Carotenoids and Co-Antioxidants in Age-Related Maculopathy: Design and Methods

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ABSTRACT

Age-related macular degeneration (AMD), is the leading cause of blind registration in the Western World among individuals 65 years or older. Early AMD, a clinical state without overt functional loss, is said to be present clinically when yellowish deposits known as drusen and/or alterations of fundus pigmentation are seen in the macular retina. Although the etiopathogenesis of AMD remains uncertain, there is a growing body of evidence in support of the view that cumulative oxidative damage plays a causal role. Appropriate dietary antioxidant supplementation is likely to be beneficial in maintaining visual function in patients with AMD, and preventing or delaying the progression of early AMD to late AMD. The Carotenoids in Age-Related Maculopathy (CARMA) Study is a randomized and double-masked clinical trial of antioxidant supplementation versus placebo in 433 participants with either early AMD features of sufficient severity in at least one eye or any level of AMD in one eye with late AMD (neovascular AMD or central geographic atrophy) in the fellow eye. The aim of the CARMA Study is to investigate whether lutein and zeaxanthin, in combination with co-antioxidants (vitamin C, E, and zinc), has a beneficial effect on visual function and/or prevention of progression from early to late stages of disease. The primary outcome is improved or preserved distance visual acuity at 12 months. Secondary outcomes include improved or preserved interferometric acuity, contrast sensitivity, shape discrimination ability, and change in AMD severity as monitored by fundus photography. This article outlines the CARMA Study design and methodology, including its rationale.

INTRODUCTION

Age-related macular degeneration (AMD), previously also known as age-related maculopathy, is the term used to describe a spectrum of ageing changes in the macular retina of the eyes of older adults. In the early stages a large proportion of older adults have yellowish deposits known as drusen and alterations of fundus pigmentation in the macular retina. This is known as early AMD. In a significant proportion of eyes exhibiting early AMD there is progression through intermediate (more severe levels of drusen and pigmentary irregularities) to late stages with dysfunction and death of photoreceptors secondary to an atrophic (geographic atrophy, GA) and/or a neovascular (choroidal neovascularization, CNV) event. These late stages are commonly referred to as late AMD. Currently, late AMD is the leading cause of blind registration in Western countries among individuals 65 years and older,[1–3] with CNV accounting for 90% of these cases.[4,5]

In the future, the prevalence and incidence of AMD is expected to rise because of an increase in life expectancy, and the consequential demographic shift towards an elderly population will have implications for the socio–economic impact of this disease.[6,7] Currently available therapeutic interventions for this condition are largely aimed at preservation of presenting visual acuity (VA). [8–11] Although a significant proportion of patients may achieve some visual gain with the novel inhibitors of vascular endothelial growth factor, complete restoration of

discriminant visual function is only achieved in small proportions of those treated.[12,13] Moreover, there is no available treatment for atrophic AMD. There is no doubt that effect of late AMD on the activities of daily living, self-care, emotional well being, and overall quality of life are important, and that these are related to disease severity.[14-19] Therefore, prevention or delay of early to late manifestations represents the best means of limiting the impact of this degenerative disorder on visual health and quality of life in the elderly population.

While the exact etiopathogenesis of the disorder remains uncertain, there is a growing body of evidence that cumulative oxidative damage may play a causal role.[20,21] Oxidative damage refers to tissue injury by reactive oxygen intermediates, which are unstable molecules generated in cells as a by-product of oxygen metabolism, and, additionally, in the retina, by photochemical reactions between light and oxygen. Consequently, antioxidant supplementation could play an important role in preventing the development of AMD, or delaying the progression from early to late disease.

STUDY RATIONALE

One of the unique features of the primate macula is the presence of high concentrations of the xanthophylls, lutein (L) and zeaxanthin (Z), which gives it its eponymous yellow color. Together, these two xanthophylls are referred to as macular pigment (MP).[22] MP peaks in the central 1 to 2 degrees of the fovea, and declines to optically negligible levels by 5 to 10 degrees radial eccentricity.[23] Of note, MP cannot be synthesized *de novo*, and is derived entirely from diet.

Macular pigment is purported to prevent or retard the development or progression of AMD because of its ability to absorb blue light at the prereceptorial level, and because of its capacity to quench reactive oxygen intermediates *via* its powerful antioxidant activity.[20,21] Furthermore, it has been observed that increased intake of dietary L and Z results in the augmentation of MP,[24] consistent with the possibility that appropriate dietary supplementation or modification would confer protection against development and/or progression of AMD.

The Age-Related Eye Disease Study (AREDS) has investigated the role of multiple antioxidants, namely vitamins A,C, and E and zinc, and demonstrated that treatment reduces the relative risk of disease progression to late AMD by 25% and results in odds reduction of 27% for visual acuity loss.[25] These beneficial effects were mainly observed in the fellow eyes of a subset of participants who had late stage AMD in the contralateral eye. Noteworthy, too, was the protective effect in those participants with intermediate stages of AMD in both eyes.

To date, five interventional studies have investigated the role of L supplementation in AMD patients (Table 1),[26-30] and the data from four of these demonstrated improvements in acuity and/or other parameters of visual function in the treatment groups.[27-30] Although the statistical power of these studies is limited by their small sample sizes (n ≤ 90), they are, nonetheless, encouraging. More recently Chong and colleagues undertook a systematic review and meta-analysis of dietary antioxidants and supplements, and questioned the value of primary prevention.[31] However, the findings of AREDS in terms of secondary prevention, particularly in the fellow eyes of patients with advanced disease in the contralateral eye, raises intriguing questions on the potential benefits of supplemental antioxidants.

Furthermore, a number of well-conducted population-based studies employing longitudinal study design have suggested that high levels of dietary antioxidants, specifically L and Z, may have protective and beneficial effects in retarding progression to advanced AMD (Table 2). Lutein and Z were not included in the AREDS formulation because they were not commercially available in supplement form at the inception of that study. Further,

Table 1. Human Interventional Studies Investigating the Role of Supplemental L on Visual Function in Age-Related Macular Degeneration

Study/Author (Year of publication)	Design	Participants (n)	Supplement given/dose	Duration of study	Outcome
Richer et al. (1999)	Case-series	AMD patients (14)	14 mg L/day	3–12 months	Improvement in visual acuity, and several other parameters of visual function
Massacesi et al. (2001)	Randomized, placebo-controlled	Early AMD patients (50)	15 mg L/day	18 months	Improvement in visual acuity
Olmedilla et al. (2001)	Case-series	AMD patients (5)	15 mg L/day	13 months	Improvement in visual acuity, and glare sensitivity
LAST (2004)	Randomized, placebo-controlled	AMD patients (90)	10 mg L/day	12 months	Improvement in visual acuity, and several other parameters of visual function
Barlett et al. (2007)	Randomized, placebo-controlled	Early and late AMD patients (25)	6 mg L/day	9 months	No statistically significant improvement in contrast sensitivity function

LAST: Lutein Antioxidant Supplementation Trial.
L: Lutein.

Table 2. Observational Studies Examining the Relationship between Dietary and/or Serum Levels of Antioxidants and Risk of AMD

Study/Author (Year of publication)	Design	No. of participants	Antioxidants examined (Diet or serum)	Conclusion
Eye-Disease Case Control Study (1994)	Case-control	356 cases 520 controls	L, Z, lycopene, α and β carotene, β cryptoxanthin, retinol, vitamin A, C, and E (diet)	Subjects with the highest quintile of carotenoid intake had a 43% lower risk of AMD when compared with those in the lowest quintile. Furthermore, of the carotenoids, L and Z, which are primarily obtained from dark green leafy vegetables, were most strongly associated with a reduced risk of AMD (p for trend = .001).
Beaver Dam Eye Study 1998)	Population-based	4926	L, Z, lycopene, α and β carotene, β cryptoxanthin, vitamin E, C, and zinc (diet)	A significant (but modest) inverse association was observed between dietary intakes of pro-vitamin A carotenoids (and vitamin E) and the incidence of large drusen. Similarly, a high level of dietary zinc was associated with lower incidence of pigmentary abnormalities.
National Health And Nutrition Examination Survey III (2001)	Cross-sectional	8,222	L, Z, and other carotenoids (diet and serum)	A higher level of L and Z in the diet was associated with lower risk of pigmentary abnormalities and of late AMD in the youngest age groups at risk for developing early or late AMD, after adjustment for age, gender, alcohol use, hypertension, smoking, and body mass index.
Snellen et al. (2002)	Case-control	72 cases 66 controls	L, Z, and other antioxidants (diet)	Subjects with low intake of dietary L and other antioxidants exhibited a higher prevalence of neovascular AMD when compared with subjects with high dietary intake of these nutrients.
Gale et al. (2003)	Cross-sectional	380	L, and Z (serum)	Subjects with lowest concentrations of serum Z had a two-fold increase in risk of AMD when compared with those with the highest concentrations of this carotenoid in the serum.
Rotterdam Study (2005)	Population-based	4170	L, Z, lycopene, α and β carotene, β cryptoxanthin, vitamin A, C, E, iron, and zinc (diet)	Dietary intake of vitamin E and zinc was inversely associated with incident AMD. Furthermore, an above-median intake of these nutrients, in combination with beta-carotene and vitamin C, was associated with a 35% reduced risk for AMD.
Pathologies Oculaires Liees a' l'Age Study (2006)	Population-based	899	L, Z, lycopene, dehydro-lutein, α and β carotene, and β cryptoxanthin (serum)	Subjects with high serum L plus Z had a 79% reduced risk of AMD when compared with subjects with low serum L plus Z, and this inverse association was particularly strong for serum Z.
The Carotenoids in Age-Related Eye Disease Study (2006)	Population-based	1787	L, Z, β carotene, omega-3 fatty acids, β cryptoxanthin, α and γ tocopherols, vitamin C and E, and zinc (diet)	There was no statistically significant difference in the prevalence of intermediate AMD among subjects with high and low intake of dietary L plus Z. However, limiting analyses to women younger than 75 years with stable intake of L plus Z (without a history of chronic diseases that are often associated with diet changes), revealed a reduced risk of this subtype of AMD in association with a high dietary intake of these antioxidants.
Age-Related Eye Disease Study (2007)	Case-control	4519	L, Z, lycopene, α and β carotene, β cryptoxanthin, retinol, vitamin A, C, and E (diet)	A high dietary intake of L and Z was independently associated with decreased likelihood of having neovascular AMD, geographic atrophy, and large and extensive drusen.
Blue Mountains Eye Study (2007)	Population-based	2454	L, Z, lycopene, α and β carotene, β cryptoxanthin, vitamin A, C, E, iron, and zinc (diet)	Subjects in the highest tertile of dietary intake of L and Z had a reduced risk of neovascular AMD, whereas those with above median intakes of these carotenoids in the diet had a reduced risk of indistinct soft or reticular drusen. In addition, a protective influence of dietary zinc against AMD was observed.

AREDS did not measure subtle differences in visual function between the various intervention groups following antioxidant supplementation. Thus there was an obvious need for controlled clinical trials with L and Z included in the test formulation. We hypothesized that reduction of oxidative stress at the level of the RPE could potentially improve function. It was our view that the detection of differences in visual function between intervention groups would provide both a better understanding of the pathogenesis, and add to the evidence base for the use of antioxidants in preventing progression of early or intermediate to late AMD.

Therefore, the Carotenoids in Age-Related Maculopathy (CARMA) Study, a randomized controlled clinical trial, was developed to test the hypothesis that a combination of antioxidant vitamins, including L and Z, would be beneficial in maintaining visual function and reducing the risk of progression to a more severe morphological state. The rationale for adding L and Z to the antioxidants (already proven to have a beneficial effect in the AREDS Study) are two-fold: first, L and Z exhibit the ideal anatomic, biochemical, and optical properties to limit (photo) oxidative damage at the macula; second, carotenoids act synergistically with other antioxidants to exert antioxidant effect.[32]

STUDY DESIGN

The CARMA Study is a randomized and double-masked clinical trial of antioxidant supplementation versus placebo in 433 Caucasian AMD participants aged 50 years and above, who are recruited at two clinical centers in Ireland (Waterford and Belfast). Participants must have either early AMD features of sufficient severity in at least one eye or any level of AMD in one eye and advanced AMD (neovascular AMD or central GA) in the fellow eye. All enrolled participants will be followed until the last participant has completed one year of follow-up or until the Data and Safety Monitoring Committee (DSMC) has recommended earlier termination of the trial.

An overview of the administrating and coordinating infrastructures of the CARMA Study is given in Figure 1. The study is approved by the Waterford Research Ethics Committee, and by the Office for Research Ethics Committees in Northern Ireland. The CARMA Study is registered with a clinical trial database (International Standard Randomized Controlled Trial Number: 9455760).

AIMS OF THE STUDY

The principal aim of the CARMA Study is to investigate the potential efficacy of L and Z, in combination with key vitamins and minerals, on psychophysical measures of vision namely distance visual acuity (DVA), contrast sensitivity, photopic interferometric acuity, and shape discrimination. Secondary aims are to examine the potential efficacy of the CARMA preparation in delaying or retarding the morphological progression of early AMD. The CARMA Study will also measure MP levels, and serum concentrations of L and Z, in response to supplementation with the macular carotenoids.

SAMPLE SIZE AND POWER CONSIDERATION

It is hypothesized that treatment with CARMA preparation will result in either maintained function or improved function while treatment with placebo will result in decreasing function. The original study design employed photopic interferometric acuity as the primary outcome variable. However, we encountered several problems in the consistency and reliability of this measurement technique, and, therefore, following the DSMC recommendations during its meeting in October 2005, the primary outcome measure was changed from photopic interferometry to DVA. The study sample size was also increased in order to take into account the change in the primary outcome measure. The revised protocol was approved by the Research Ethics Committee at both the clinical centers.

The revised sample size calculations estimated that a sample size of 500 participants with 5% alpha (2 sided test) will have 90% power to detect a 0.04 (2 logMAR letters) difference in DVA assuming that standard deviation of the change in DVA within a participant is 0.13 (6.5 logMAR letters) and that there would be a 10% attrition rate. The study duration was extended as a result of increase in the sample size.

RECRUITMENT STRATEGIES

Study awareness campaigns consisting of the distribution of leaflets and posters along with presentations describing CARMA are conducted at both sites. Potential participants are sent a letter of invitation along with the study information leaflet (explaining the background and rationale for the CARMA Study) containing contact details of the study staff. No financial incentives are offered to the participants;[33] however, costs of transportation to and from the study center are provided, where requested.

All potential participants who expressed a willingness and desire to participate in the CARMA Study are screened at a study clinic where informed consent is obtained. Following verification of eligibility (Table 3), all participants are randomized to one of the two groups. The CARMA preparation is provided in numbered containers and the participant is instructed to take one tablet twice daily. The first participant in the CARMA Study was enrolled in July 2004, and new recruitments continued until April 2007 to achieve the revised sample size. All enrolled participants will be followed until the last participant has completed 1 year of follow-up, and, therefore, the study concluded on April 2008. Also, participants who are recruited early on will have the opportunity to be followed up for a maximum of 36 months.

The baseline/screening visit consists of four separate parts (informed consent, clinical examination, questionnaire, and randomization), and takes approximately 2–3 hours per participant. The study is conducted in accordance with the principles set forth in the Declaration of Helsinki.
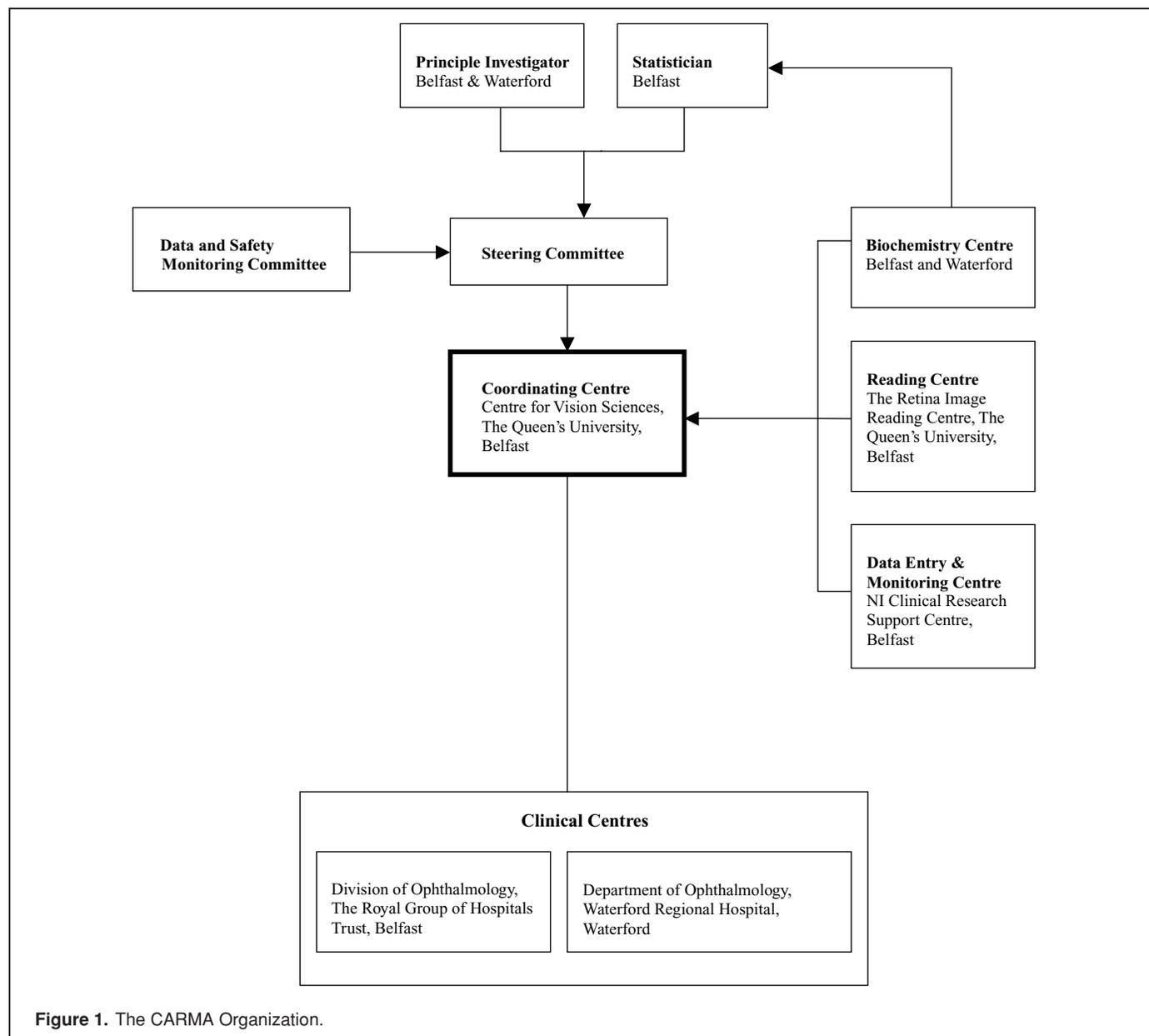
Figure 1. The CARMA Organization.

CLINICAL EXAMINATION

The clinical assessment was conducted by either an ophthalmologist or optometrist, supported by a study nurse, and consisted of ophthalmic and systemic examination.

Ophthalmic examination

Distance visual acuity

Best-corrected DVA is determined using retro-illuminated Early Treatment Diabetic Retinopathy Study (ETDRS, Precision Vision, USA) charts viewed at 4-m distance with room lights turned off. After refracting the participant with ETDRS "R" chart, using a combination of objective and subjective refraction, the best corrected VA for each eye is measured with the standard ETDRS protocol.[34,35] If VA is worse than 0.8 logMAR, i.e. fewer than 15 letters are read correctly at 4-m distance, measurement of VA is repeated at 1-m distance. During recording of vision on a score-sheet, each letter read correctly by the participant is encircled whereas those letters identified incorrectly are crossed.

Photopic interferometric acuity (Retinal acuity)

Interferometry is performed under photopic conditions using a white light interferometer (Lotmar Visometer, Bern, Switzerland).[36] A total of 20 readings of the threshold spatial frequency are made (5 for each orientation with the stimuli presented randomly) and the mean value calculated. The Purkinje image of the green light on the cornea is monitored throughout

Table 3. Inclusion and Exclusion Criteria for the CARMA Study

Inclusion criteria

- Age 50 years or older.
- Either gender.
- Able to give informed consent, make the required study visits, and adhere to the protocol.
- Fundus characteristics:

Group 1-Participants with CNV or central GA in one eye, and any level of AMD in the fellow eye with a best-corrected VA of 0.3 logMAR or better.

Group 2-Participants with early AMD features of sufficient severity in at least one eye. AMD features of sufficient severity are soft distinct or indistinct drusen > 20 or if fewer than 20 soft drusen, focal hyper pigmentation must be present. Best-corrected VA in the study eye must be 0.3 logMAR (Snellen equivalent: 6/12) or better. Both eyes of a participant may be eligible for inclusion into the study.

Exclusion Criteria

Systemic conditions

- History of any life threatening or terminal medical condition, such as malignant disorders and cerebrovascular accidents, which may prevent follow-up visits.
- A history of major psychiatric illness and/or poor cognitive function precluding the giving of informed consent.
- History of severe gastro-intestinal disease and/or malabsorption that may hinder the absorption of active ingredients from the gastro-intestinal tract.
- Known allergy to one of the active ingredients, or other excipients in the study preparation.

Ophthalmic conditions

- History of ophthalmic disease in the study eye, such as glaucoma and diabetic maculopathy.
- History of retinal laser therapy in the proposed study eye.

Supplement use

- Prior and concurrent use of non-study preparation supplements containing vitamin C and E, Zn, L and Z.*

*Participants consuming vitamins or natural/herbal remedies that are not constituents of the CARMA preparation are eligible to participate in the CARMA Study. The screening/baseline examination is conducted after a washout period of 3 months.

the test to ensure correct alignment was maintained, as this can influence target visibility.

Contrast sensitivity

Contrast sensitivity is measured for each eye using Pelli-Robson contrast sensitivity charts (Clement Clarke International, Harlow, UK) viewed at a 1-m distance, and with + 0.5 diopter addition to the participant's refractive correction. The right and left eye is tested with the chart having V, R, S and H, S, Z as the first triplet, respectively. Care is taken to ensure uniform illumination of the chart, with luminance ranging from 60 to 120 foot-candles, and to conceal the chart from viewing until the participant is ready for testing. Recording of contrast sensitivity on a score-sheet is done in a manner similar to the recording of VA.

Hyperacuity

Hyperacuity is assessed using the recently developed shape discrimination charts consisting of radial frequency patterns as the test targets.[37] The test is performed under room lighting conditions at standard viewing distance (38 cm) with reading correction in place. As with contrast sensitivity, the participant is encouraged to guess the deformed circle when the task is perceived as being too difficult.

Anterior and posterior segment examination

A comprehensive slit lamp biomicroscopic examination is performed after pupillary dilation. Anterior segment examination consists of a standardized detailed examination of the cornea, the anterior chamber, and the lens. Lenticular opacities are graded using Lens Opacities Classification Systems II.[38] Fundus examination involves identification of AMD lesions, such as drusen (type, number, and location) and/or pigmentary changes (type, location, and nature). Participants requiring intervention for recent evidence of neovascular disease and any detected eye diseases (such as advanced cataract) are referred to the principal investigator at the site for further management.

Macular pigment measurement

Macular pigment is measured using Raman spectroscopy, which is an objective and non-invasive technique of measuring MP *in vivo*.[39,40] Detailed information of principle, apparatus, and procedure, has been described elsewhere.[41]

Anterior and posterior segment photography

The anterior and posterior segments are photographed using a digital Topcon Fundus Camera (Topcon TRC-50EX, Topcon, Newbury, UK). Anterior segment images are graded for iris color and lenticular opacities. The posterior segment images, which consist of two non-simultaneous stereoscopic pairs centered on the macula with a 35-degree field, are obtained after full pupillary dilation. All images are stored as uncompressed tagged image files, copied to compact discs and send to the grading center, on a monthly basis.

Reading center fundus grading

Stereoscopically captured color retinal images are classified and graded in a masked fashion by any one of the five graders (who have completed the grader training and concordance exercises for the CARMA Study) using definitions of Wisconsin Age-Related Maculopathy Grading System (WARMGS) for early and late AMD features.[42] The training for graders was provided in-house using a structured training program along with external qualified photo graders from the Wisconsin Fundus Photograph Reading Centre and the Complications of AMD Treatment Reading Centre. Concordance exercises were carried out between Reading Centre Graders and representatives from the above institutions. Ongoing quality assurance assessments demonstrate that our inter-observer agreement values are

comparable to past studies[43] including one from our Reading Centre.[44]

Fundus photographs are sent to the senior grader and Reading Centre Clinician for adjudication in cases where a grader is not 100% confident of the AMD features to be graded. Ten percent of gradings are randomly selected to be re-graded, and any discrepancies are arbitrated by the Reading Centre clinician. As the CARMA Study is a longitudinal study, changes in morphological features of AMD are monitored over time, and in any fundus photograph where progression or regression to a different stage of AMD is deemed to have occurred, the images are routinely checked by a senior grader.

General examination

The general physical examination consisted of measurement of ambulatory blood pressure and body mass index.

Blood sample collection

For each participant, a peripheral venous blood sample of 20 ml is obtained. The participants are non-fasting; however, they are requested not to take study medication, fruit or fruit juices on the day of blood sample collection. Blood samples are centrifuged within four hours of collection (3000 rpm, 15 minutes), and the resultant serum and plasma layers are aliquoted into 12 micro centrifuge tubes and stored at −70 degree Celsius (serum tubes: 4; plasma tubes: 4; vitamin C tubes: 2; DNA tubes: 2). The two micro centrifuge tubes, which are used for estimation of vitamin C, consist of 100 μl plasma stabilized with 900 μl of 5% metaphosphoric acid (which is freshly prepared weekly). Buffy coats (collected at a single visit only) are stored in 2 micro centrifuge tubes for future genetic studies. Serum samples packed in dry ice are shipped to the central laboratory on a 3-monthly basis where they are stored at −70 degree Celsius until analyzed by reverse-phase high pressure liquid chromatography.

Questionnaires

The general life-style and food frequency questionnaire are administered during the baseline visit. The former gathers information relating to socio-demographic details, smoking habits, past medical history, use of aspirin and other prescription/or over-the-counter medications, and in women a reproductive history including hormone replacement therapy is included.

A validated semi-quantitative food frequency questionnaire evaluates the dietary intake for each participant for the 12 months immediately preceding the date of randomization(Willet W. *Nutritional Epidemiology*. Oxford: Oxford University Press, 1990). The questionnaire comprises a list of 147 food items which are divided into 12 sections: meat, fish,bread and savory biscuits, cereals, rice and pasta, dairy products, fats and dressings, sweets and snacks, soups and sauces, drinks, fruits, vegetables. The options for frequency of consumption are provided for each food item, and quantified in appropriate units with reference to standard portion sizes. At baseline, the dietary intake of L and Z will be assessed in both the intervention and in the control group.

Randomization

Each participant enrolled in the CARMA Study is allocated a unique number, which determines treatment allocation according to the computerized randomization database. This unique number exists on the identification label of each study-preparation box. The masked study-preparation boxes are kept in the hospital pharmacy, and released in a sequential manner by the pharmacist on randomization of each participant, beginning with the first in the numerical series assigned to each clinical center. The participants are advised to take 1 tablet twice daily with a meal.

The CARMA Study is strictly a double-masked clinical trial in that neither the CARMA participants nor the study staff, including the study investigator, are aware of the nature of study preparation allocated to the participants. To ensure masking, the study-preparation boxes are labeled with pre-assigned numbers at the site of manufacturing, and then shipped to both clinical centers for distribution. A single pharmacist involved with manufacturing of the study preparation holds the key to randomization of the CARMA supplements.

Summary of the baseline data

In the CARMA Study, we have enrolled 433 participants, with 237 (55%) and 196 (45%) in groups 1 and 2, respectively. The mean age of the study population ($\pm$ SD) was 75.93 $\pm$ 7.74 years, ranging between 50 and 95 years. Of the 433 participants, 248 (57%) were females and 185 (43%) were males.

At baseline visit, the mean ($\pm$ SD) logMAR VA was comparable in the two groups [Group 1 = 0.08 $\pm$ 0.12 (range: −0.3 to 0.3); Group 2 = 0.08 $\pm$ 0.14 (range: −0.2 to 0.5)]. Similarly, there was no significant difference in total number of logMAR letters recognized by the participants belonging to the two groups [Group 1: = 50.80 $\pm$ 6.43 (range: 36 to 67); Group 2 = 50.60 $\pm$ 7.44 (range: 29 to 65)].

Follow-up visits

Follow-up visits occur at 6 monthly intervals, with telephone contact occurring at 2-weeks and at 3-months. The scheduling of the follow-up visit at the study center is determined by the date of randomization $\pm$ 7 days. At repeat study visits, the clinical and ophthalmic assessments are repeated (Fig. 2). Information on intercurrent illness, changes in regular medication and potential adverse events, are recorded on a standardized proforma. At the conclusion of the study visit, a further 6-month supply of the study preparation is provided, and the importance of adherence with the study protocol is re-emphasized to participants.

The compliance with study-preparation is assessed as follows: self-reporting the precise number of left-over tablets by 2-weekly and 3-monthly telephone contacts; tablet count of the returned study-preparation box at each follow-up visit.
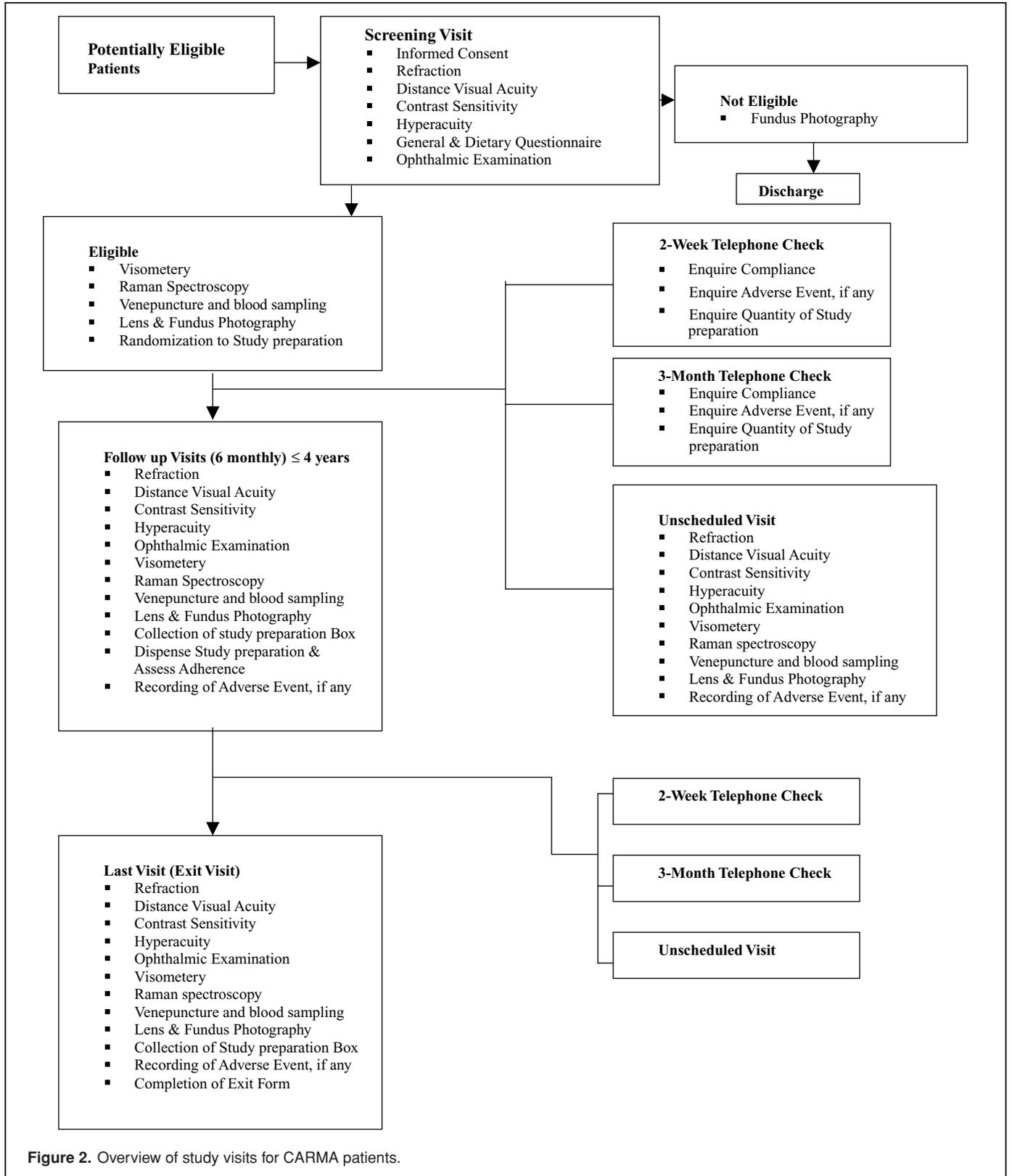
Potentially Eligible Patients

Screening Visit
- Informed Consent
- Refraction
- Distance Visual Acuity
- Contrast Sensitivity
- Hyperacuity
- General & Dietary Questionnaire
- Ophthalmic Examination

Not Eligible
- Fundus Photography

Discharge

Eligible
- Visometery
- Raman Spectroscopy
- Venepuncture and blood sampling
- Lens & Fundus Photography
- Randomization to Study preparation

2-Week Telephone Check
- Enquire Compliance
- Enquire Adverse Event, if any
- Enquire Quantity of Study preparation

3-Month Telephone Check
- Enquire Compliance
- Enquire Adverse Event, if any
- Enquire Quantity of Study preparation

Follow up Visits (6 monthly) ≤ 4 years
- Refraction
- Distance Visual Acuity
- Contrast Sensitivity
- Hyperacuity
- Ophthalmic Examination
- Visometery
- Raman Spectroscopy
- Venepuncture and blood sampling
- Lens & Fundus Photography
- Collection of study preparation Box
- Dispense Study preparation & Assess Adherence
- Recording of Adverse Event, if any

Unscheduled Visit
- Refraction
- Distance Visual Acuity
- Contrast Sensitivity
- Hyperacuity
- Ophthalmic Examination
- Visometery
- Raman spectroscopy
- Venepuncture and blood sampling
- Lens & Fundus Photography
- Recording of Adverse Event, if any

2-Week Telephone Check

3-Month Telephone Check

Unscheduled Visit

Last Visit (Exit Visit)
- Refraction
- Distance Visual Acuity
- Contrast Sensitivity
- Hyperacuity
- Ophthalmic Examination
- Visometery
- Raman spectroscopy
- Venepuncture and blood sampling
- Lens & Fundus Photography
- Collection of Study preparation Box
- Recording of Adverse Event, if any
- Completion of Exit Form

Figure 2. Overview of study visits for CARMA patients.

Withdrawal and termination of followup visits

Participants are encouraged to adhere to the study protocol. However, where a participant wishes to discontinue his/her participation in the CARMA Study, whether voluntarily or because of intercurrent medical indication (such as development of severe systemic illness which preclude the participant from continuing the study preparation or attending the follow-up visits), an exit visit is scheduled. During this visit, a clinical and ophthalmological assessment with photography is undertaken and an exit form (stating the reason for his/her premature exit from the study) is completed. Participants who are withdrawn from the study are not replaced, and their identification numbers are not reassigned.

Unscheduled visits

Unscheduled visits are conducted at the discretion of the investigator, and the information collected during such visits is recorded on the Unscheduled Visit Forms. If a participant wishes to discontinue at an unscheduled visit, the exit form is also completed.

Study preparation

The study preparation, manufactured by Bausch and Lomb (Berlin), consists of either placebo or L (6 mg), Z (0.3 mg), and co-antioxidants (vitamin E: 7.5 mg; vitamin C: 75 mg; zinc: 10 mg; copper: 0.2 mg) as main ingredients in a tablet. Beta-carotene, a carotenoid with antioxidant properties, is not included in the study preparation, as there is an increased risk of lung cancer in smokers who take supplements containing beta-carotene.[45,46] The placebo preparation consists of cellulose microcrystalline, lactose, and magnesium stearate, and are indistinguishable from the intervention preparation in size, color, smell, and taste. The study preparation is produced according to Good Medical Practice as laid down in relevant European directives, and currently has a shelf life of 24 months.

All the main ingredients of the study preparation belong to the vitamin/micronutrient group, which are found in a typical diet. Therefore, it is expected that the study preparation should be well tolerated, and no side effects are anticipated when used according to the study protocol, although mild gastro-intestinal irritations may occur in a small number of participants. Allergic reactions against the main ingredients or other excipients of the study preparation may occur.

Safety of lutein and zeaxanthin

Three studies have investigated L toxicity in animal models, and the data from these studies demonstrate that there have been no adverse clinical or histopathological changes suggestive of toxicity, following administration of high doses of L (up to 20 mg L/kg body weight/day for 52 weeks).[47−49] Furthermore, a number of human interventional studies, involving supplementation with high doses of L for extended periods of time, have failed to demonstrate any toxicity in ocular and non-ocular tissues (20 mg of L per day for 6 months).[50−53] The only side effect reported as a result of L supplementation in humans has been carotenedermia, a harmless and reversible cutaneous hyperpigmentation, which is not associated with any specific adverse effect on health.[54−56]

Adverse events

If an adverse event is detected or reported at any stage in the study, an adverse event form is completed within five days of the investigator being made aware of such an event. This form contains information on the event, with particular attention directed towards the date of onset, description of event, severity, possible explanation, date of resolution (if any), relationship to the study preparation, and the action taken with the study preparation. If an adverse event is believed to be attributable to the study preparation (such as an allergic reaction), the participant is advised to discontinue the study preparation, immediately and permanently.

Every attempt is made to classify the event into a severe adverse event or an adverse event, this categorization being consistent with the ICH guidelines (Definitions and Standards for Expedited Reporting, 2003). In the event of death, the medical records, including the details of death certification, are obtained, where available, to allow determination of the cause of death and its relationship, if any, with the study preparation. All adverse events are reported to the health authorities concerned. It is mandatory to report serious adverse events within a timeframe of 7 days of the principal investigator being notified of such an event.

Outcome measures

The primary outcome variable is a change in the DVA of the study eye at 12 months of antioxidant supplementation. The level of significance for a change in DVA was set at 0.05. The secondary outcome measures include retinal visual acuity, morphological progression of AMD, MP levels, and serum levels of antioxidants. Morphological status of AMD is based on detailed grading of stereoscopic color fundus photographs.

The development of CNV or central GA is considered end stage disease, and if reached, will be handled as follows: In group 1, participants will exit the study if CNV or central GA develops in the sole study eye. In group 2, where both eyes are study eyes, the development of CNV or central GA in one eye only is not a reason to exit the study, and the participant remains in the study unless the second eye also develops end stage disease. Participants who develop CNV during the course of study are referred to the principle investigators for appropriate management.

DATA ANALYSIS

In the present study, the main outcome DVA (expressed in units which are the logarithm of the minimum angle of resolution) will be measured as the average change in DVA across the number of eyes recruited to the study. The weights assigned to each participant will be obtained from

the formula suggested in the study protocol, and is expanded below.

Handling of data from Group 2 participants

Several papers mention the lack of statistical independence when dealing with outcomes in paired organs. This is a particular problem in ophthalmology as the outcomes measured in both eyes of an individual are likely to be more closely related than outcomes measured on eyes from different individuals. Earlier reports have examined dichotomous outcomes, and so the solution becomes a particular application of *intra-class correlation*. In this study, the main outcome, DVA, will be treated as continuously distributed. One possible solution is simply to use data from one eye only from participants in whom both eyes fit the entry criteria. This method imposes a strict one eye per participant regime. The disadvantage of this approach is that it does not utilize all readily available data, and is therefore cost inefficient. Collecting all data and treating them as independent outcomes can also be flawed for reasons stated above. A compromise solution is suggested that follows parallel arguments to that for intra-class correlation.[57]

Group 1 participants will only have one study eye (the fellow eye having already been lost to exudative AMD). Let the variance associated with change in DVA in this group be σ_1^2. Group 2 participants may have both eyes eligible for the study, and thus have two components of variance that can be estimated. That between participants will be denoted σ_{2B}^2 and that between eyes within the same participant be σ_{2W}^2. In practice the study will yield some large sample estimators s_1^2, s_{2B}^2 and s_{2W}^2. A reasonable prior expectation might be for homogeneity of variance across all participants, where $\sigma_1^2 = \sigma_{2B}^2 + \sigma_{2W}^2$.

However, the approach suggested above will permit heterogeneity of variance between groups. It is usual, in situations of heterogeneity of variance, to weight cases by the reciprocal of the variation associated with each case. We propose, therefore, that all data be collected and the variance estimates described above calculated. Data will be aggregated to one result per participant—the sole result will stand for group 1 participants, and the mean of the two results will be applied to group 2 participants. The variance associated with group 1 participants will be s_1^2, while that associated with the mean of group 2 participants will be $s_{2B}^2 + s_{2W}^2/2$. Therefore, in the main analysis, group 1 participants will be given a weight of 1, whereas group 2 participants will be given a weight: $w_2 = s_1^2/(s_{2B}^2 + s_{2W}^2/2)$.

We note that if most of the variation lies between participants, then w_2 will tend to be close to 1. Here, the second eye from group 2 participants provides very little additional information. However, if most of the variation lies within participants, then w_2 will tend to be close to 2. In this situation, the second eye from participants in group 2 contributes additional information, and we are moving towards a situation where both eyes of a participant have been entered as independent observations. It is important to note this approach is essentially weighted by reciprocal of the variation associated with each observation, which is among the most common weighting strategies.

Analysis of primary outcome

These data will be analyzed by an independent samples t-test with arm of the study as grouping variable. All tests will be performed as 2-tailed hypotheses, and a p-value of 0.05 will be regarded as statistically significant. For the purposes of analysis, eyes with extremely poor vision i.e., worse than 1.6 logMAR will be allocated a score of 1.7, and we do not intend to differentiate between hand movements and light perception.

Adjustment for covariates

Distance VA, as defined above at 12 months after randomization into the trial, will be regressed on DVA at baseline, with study group to which randomized as main factor, and linear adjustments allowed for variables (variable types) defined below:

- Gender (dichotomous)
- Age (continuous)
- Bilateral involvement (dichotomous)
- Smoking status (categorical)
- Eye color (categorical, but probably dichotomized)
- Use of vitamin supplements and/or diet at baseline (continuously distributed).

ANALYSIS OF SECONDARY OUTCOMES

This will depend on the type of variable. Categorical variables will be analyzed by chi-squared or Fisher's Exact tests (the latter being employed when expected counts drop close to or below 5.0). Continuously distributed variables will be analyzed by independent sample t-tests, after assessment of whether equal or unequal variances can be assumed. Adjustment for covariates will be performed when appropriate, and by logistic regression for dichotomized categorical variables, and linear regression for continuously distributed outcomes. Tests for heterogeneity of variance and parallelism will be employed to check the validity of these models. Transformations will be attempted when the usual assumptions fail.

In this study, it is not expected that many outcomes will be of an ordinal nature. However, if because of failed distributional assumptions, continuously distributed variables have to be transformed to ordinal scales, then the module for ordinal regression will be employed along with tests for parallelism. Although some secondary outcomes such as CS, like distance VA, will be assessed at the level of eye, such outcomes will be combined by the method suggested above, and the analysis will continue with the experimental unit as a single participant and using appropriate statistical methods.

QUALITY CONTROL

Various steps are taken to ensure adequate quality control for the CARMA Study. First, a comprehensive study manual, with detailed and clear instructions for carrying out the study procedures is available. Second, study staff at the two centers work to agreed protocols. Third, both centers are equipped with

identical instruments which are calibrated to agree preset standards. Fourth, the eligibility criteria are verified by an examining ophthalmologist at the screening visit before randomization. Where eligibility is in question, adjudication by both principal investigators is used to determine the ultimate eligibility of the patient. Fifth, centralized data management facilitates scrutiny of the CRF for completeness and consistency, with queries resolved rapidly. Finally, regular monitoring and quality control is undertaken by the coordinating center.

SAFETY AND DATA MONITORING COMMITTEE

An independent DSMC scrutinizes the unblinded data for evidence of safety and efficacy every year. This committee comprises of three internationally recognized scientific and clinical members (two ophthalmologists and one physician epidemiologist), and has the power to recommend any of the following; continuation of the study uninterrupted, alteration of the study or any arm of the study, or termination of the study or any arm of the study.

DISCUSSION AND EXPECTED CONTRIBUTION

The CARMA Study is an important interventional study of antioxidant supplementation in AMD patients. Similar to AREDS II,[58] the CARMA Study is a randomized, double masked, and placebo controlled clinical trial of L and Z along with co-antioxidants in AMD participants with a reasonably good sample size. However, the CARMA supplement does not include omega 3 poly-unsaturated fatty acids, which are increasingly believed to play a protective role in AMD.

Furthermore, and similar to the LAST trial,[30] the CARMA Study is examining the effect of antioxidant supplementation on psychophysical visual function, such as DVA, contrast sensitivity, and retinal visual acuity. However, in the LAST trial, the study population consisted of 90 male participants with advanced AMD (atrophic) and at least one vision-degrading psychophysical abnormality. Whereas, in the CARMA Study, 248 females and 185 males with either early AMD features of sufficient severity in at least one eye or any level of AMD in one eye and advanced AMD in the fellow eye have participated. Also, the OcuPower supplement in the LAST trial consisted of a large number of nutrients in contrast to the CARMA supplement, which contains 5 major antioxidants, including L and Z that constitute MP.

The CARMA Study will provide invaluable and timely data on the potential role of antioxidants, including L and Z, in delaying AMD progression. An observed beneficial effect of such intervention will complement evidence from other completed and ongoing randomized clinical trials, and inform current practice with respect to the role of dietary modification and/or supplementation in patients with AMD. The CARMA Study will also yield valuable information with respect to visual function

and MP levels following supplementation with L, Z, and antioxidants in eyes with AMD. Finally, the CARMA Study will provide data on the safety and toxicity of L, Z, and antioxidant supplementation in human subjects.

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ACKNOWLEDGMENT

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