonstudy eyes. The concordance correlation coefficients[32] for MP0.23° are 0.98 in the nonstudy eyes but only 0.50 in the study eyes; the corresponding indices for MP volume are 0.93 in the nonstudy eyes and 0.84 in the study eyes.

Table 3 provides summary statistics underpinning this graphical representation. For study eyes, the average V2/V1 ratios are significantly different from 1 in all cases ($P < 0.0005$ at 0.23°, 0.47°, and 0.98°; $P = 0.007$ at 1.72°; and $P = 0.014$ for MP volume; 1-sample *t*-test). Thus, statistically significant percentage increases in measured MP following cataract surgery are seen at each eccentricity and for MP volume. Of note, the greatest average apparent increase in MP readings was seen centrally (i.e., 35% at 0.23°), which is approximately double the increase observed at outer eccentricities and for overall MP volume (16%–18%).

In contrast, in nonstudy eyes, none of the average V2/V1 ratios (lower part of Table 3) is significantly different from 1 ($P > 0.05$ for all; 1-sample *t*-test). It is even more revealing to compare, side by side, the V2/V1 ratios in the study versus nonstudy eyes for individual patients' measures of MP at 0.23°

Table 2. Demographic, Lifestyle, Cataract, Dietary, and Serum Carotenoid Characteristics of Patients Before Cataract Surgery

Variable	*n* (%)	Mean ± SD	Range
Age, y	36 (100)	72.92 ± 7.47	54 to 87
Sex			
Male	11 (30.56)		
Female	25 (69.44)		
Body mass index, kg/m^2	36 (100)	26.26 ± 3.68	20.40 to 34.20
Spectacle prescription SE, diopters	34 (94.44)	0.86 ± 2.04	−5.25 to 3.88
Visual acuity SE, VAR	36 (100)	87.92 ± 8.11	75 to 101
Diet score	36 (100)	15.44 ± 11.01	0 to 42
Serum carotenoids, μM			
Lutein	35 (97.22)	0.79 ± 0.41	0.26 to 2.18
Zeaxanthin	35 (97.22)	0.15 ± 0.08	0.02 to 0.33
Foveal thickness SE, μm			
Minimum	36 (100)	225.08 ± 26.31	146 to 277
Mean	36 (100)	292.75 ± 80.25	219 to 742
Axial length SE, mm	30 (83.33)	22.19 ± 0.87	22.19 to 25.89
Cataract SE			
Nuclear opalescence	36 (100)	3.42 ± 1.22	1.2 to 5.9
Nuclear color	36 (100)	4.14 ± 1.14	1.8 to 6.4
Cortical	36 (100)	1.91 ± 1.54	0.1 to 5.1
Posterior subcapsular	36 (100)	0.70 ± 0.70	0.1 to 3.2
Cataract NSE			
Nuclear opalescence	22 (61.11)	3.43 ± 1.07	1.8 to 5.5
Nuclear color	22 (61.11)	4.11 ± 1.07	2.2 to 6.0
Cortical	22 (61.11)	1.45 ± 1.43	0.1 to 4.3
Posterior subcapsular	22 (61.11)	0.69 ± 0.99	0.1 to 4.2
MPOD SE			
0.23°	35 (97.22)	0.48 ± 0.17	0.15 to 0.83
0.47°	35 (97.22)	0.43 ± 0.14	0.12 to 0.73
0.98°	35 (97.22)	0.35 ± 0.11	0.10 to 0.55
1.72°	35 (97.22)	0.16 ± 0.07	0.06 to 0.35
MP volume	35 (97.22)	5,880.57 ± 2,332.97	1,807 to 12,809
MPOD NSE			
0.23°	33 (91.67)	0.56 ± 0.24	0.17 to 1.44
0.47°	33 (91.67)	0.48 ± 0.20	0.14 to 1.14
0.98°	33 (91.67)	0.36 ± 0.15	0.17 to 0.95
1.72°	33 (91.67)	0.17 ± 0.09	0.08 to 0.55
MP volume	33 (91.67)	6,215.42 ± 3,062.16	1,502 to 18,964

Data displayed are mean ± standard deviation (SD) for interval data and percentages for categorical data: SE, study eye; NSE, nonstudy eye; diet score, estimated dietary intake of lutein (L) and zeaxanthin (Z) using the L/Z screener; visual acuity measured with Thompson Test Chart 2000 Pro and recorded in visual acuity rating (VAR); spectacle prescription reported in spherical equivalent refraction (SER); serum carotenoids analyzed using high-performance liquid chromatography (HPLC); axial length measured using the IOLMaster, Version 5 (Carl Zeiss Meditec, AG, Jena, Germany); foveal thickness and macular pigment optical density at 0.23°, 0.47°, 0.98°, and 1.72° eccentricity obtained using the Heidelberg Spectralis HRA+OCT MultiColor; cataracts graded using the Lens Opacities Classification System (LOCS) III; $n = 36$ for all tests/measures because certain tests/measures could not be obtained.

eccentricity (Table 4). The V2/V1 ratios in Table 4, with few exceptions ($n = 3$; 11.5%), are closer to 1 in the nonstudy eye than in the study eye. Indeed, the interocular disparity in V2/V1 ratios is dramatic in many cases.

Other Results for Study Eyes

Uniformity of Disagreement in Measured MP at Different Eccentricities, Before and After Surgery (Study Eyes). Disparities in measures of MP before and after surgery hold true across the different retinal eccentricities in a given eye. For example, the pairwise correlations of the V2/V1 ratios at the different eccentricities were statistically significant ($P < 0.001$ for all) and very close to 1 for eccentricities 0.23°, 0.47°, and 0.98° (between 0.94 and 0.97). The pairwise correlations of these ratios at 1.72° were statistically significant ($P < 0.001$ for all) and still high (between 0.68 and 0.79). Thus, there is strong evidence that observed agreement/disagreement within a given eye is maintained across eccentricities for that eye.

Is Disagreement in Measured MP (Study Eyes) Related to V1 Cataract Scores? Preliminary analysis, based on Pearson correlations, showed that NO and NC were positively and significantly associated with V2/V1 ratios at all retinal eccentricities, and with the MP volume ratio ($P < 0.05$ for all). Posterior subcapsular cataract was positively and significantly associated with ratios at eccentricities from 0.23° up to 0.98° ($P < 0.05$ for all). However, C (cortical cataract) was not significantly associated with any ratio ($P > 0.05$ for all).

Nuclear opalescence and NC cataract scores are themselves highly correlated ($r = 0.889$, $P < 0.0005$). When we proceeded

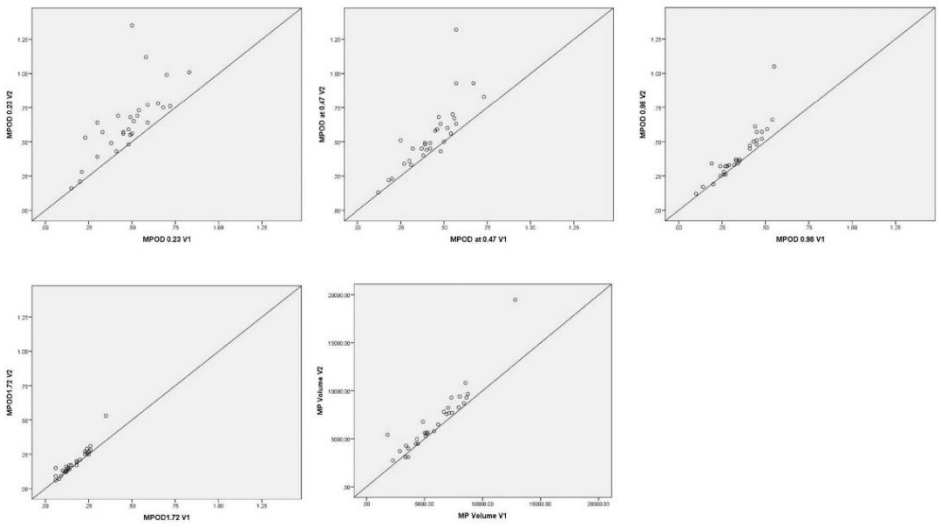

FIGURE 2. Scatter plots with the line $y = x$ superimposed comparing macular pigment optical density (MPOD) measurements in the study eye using the Heidelberg Spectralis HRA+OCT MultiColor before cataract surgery (V1) and after cataract surgery (V2).

to fit general linear models for the V2/V1 ratio (such as ratio ~ NO+NC+P, where " ~ " means "is modeled as depending on"), the effect of this high correlation, in all cases, was that either NO or NC became redundant in any model already containing the other variable. Selecting from just these three cataract variables, the following models emerged as best: ratio ~ NC+P at 0.23° and 0.47°, ratio ~ NO+P at 0.98°, ratio ~ NC at 1.72° (or ratio ~ NO, i.e., NC and NO are equally strongly related to the V2/V1

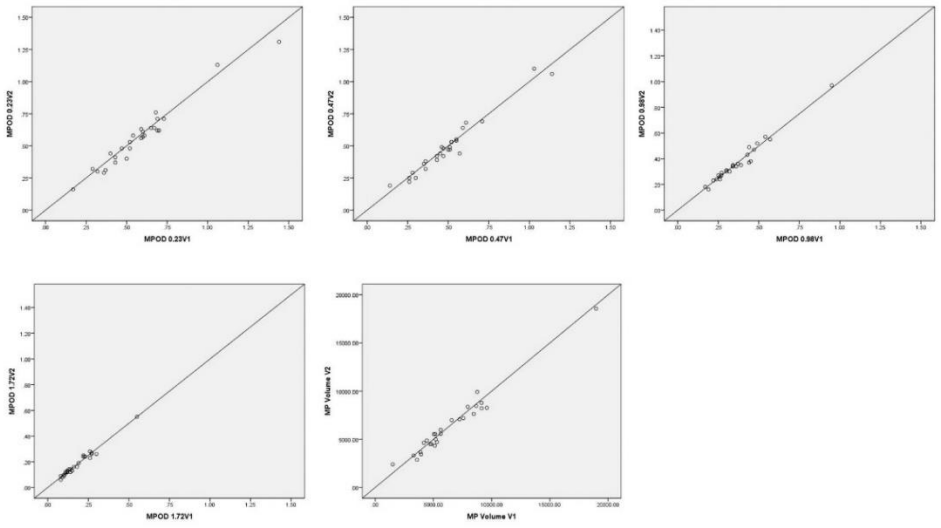

FIGURE 3. Scatter plots with the line $y = x$ superimposed comparing macular pigment optical density (MPOD) measurements in the nonstudy eye using the Heidelberg Spectralis HRA+OCT MultiColor before cataract surgery (V1) and after cataract surgery (V2).

Table 3. Change in Measured Macular Pigment, Before and After Surgery, Based on the Ratio of Measured Macular Pigment After Cataract Surgery (V2) to Before Cataract Surgery (V1)

MP Ratio (V2/V1)	*n*	Mean ± SD	Range
SE 0.23°	29	1.35 ± 0.37	1.00–2.70
SE 0.47°	29	1.27 ± 0.30	0.90–2.32
SE 0.98°	29	1.17 ± 0.21	0.95–1.91
SE 1.72°	29	1.16 ± 0.29	0.88–2.50
SE MP volume	29	1.18 ± 0.37	0.86–2.99
NSE 0.23°	26	0.97 ± 0.08	0.80–1.12
NSE 0.47°	26	0.99 ± 0.10	0.83–1.36
NSE 0.98°	26	0.99 ± 0.07	0.84–1.11
NSE 1.72°	26	0.98 ± 0.10	0.75–1.14
NSE MP volume	26	1.00 ± 0.15	0.80–1.60

SD, standard deviation; SE, study eye; NSE, nonstudy eye.

ratio at this eccentricity), and ratio ~ NO for MP volume. In all models, coefficients of explanatory variables are positive, indicating that, in all cases, more severe cataract in the study eye (higher NC, NO, or P scores) is associated with higher ratios, that is, with greater disagreement. R^2 values for these fitted models (the proportion of variance in the V2/V1 ratio explained by the cataract scores) ranged from 0.18 up to 0.38, the higher R^2 values being found at central eccentricities.

Is Observed Disagreement in Measured MP (Study Eyes) Related to Other V1 Variables? We examined a wide range of other study variables (including all V1 variables listed in Table 2) in relation to observed disagreement in measures of MP before and after surgery. Statistically significant relationships with disagreement were found for six of these variables: age, VA, axial length, serum L, serum Z, and V1 MP at 1.72° eccentricity. We also found a significant negative correlation between change in serum Z (V2 − V1) and disagreement in measurement of MP volume ($r = -0.40$, $P = 0.031$).

Just two of these variables, however, remained significant when included alongside the cataract variables in the earlier fitted models. When V1 MP1.72° is included in some of these models, it has the effect of substantially increasing R^2 values for these models, and also makes the cataract variable P redundant. This is also true of V1 serum L, which could replace P (and increase R^2 to 0.43, from 0.33) in model iii below. However, as measurement of serum L requires specialized laboratory facilities and personnel, model iii below (which requires only cataracts to be measured) is presented as being more practical for the purpose of estimating postsurgery MP from measured presurgery MP.

The final fitted models, therefore, are as follows:

(i) Ratio at 0.23° = 0.222 + 0.184 × NC + 2.193 × MP1.72° V1, $R^2 = 0.49$
(ii) Ratio at 0.47° = 0.41 + 0.14 × NC + 1.677 × MP1.72° V1, $R^2 = 0.45$
(iii) Ratio at 0.98° = 0.866 + 0.074 × NO + 0.085 × P, $R^2 = 0.33$
(iv) Ratio at 1.72° = −0.690 + 0.113 × NC, $R^2 = 0.19$
(v) Ratio for MP volume = 0.764 + 0.123 × NO, $R^2 = 0.18$.

All coefficients of explanatory variables in these models were positive, indicating that greater disagreement is associat-

Table 4. Comparison of Disagreement, Before and After Surgery, in Measured Macular Pigment Between Study and Nonstudy Eye for Individual Patients, Based on the Ratio of Measured Macular Pigment After Cataract Surgery (V2) to Before Cataract Surgery (V1) at 0.23° Eccentricity

	Nonstudy Eye				Study Eye								
ID	0.23V1	0.23V2	Diff0.23	0.23V2/V1	0.23V1	0.23V2	Est.0.23V2*	Diff0.23	0.23V2/V1	NO	NC	C	P
SS002	0.36	0.29	−0.07	0.81	0.83	1.01	1.07	0.18	1.22	1.3	2.8	0.1	0.3
SS003	0.69	0.71	0.02	1.03	0.59	0.77	0.78	0.18	1.31	1.7	3.2	0.7	0.1
SS004	0.52	0.53	0.01	1.02	0.49	0.55	0.55	0.06	1.12	2.8	3.3	2.4	0.1
SS005	0.68	0.76	0.08	1.12	0.49	0.68	0.67	0.19	1.39	2.7	3.3	2.3	1.4
SS006	0.29	0.32	0.03	1.10	0.21	0.28	0.22	0.07	1.33	2.8	3.8	1.3	1.2
SS008	1.06	1.13	0.07	1.07	0.50	1.35	0.95	0.85	2.70	4.8	4.9	0.4	1.8
SS010	0.73	0.71	−0.02	0.97	0.70	0.99	1.14	0.29	1.41	4.3	4.8	2.4	1.2
SS011	0.61	0.58	−0.03	0.95	0.59	0.64	0.78	0.05	1.08	3.7	4.4	0.1	1.2
SS013	0.54	0.58	0.04	1.07	0.45	0.57	0.60	0.12	1.27	2.8	4.3	1.3	1.2
SS015	0.43	0.37	−0.06	0.86	0.45	0.56	0.62	0.11	1.24	3.1	3.2	2.2	1.1
SS018	0.69	0.62	−0.07	0.90	0.48	0.59	0.62	0.11	1.23	3.6	4.4	0.8	0.2
SS019	0.40	0.44	0.04	1.10	0.49	0.55	0.81	0.06	1.12	5.1	5.4	0.6	0.6
SS020	0.70	0.62	−0.08	0.89	0.72	0.76	0.89	0.04	1.06	2.9	3.4	0.1	0.1
SS021	0.52	0.48	−0.04	0.92	0.50	0.56	0.47	0.06	1.12	1.4	1.8	3.4	0.1
SS024	0.32	0.30	−0.02	0.94	0.30	0.39	0.41	0.09	1.30	4.8	4.7	5.1	1.4
SS026	0.60	0.61	0.01	1.02	0.51	0.65	0.77	0.14	1.27	3.8	4.3	0.8	0.8
SS029	0.43	0.41	−0.02	0.95	0.48	0.48	0.52	0.00	1.00	2.4	3.3	0.1	0.1
SS030	0.50	0.40	−0.10	0.80	0.42	0.69	0.75	0.27	1.64	4.7	5.4	2.8	1.2
SS031	0.59	0.56	−0.03	0.95	0.53	0.69	0.64	0.16	1.30	2.8	3.7	2.4	0.1
SS032	0.59	0.63	0.04	1.07	0.65	0.78	0.80	0.13	1.20	2.3	3.8	2.2	0.1
SS033	0.60	0.57	−0.03	0.95	0.54	0.73	0.61	0.19	1.35	1.2	2.8	4.7	0.5
SS034	0.37	0.31	−0.06	0.84	0.20	0.21	0.26	0.01	1.05	2.9	4.8	3.7	0.1
SS035	0.67	0.64	−0.03	0.96	0.33	0.57	0.53	0.24	1.73	5.9	6.4	4.3	1.3
SS036	0.47	0.48	0.01	1.02	0.41	0.43	0.43	0.02	1.05	3.8	3.4	4.3	0.1
SS037	1.44	1.31	−0.13	0.91	0.58	1.12	1.10	0.54	1.93	4.9	6.2	4.3	0.9
SS038	0.65	0.64	−0.01	0.98	0.68	0.75	0.73	0.07	1.10	2.7	3.3	0.1	0.1

Diff0.23, difference in macular pigment optical density (V2 − V1); NO, NC, C, P refer to cataract grades in the study eye before cataract surgery based on the Lens Opacities Classification System III (LOCS III).

* Est0.23V2 is the product of the estimated macular pigment 0.23° V2/V1 ratio (using the model: ratio = 0.222 + 0.184 × NC + 2.193 × MP1.72° V1) and macular pigment optical density at 0.23° V1 in the study eye.

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Table 5. Comparison of Measured and Estimated Macular Pigment Measurement Ratios Before and After Cataract Surgery at 0.23° Eccentricity

Variable	*n*	Mean ± SD	Range
Measured MP0.23° V2/V1 ratio	29	1.35 ± 0.37	1.0–2.7
Estimated MP0.23° V2/V1 ratio*	29	1.00 ± 0.18	0.68–1.46

* Estimated macular pigment 0.23° V2/V1 ratio based on the model: ratio = 0.222 + 0.184 × NC + 2.193 × MP1.72 V1; *n* = number included in analysis.

ed with more severe cataracts and with higher measures of presurgery MP1.72°.

Using the Fitted Models (Study Eyes) to Adjust MP Measures at V1. The fitted models, in addition to describing the relationship between measured MP and cataracts, may be used to adjust the presurgery measure of MP (at any eccentricity or MP volume) for cataract in a given eye. To illustrate: Suppose we measure MP0.23° as 0.6 at V1 for a patient whose cataract score at V1 is NC = 4.0 and whose MP1.72° is measured as 0.2 at V1. Then, the V2/V1 ratio for this patient (at 0.23°) is estimated from the model as follows: 0.222 + 0.184 × 4.0 + 2.193 × 0.2 = 1.4 approximately. Thus, for this patient, we would estimate postsurgery MP0.23° as 1.4 × 0.6 = 0.84.

Table 5 compares the measured V2/V1 ratio for measured MP0.23° with the ratio V2 MP0.23°/predicted MP0.23°, where predicted MP0.23° uses the above model to predict MP0.23° post surgery from each patient's NC and MP1.72° scores presurgery. It is apparent from Table 5 that the predicted V2 MP0.23° is, generally, much closer to the measured V2 MP0.23° than is the V1 MP0.23°. The mean ratio is now 1, as it should be, and the variation around this ratio (measured by the standard deviation or the range) is much less. Nevertheless, there is still considerable discrepancy between the measured and predicted MP0.23° for some patients. For one patient (minimum ratio = 0.68), predicted MP0.23° is 32% below measured MP0.23°, and for another (maximum ratio = 1.46), the predicted MP0.23° is 46% above measured MP0.23°.

Analysis of Outliers (Study Eyes). Six patients had a V2/V1 ratio in excess of 1.4 at 0.23° eccentricity; that is, their MP0.23° measurement post surgery was at least 40% higher than the presurgery measurement. The lowest of the six NO scores, before surgery, for these patients was 4.3 (which is the 74th percentile for presurgery NO in this study), and the lowest NC score was 4.8 (77th percentile). Thus, all six patients with very poor agreement in measured MP0.23° also exhibited NO and NC grades very much at the upper end of the range. Given that there was high correlation between agreement/disagreement in MP readings at different eccentricities, it is reasonable to hypothesize that these findings extend to other eccentricities. Thus, in general, we report that the greatest discrepancy between pre- and postoperative measures of MP (whether centrally or peripherally) was seen in eyes with more severe lens opacification.

Does the Parafoveal Reference Location Influence the Effect of Cataract on MP Measures Before (V1) and After Cataract Surgery (V2) in the Study Eye? Table 6 presents

Table 6. Location of Parafoveal Reference and Its Impact on Macular Pigment Measurement at 0.23° Eccentricity Before (V1) and After Cataract Surgery (V2) in the Study Eye

	Parafoveal Reference at 5°				Parafoveal Reference at 7°				Parafoveal Reference at 10°			
ID	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23	MP0.23V1	MP0.23V2	0.23V2/V1	Diff0.23
SS002	0.79	0.97	1.23	0.18	0.83	1.01	1.22	0.18	0.86	1.04	1.21	0.18
SS003	0.55	0.73	1.33	0.18	0.59	0.77	1.31	0.18	0.62	0.81	1.31	0.19
SS004	0.46	0.51	1.11	0.05	0.49	0.55	1.12	0.06	0.51	0.57	1.12	0.06
SS005	0.45	0.63	1.40	0.18	0.49	0.68	1.39	0.19	0.52	0.72	1.38	0.20
SS006	0.20	0.26	1.30	0.06	0.21	0.28	1.33	0.07	0.22	0.30	1.36	0.08
SS008	0.42	1.24	2.95	0.82	0.50	1.35	2.70	0.85	0.57	1.44	2.53	0.87
SS009	0.14	0.15	1.07	0.01	0.15	0.16	1.07	0.01	0.17	0.18	1.06	0.01
SS010	0.68	0.96	1.41	0.28	0.70	0.99	1.41	0.29	0.72	1.01	1.40	0.29
SS011	0.57	0.62	1.09	0.05	0.59	0.64	1.08	0.05	0.61	0.66	1.08	0.05
SS013	0.43	0.54	1.26	0.11	0.45	0.57	1.27	0.12	0.47	0.59	1.26	0.12
SS015	0.40	0.51	1.28	0.11	0.45	0.56	1.24	0.11	0.48	0.58	1.21	0.10
SS018	0.45	0.57	1.27	0.12	0.48	0.59	1.23	0.11	0.50	0.61	1.22	0.11
SS019	0.45	0.51	1.13	0.06	0.49	0.55	1.12	0.06	0.52	0.58	1.12	0.06
SS020	0.67	0.71	1.06	0.04	0.72	0.76	1.06	0.04	0.77	0.80	1.04	0.03
SS021	0.48	0.52	1.08	0.04	0.50	0.56	1.12	0.06	0.53	0.60	1.13	0.07
SS023	0.36	0.47	1.31	0.11	0.38	0.49	1.29	0.11	0.39	0.50	1.28	0.11
SS024	0.28	0.37	1.32	0.09	0.30	0.39	1.30	0.09	0.33	0.41	1.24	0.08
SS025	0.29	0.61	2.10	0.32	0.30	0.64	2.13	0.34	0.31	0.67	2.16	0.36
SS026	0.47	0.62	1.32	0.15	0.51	0.65	1.27	0.14	0.54	0.68	1.26	0.14
SS029	0.44	0.44	1.00	0.00	0.48	0.48	1.00	0.00	0.51	0.51	1.00	0.00
SS030	0.37	0.63	1.70	0.26	0.42	0.69	1.64	0.27	0.46	0.74	1.61	0.28
SS031	0.49	0.65	1.33	0.16	0.53	0.69	1.30	0.16	0.56	0.72	1.29	0.16
SS032	0.61	0.75	1.23	0.14	0.65	0.78	1.20	0.13	0.68	0.80	1.18	0.12
SS033	0.50	0.69	1.38	0.19	0.54	0.73	1.35	0.19	0.57	0.76	1.33	0.19
SS034	0.17	0.19	1.12	0.02	0.20	0.21	1.05	0.01	0.22	0.23	1.05	0.01
SS035	0.32	0.56	1.75	0.24	0.33	0.57	1.73	0.24	0.35	0.58	1.66	0.23
SS036	0.39	0.42	1.08	0.03	0.41	0.43	1.05	0.02	0.42	0.44	1.05	0.02
SS037	0.55	1.09	1.98	0.54	0.58	1.12	1.93	0.54	0.61	1.15	1.89	0.54
SS038	0.65	0.73	1.12	0.08	0.68	0.75	1.10	0.07	0.70	0.77	1.10	0.07

Diff0.23, difference in macular pigment optical density (V2 − V1); macular pigment optical density at 0.23° eccentricity obtained using the Heidelberg Spectralis HRA+OCT MultiColor.

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data on MP0.23° (study eye) obtained for three parafoveal reference locations (5°, 7°, and 10°). It is evident from this table that the absolute values of MP at 0.23° eccentricity differ slightly for the three reference locations, with the farther-out reference eccentricities producing slightly higher estimates of MP. However, there is very strong consistency between the MP0.23° V2/V1 ratios for the three parafoveal reference locations. In fact, the intraclass correlation coefficient for the three ratio columns in Table 6 is 0.98 (95% confidence interval 0.97, 0.99); that is, 98% of the variation in ratios in these three columns is between subjects, and only 2% is between columns.

Macular Pigment Profile Assessment

Visual assessment of the MP spatial profile was performed for each patient in the study eye before and after cataract surgery. Of note, 58% of patients had typical profile types (exponential decline from center), and 42% exhibited atypical profile types (e.g., ring-like structures). Profile type did not change following cataract surgery in these patients.

Discussion

We investigated the impact of cataract on measures of MP using the Spectralis, and found statistically significant disagreement between MP readings before and after cataract surgery in our primary analyses (study eyes), with postsurgery measures being higher than those acquired prior to surgical intervention (Fig. 2). Disagreement was statistically significant at all eccentricities, the greatest disagreement being observed centrally; and disagreement was related to severity of lens opacification. In nonstudy eyes (secondary analyses), and in contrast, we found no statistically significant disagreement between measures of MP taken before and after cataract surgery (Fig. 3); in fact, given that test–retest measurements were taken an average of 38 days apart, we report concordance correlation coefficients that are remarkably high in these nonstudy eyes.

In study eyes, we investigated the use of general linear models to adjust for the impact of cataract on MP measurement. While patient cataract scores (LOCS III), as expected, featured prominently as explanatory variables in these models, it was a surprise that presurgery measure of MP, at 1.72° eccentricity, was also a significant predictor of disagreement between pre- and postsurgery measures of MP centrally. A possible explanation, supported by our statistical observations for the different eccentricities, is that cataract affects presurgery MP less at this outer eccentricity; in other words, high presurgery measures of MP at 1.72° reflect high presurgery MP at central eccentricities, the latter being disproportionately affected by cataract. Of note, three of four cataract scores (NO, NC, and P, but not C) were significantly and positively associated with disagreement in measured MP before and after surgery. Nuclear opalescence, NC, and P reflect opacification in the nucleus and posterior subcapsular region of the lens, and are dominant centrally (along the visual axis), whereas C reflects opacification of the cortical region of the lens (and is distributed radially, in a manner that is not dominant along the visual axis).[27] Our findings are therefore not counterintuitive, given that disagreement was greater at central eccentricities (and that disagreement was related to those measures of opacification that are dominant centrally).

Predicting postsurgery central MP with a general linear model, including the MP1.72° variable alongside the cataract variable NC, did go some way toward addressing the observed downward bias in presurgery measures of MP centrally. Our final models (e.g., estimated MP0.23° V2/V1 ratio = 0.222 + $0.184 \times$ NC + $2.193 \times$ MP1.72° V1) may therefore be useful for addressing the impact of lens opacification on MP using the Spectralis.

Furthermore, we found that MP volume is less affected by cataract, in line with the observation that MP values at outer eccentricities are less affected than those yielded for central MP. Thus, in an older population with varying severity of cataract, MP volume would appear to be a more appropriate surrogate of overall MP than, say, MP at central retinal eccentricities (e.g., 0.23°). Moreover, central MP does not always predict total amount of MP because of variability in MP spatial profile (e.g., narrow peak versus broad peak, with same central value).

Our results are consistent with a study by Sasamoto et al.,[23] which examined the effect of cataract on MP measurement using the dual-wavelength AF technique. In that study, MP was measured before and after cataract surgery in 45 eyes of 41 subjects using the Heidelberg Retina Angiograph (HRA; Heidelberg Engineering, Dossenheim, Germany), but at only one eccentricity (0.5°) and utilizing the wavelengths 488 and 514 nm; the authors concluded that MP measurements are affected by cataracts (especially by nuclear cataracts). Of note, the fellow eye was not used as control in the study by Sasamoto et al.,[23] which represents a limitation of that study.

Our secondary analyses (nonstudy eyes) demonstrate that, in the absence of cataract surgery or cataract progression, MP measurement using the Spectralis is robust to test–retest variability over short periods of time, and this observation is consistent with a study by You et al.[33] and will have important implications for clinical practice in the future, as well as for those research studies measuring MP over time.

We could not obtain MP measurements in some patients because the HEYEX software could not compute the MP spatial density profile from the acquired video. Possible explanations include too much eye movement during MP measurement acquisition and poor image quality, which may be related to cataract severity. For example, the cataract severity grade (LOCS III) in one of these patients was NO: 5.5; NC: 5.5; C: 1.4; P: 0.2.

In the current study, we examined the effect of the parafoveal reference point on the discrepancy between pre- and postoperative measures of MP (Table 6). In addition to the 7° reference point (standard device reference point), we examined the effect of the parafoveal reference location at 5° and 10° on V2/V1 ratios (study eye) for MP0.23° eccentricity. We found that the choice of parafoveal reference location had very little influence on the ratio data for our analysis, and so we would have arrived at the same conclusions (about the effect of cataract on MP measurement with the Spectralis) whichever reference point had been chosen.

It is known that central retinal thickness is positively correlated with MP optical density.[34] We investigated whether central foveal thickness could also help explain the discrepancy between measures of MP before and after cataract surgery. We report that discrepancy between MP measurements, before and after cataract surgery, was not associated with baseline central retinal thickness.

Another important point is that detector sensitivity remained unchanged throughout the current study and therefore the effect of different detector settings on pre- and postoperative measures of MP was not examined. Future studies should examine the effect of different detector settings (high versus low) on measures of MP using the Spectralis.

The Spectralis uses the dual-wavelength AF technique, which rests on the assumption of a relatively clear ocular media. The optical density of the crystalline lens is particularly variable among persons at any age.[35] This should be borne in mind in interpreting the results of the current study.

Assumptions and possible mechanisms that could contribute to the observed discrepancy in macular pigment optical density (MPOD) measurements before and after cataract surgery are discussed below. First, the basic idea to overcome the effect of the lens scattering is the following: The ratio of green AF to blue AF (GAF/BAF) for the center is referenced to the ratio GAF/BAF in the periphery (5°, 7°, and 10°). Here the following assumptions were made:

a) The scattering effect (for the ratio green excitation light to blue excitation light) is similar for light entering the pupil at an angle of 0° central to the fovea as for light entering the pupil at 5°, 7°, or 10°.
b) The MP density is negligible in the periphery (5°, 7°, 10°); therefore the periphery can be used as a reference point.
c) Bleaching of the photopigment leads to a stable ratio (green excitation light to blue excitation light) in the periphery as well as in the center.
d) Fluorescence signal from the lens does not contribute significantly since the signal is suppressed by the confocal detection (pinhole).

All assumptions are reasonable, although it is difficult to say to what extent they are really valid especially in severe cataracts. Assumption a) is most likely better fulfilled for smaller angles 5° vs. 10°, whereas assumption b) is most likely better fulfilled for the 10° reference compared to 5° reference. Assumptions a), c), and d) could depend on the extent of the cataract, whereas assumption b) does not. Especially assumption d) could contribute, since severe cataract will reduce the signal from the retina and at the same time fluorescence from the lens can be increased. The detection unit in the Spectralis is designed to detect light simultaneously from several layers (e.g., choroidal and retinal blood system); therefore the pinhole is larger than the diffraction limit and confocal suppression is not optimum. This could be possibly the major effect for erroneous MP measurements in patients with cataract.

Bleaching of the retina (photopigment) could be different for fovea compared with the periphery, since for the center, due to the presence of MP, a higher luminance is required to bleach the photopigments. It is possible that for eyes with cataracts, where less light reaches the retina, the bleaching effect is still sufficient in the periphery but not complete within the fovea. This could explain larger gray value changes in the fovea compared with changes in the periphery after cataract surgery.

Second, regarding sensor sensitivity, the higher the sensitivity, the broader the noise distribution for a given AF value, and clipping could occur during the averaging procedure. However, this effect has been carefully considered in the design of the Spectralis MP software. The Spectralis has a sensitivity wheel (next to the touch screen), which can be adjusted between 31 and 107 in arbitrary sensitivity units. These sensitivity units are adjusted according to the intensity of light; that is, for IR reflection, very low sensitivity settings (e.g., 50) are used, whereas for AF images the sensitivity increases to >90 (maximum 107). However, when measuring MP, the Spectralis limits the sensitivity to 90 (i.e., the very high sensitivity settings are blocked for MP measurements), and by shifting of the digitization range, it is guaranteed that the complete zero light distribution is measured and no clipping occurs. The offset level is very carefully analyzed from laser offset measurements acquired during the resetting period of the Y-scanners. Therefore, the high-sensitivity setting should have no or only minor effects on MP measurements with the Spectralis; this could differ to some extent in the older HRA devices.

Other mathematical assumptions and potential explanations:

1. Wavelength-dependent absorption of lens: Assume that Beer's law is valid for the lens. In this case the intensity of blue and green light after passing the lens becomes:

$$B(x) = B_0 \times \exp(-b \times x)$$
$$G(x) = G_0 \times \exp(-g \times x)$$

with

B_0 = intensity of incoming blue laser light
G_0 = intensity of incoming green laser light
b = absorption coefficient of the lens for blue laser light
g = absorption coefficient of the lens for green laser light
x = thickness of the lens
B = intensity of the blue laser light after passing the lens
G = intensity of the green laser light after passing the lens

Assume $B_0 = G_0 = 1$, density of fluorophores is constant, and there is no MP. In this case the measured optical density would be related to

$$\log\Big(B(x)/G(x)\Big) = (g - b) \times x \neq 0.$$

If b, g, and x vary with the scanning angle, then this variation in addition to the optical density of the retina is measured.

If this is the case, then there should be a similar effect in the cHFP. The optical density of MP after cataract operation differs from the optical density before cataract operation. However, the changes may be different due to different wavelengths of the devices.

2. Fluorescence of the lens: If this is the case, the detected optical density would be:

$$\mathrm{OD} = \log\Big((B + BAF)/(G + GAF)\Big) \neq \log(B/G)$$

with

B = intensity of fluorescence light of the retina excited with the blue laser light
G = intensity of fluorescence light of the retina excited with the green laser light
BAF = intensity of fluorescence light of the lens excited with the blue laser light
GAF = intensity of fluorescence light of the lens excited with the green laser light

Please note that this effect would occur in cHFP.

3. Little fluorescence of the retina: If there is only little fluorescence of the retina, then the signal-to-noise ratio becomes poor. Small errors in the measurement of the underground or the signal may have a large effect. There could be a problem with averaging optical densities if the signal-to-noise ratio is poor. For example, if the blue and green signals have a value of 1 and noise is 0.01, the quotient of blue and green will be somewhere between 0.99/1.01 and 1.01/0.99. The average is close to 1.00. However, if the blue and green signals have a value of 1 and noise is 0.25, the quotient of blue and green will be somewhere between 0.75/1.25 and 1.25/0.75. The average is 1.13.

The strengths of this study include the following: All cataract surgery procedures were performed by a single surgeon using a single model of non–blue-blocking intraocular lens, thereby eliminating potential bias in these respects; cataract grading was conducted by a trained and certified LOCS

III grader; one trained examiner performed MP measurements before and after cataract surgery, thereby eliminating interexaminer bias and variability; dietary and serum carotenoid assessment was performed to control for any variability in MP measurement attributable to these parameters; and the fellow eye was used as a control (i.e., in the absence of cataract surgery). Limitations of this study include its small sample size and a large number of potential study patients who were ultimately unable to participate due to the need for an accompanying person for transport purposes (because of the need to pharmacologically dilate the pupils at the study visits, a measure that would not be part of routine clinical evaluation of an eye before or after cataract surgery at the IOES[36]).

In conclusion, we recommend that cataract be graded as a matter of routine during measurement of MP in older adults using currently available AF techniques, and suggest that such grading may be useful to correct for the impact of cataract on MP readings using such devices. However, over short periods of time, the Spectralis device does yield reliable and reproducible MP values in patients with cataracts that have not been surgically removed.

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References

1. Hirsch J, Curcio CA. The spatial resolution capacity of human foveal retina. *Vis Res*. 1989;29:1095–1101.
2. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. 1. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
3. Bone RA, Landrum JT, Cains A. Optical-density spectra of the macular pigment in vivo and in vitro. *Vis Res*. 1992;32:105–110.
4. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys*. 1999;371:301–307.
5. Li B, Ahmed F, Bernstein PS. Studies on the singlet oxygen scavenging mechanism of human macular pigment. *Arch Biochem Biophys*. 2010;504:56–60.
6. Wrona M, Rozanowska M, Sarna T. Zeaxanthin in combination with ascorbic acid or alpha-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radic Biol Med*. 2004;36:1094–1101.
7. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. Lutein supplementation leads to decreased soluble complement membrane attack complex sC5b-9 plasma levels. *Acta Ophthalmol*. 2015;93:141–145.
8. Tian Y, Kijlstra A, van der Veen RL, Makridaki M, Murray IJ, Berendschot TT. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a and C3d. *PLoS One*. 2013;8:e73387.
9. Li SY, Fung FK, Fu ZJ, Wong D, Chan HH, Lo AC. Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: in vivo and in vitro studies. *Invest Ophthalmol Vis Sci*. 2012;53:5976–5984.
10. Firdous AP, Kuttan G, Kuttan R. Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. *Pharm Biol*. 2015;53:961–967.
11. Liu R, Wang T, Zhang B, et al. Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:252–258.
12. Akuffo KO, Nolan JM, Howard AN, et al. Sustained supplementation and monitored response with differing carotenoid formulations in early age-related macular degeneration. *Eye (Lond)*. 2015;29:902–912.
13. Ma L, Yan SF, Huang YM, et al. Effect of lutein and zeaxanthin on macular pigment and visual function in patients with early age-related macular degeneration. *Ophthalmology*. 2012;119:2290–2297.
14. Loughman J, Nolan JM, Howard AN, Connolly E, Meagher K, Beatty S. The impact of macular pigment augmentation on visual performance using different carotenoid formulations. *Invest Ophthalmol Vis Sci*. 2012;53:7871–7880.
15. Hammond BR, Fletcher LM, Roos F, Wittwer J, Schalch W. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on photostress recovery, glare disability, and chromatic contrast. *Invest Ophthalmol Vis Sci*. 2014;55:8583–8589.
16. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*. 2013;309:2005–2015.
17. Bone RA, Landrum JT. Heterochromatic flicker photometry. *Arch Biochem Biophys*. 2004;430:137–142.
18. Delori FC. Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. *Arch Biochem Biophys*. 2004;430:156–162.
19. Wooten BR, Hammond BR. Spectral absorbance and spatial distribution of macular pigment using heterochromatic flicker photometry. *Optom Vis Sci*. 2005;82:378–386.
20. Stringham JM, Hammond BR, Nolan JM, et al. The utility of using customized heterochromatic flicker photometry (cHFP) to measure macular pigment in patients with age-related macular degeneration. *Exp Eye Res*. 2008;87:445–453.
21. Dennison JL, Stack J, Beatty S, Nolan JM. Concordance of macular pigment measurements obtained using customized heterochromatic flicker photometry, dual-wavelength autofluorescence, and single-wavelength reflectance. *Exp Eye Res*. 2013;116:190–198.
22. Akuffo KO, Beatty S, Stack J, et al. Concordance of macular pigment measurement using customized heterochromatic flicker photometry and fundus autofluorescence in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2015;56:8207–8214.
23. Sasamoto Y, Gomi F, Sawa M, Sakaguchi H, Tsujikawa M, Nishida K. Effect of cataract in evaluation of macular pigment optical density by autofluorescence spectrometry. *Invest Ophthalmol Vis Sci*. 2011;52:927–932.
24. Akuffo KO, Beatty S, Stack J, et al. Central Retinal Enrichment Supplementation Trials (CREST): design and methodology of the CREST randomized controlled trials. *Ophthalmic Epidemiol*. 2014;21:111–123.
25. Nolan JM, Loskutova E, Howard A, et al. The impact of supplemental macular carotenoids in Alzheimer's disease: a randomized clinical trial. *J Alzheimers Dis*. 2015;44:1157–1169.
26. Nolan JM, Loskutova E, Howard AN, et al. Macular pigment, visual function, and macular disease among subjects with

Alzheimer's disease: an exploratory study. *J Alzheimers Dis*. 2014;42:1191–1202.

27. Chylack LT Jr, Wolfe JK, Singer DM, et al. The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. *Arch Ophthalmol*. 1993;111:831–836.
28. Wolf-Schnurrbusch UE, Ceklic L, Brinkmann CK, et al. Macular thickness measurements in healthy eyes using six different optical coherence tomography instruments. *Invest Ophthalmol Vis Sci*. 2009;50:3432–3437.
29. Charalampidou S, Loughman J, Nolan J, et al. Prognostic indicators and outcome measures for surgical removal of symptomatic nonadvanced cataract. *Arch Ophthalmol*. 2011; 129:1155–1161.
30. Kirwan C, Nolan JM, Stack J, Moore TC, Beatty S. Determinants of patient satisfaction and function related to vision following cataract surgery in eyes with no visually consequential ocular co-morbidity. *Graefes Arch Clin Exp Ophthalmol*. 2015;253: 1735–1744.
31. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2008.
32. Lin L, Hedayat AS, Wu W. *Statistical Tools for Measuring Agreement*. New York: Springer; 2012.
33. You QS, Bartsch DG, Espina M, et al. Reproducibility of macular pigment optical density measurement by two-wavelength autofluorescence in a clinical setting [published online ahead of print December 11, 2015]. *Retina*. doi:10.1097/IAE.0000000000000893.
34. Liew SH, Gilbert CE, Spector TD, et al. Central retinal thickness is positively correlated with macular pigment optical density. *Exp Eye Res*. 2006;82:915–920.
35. Norren DV, Vos JJ. Spectral transmission of the human ocular media. *Vis Res*. 1974;14:1237–1244.
36. Saeed A, Guerin M, Khan I, et al. Deferral of first review after uneventful phacoemulsification cataract surgery until 2 weeks: randomized controlled study. *J Cataract Refract Surg*. 2007;33:1591–1596.

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