

Mediterranean Diet Score and its association with Age-related macular degeneration: The European Eye Study (EUREYE).

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Meeting Presentation: Some of this material was accepted for presentation at ARVO 2015 however the first author had to withdraw due to illness.

Financial support: European Commission Vth Framework, Brussels, Belgium

(contract no. QLK6-CT-1999-02094). Additional funding for cameras was provided by the Macular Disease Society, Andover, United Kingdom. Prof Rahu was financed by the Ministry of Education and Science, Tallinn, Estonia (target funding no. 01921112s02). Additional funding in Alicante was received from the Fondo de Investigacion Sanitaria, Madrid, Spain (grant nos. FIS 01/1692E, RCESP C 03/09), and Oficina de Ciencia y Tecnologia Generalitat Valenciana, Valencia, Spain (grant no. CTGCA/2002/06). Thomas Pocklington Trust funded conversion of dietary data to nutrients.

Running title : Mediterranean diet and AMD in the EUREYE study.

Abstract

Purpose: To examine associations between adherence to a Mediterranean diet and prevalence of AMD in countries ranging from Southern to Northern Europe.

Design: Cross-sectional population based epidemiological study.

Participants: Of 5060 randomly sampled people aged 65 or over from 7 study centers across Europe (Norway, Estonia, United Kingdom, France, Italy, Greece, and Spain) full dietary data were available in 4753. The mean age of participants was 73.2 (SD, 5.6) and 55% were women.

Methods: Participants underwent an eye examination and digital retinal color photography. The images were graded at a single center. Dietary intake during the previous 12 months was assessed by using a semi-quantitative food-frequency questionnaire (FFQ). A previously published Mediterranean Diet Score (MDS) was used to classify participants according to their responses on the FFQ. Multivariable logistic regression was used to investigate the association of MDS score and AMD, taking account of potential confounders and the multicentre study design.

Main outcome measures: Photographic images were graded according to the International Classification System for age-related maculopathy and stratified using the Rotterdam staging system into 5 exclusive stages (AMD 0-4) and a separate category of large drusen ($\geq 125 \mu\text{m}$). AMD4 included neovascular AMD (nvAMD) or geographic atrophy (GA).

Results: Increasing MDS was associated with reduced odds of nvAMD in unadjusted and confounder adjusted analysis. Compared with the lowest MDS adherence (≤ 4 score) those in the highest category MDS adherence (> 6 score) showed lower odds of nvAMD, OR=0.53 (0.27-1.04), p-trend=0.01). The association with MDS did not differ by Y204H (p=0.89). For all early AMD (grade 1-3), there was no relationship with MDS, p trend=0.9. There was a weak trend (p=0.1) between MDS and large drusen, those in the highest category of MDS had a 20% reduced odds compared to those in the lowest, p=0.05.

Conclusions: This study adds to the limited evidence of the protective effect of adherence to a Mediterranean dietary pattern and late AMD though does not support previous reports of a relationship with genetic susceptibility. Interventions to encourage the adoption of the Mediterranean diet should be developed and methods by which such behaviour change can be achieved and maintained investigated.

Introduction

Age-related macular degeneration (AMD) is the predominant cause of blindness in high income countries¹. It is of growing importance in other settings, in association with increasing longevity.² AMD is considered to be a complex multi-factorial disorder, involving an interplay between genetic, environmental and lifestyle factors, such as smoking,³ obesity,⁴ cardiovascular disease,⁵ macular pigment,⁶ sunlight exposure,⁷ diet,⁸ high BMI⁹ and physical activity¹⁰.

It has been known for some time that dietary factors can modulate AMD risk^{11 12}. Epidemiological studies have demonstrated that diets high in antioxidant nutrients (vitamins C and E, carotenoids such as lutein and zeaxanthin and fruit and vegetables rich in these nutrients) or zinc are associated with a decreased occurrence of AMD. Studies have also shown that a high dietary intake of trans fats is a risk factor for late AMD,¹³ while a higher intake of fish or ω -3 fatty acids is protective against AMD¹³. However the evidence from clinical trials is less consistent. While high dose multivitamin supplementation slowed the progression of AMD,¹⁴ a trial which added lutein and zeaxanthin supplements with or without omega-3 fatty acids to the AREDS supplements showed no substantial added beneficial effect of the AREDS supplements on AMD progression (AREDS2).¹⁵ However, in the pre-specified analyses of those persons with the lowest dietary intake of lutein, there was a beneficial effect of lutein for retarding the progression to late AMD, especially the neovascular form.¹⁶ Most studies to date have focused on individual food groups or nutrients, yet it is known that diet is a multifactorial lifestyle behaviour, with particular foods frequently consumed together, depending on the cultural, geographical and economic context of the individual. Therefore researchers are increasingly attempting to analyse

relationships between dietary patterns or overall diet and disease, rather than specific foods or nutrients^{17 18 20}. To date these analyses have been undertaken in populations living in defined geographical locations such as Melbourne, Australia,¹⁷ US CAREDS participants¹⁹ or in US AREDS participants^{18 20} consequently, there is a greater likelihood that the dietary patterns of the individuals in these studies was similar. This was particularly evident in the application of a Mediterranean diet score to CAREDS participants¹⁹ in that high scores were uncommon in this sample (0.04%). As dietary patterns vary by culture, and availability of local food is subject to strong regional influences, a lack of heterogeneity of populations in the prior studies may have reduced the power to detect associations with AMD outcomes. By contrast the European Eye (EUREYE) Study enrolled participants from 7 countries across Europe with widely differing cultures and dietary patterns, thus providing an interesting context in which to investigate links between diet and AMD. Notably the dietary questionnaire was modified to reflect locally available foods. For these reasons we aimed to examine the association between adherence to a Mediterranean diet and prevalence of AMD in countries ranging from Southern to Northern Europe.

Methods

Study Population

We recruited participants between 2001 and 2002 from seven European countries (Bergen, Norway; Tallinn, Estonia, Belfast, UK; Paris, France; Verona, Italy; Thessaloniki, Greece; and Alicante, Spain) using random sampling of the population aged over 65 years. Written informed consent was obtained from all study participants. Ethical approval was obtained for each country from the relevant ethics

committee and the study adhered to the Declaration of Helsinki on research into human volunteers.

The study design and methodology has been published elsewhere ^{7 21-26}. Participants attended the examination center, where they were first interviewed by trained fieldworkers, underwent an ophthalmological examination and gave a blood sample. Information collected by the interviewers included quantified smoking and alcohol use and a brief medical history, detailed questionnaire on outdoor exposure throughout working life and in retirement, and a dietary assessment (described in detail below).

Dietary Assessment Methodology

During the interview, dietary intake during the previous 12 months was assessed by using a semi-quantitative food-frequency questionnaire (FFQ). We used the UK European Prospective Investigation into Cancer and Nutrition (EPIC) Study FFQ²⁷, which was derived from the original FFQ devised by Willett²⁸. For each non-United Kingdom country in the study, we modified the FFQ for food items that were redundant or relevant using the EPIC country-specific questionnaires to identify additional food items or the local variety of a food item. In Estonia, where there was no equivalent EPIC questionnaire, we devised the FFQ after consultation with local nutrition researchers. Validation studies have been carried out on country-specific EPIC questionnaires, e.g., for the United Kingdom²⁷. In addition, among the Spanish participants, we explored the relation between energy-adjusted dietary carotenoid and vitamin C intakes and serum concentration. Significant Pearson correlations were observed for alpha-carotene 0.21, β -carotene 0.19, lycopene 0.18, β -cryptoxanthin 0.20 and vitamin C 0.36, which support an acceptable performance of the dietary intakes estimated from the FFQ.²⁹ Information on habitual consumption

of foods during the previous year included portion size and was recorded in 9 different frequency categories, from never or less than once a month to 6 times/day or more. Intake of food items was recorded into portions per day. Nutrient intake was estimated using the food-composition tables of McCance and Widdowson³⁰ and EPA and DHA intake using US Department of Agriculture tables³¹, because EPA and DHA were not available in the table of McCance and Widdowson³⁰. For each study participant, the nutrient intake was calculated by multiplying the intake frequency for each food item by the nutrient content for the portion size. Dietary intakes were adjusted by energy using the residual method.

Assessing adherence to Mediterranean Diet Score (MDS)

We used a previously published MDS to classify participants according to their responses on the FFQ.³² The composite score (range 0-9) captures consumption of key food items, such as olive oil (1 point for ≥ 1 spoon/day), wine (1 point for ≥ 1 glass/day), fruit (1 point for ≥ 1 serving/day), vegetables or salad (1 point for ≥ 1 serving/day), fish (1 point for ≥ 3 servings/week), legumes (1 point for ≥ 2 servings/week) and low consumption of meat or meat products (1 point for < 1 serving/day). A further point was awarded for a daily serving or more of both fruits and vegetables, and a final point was awarded when either consumption of both white bread (< 1 serving/day) and rice (< 1 serving/week) was low or when consumption of whole-grain bread was high (> 5 servings/week).

Assessment of age-related macular degeneration.

After pupillary dilation with tropicamide 0.5% and phenylephrine 5%, two 35° non-simultaneous stereoscopic digitized color fundus images were obtained of each eye, centered on the fovea.

The fundus images were sent to a single reading centre (Erasmus University Rotterdam) and graded using the International Classification System for Age-Related Maculopathy³³ and then categorised into five mutually exclusive grades. Grade 0 was defined as a macula free of drusen or pigmentary irregularities or with hard drusen (<63 µm) only. Early AMD was subdivided in grade 1, defined as soft distinct drusen (≥63 µm) or pigmentary abnormalities; grade 2 was defined as soft indistinct drusen (≥125 µm) or reticular drusen only or soft distinct drusen (≥63 µm) with pigmentary abnormalities; and grade 3 was defined as soft indistinct drusen (≥125 µm) or reticular drusen with pigmentary abnormalities. Grade 4 was defined as presence of either neovascular (nvAMD) [presence of any of the following: serous or hemorrhagic retinal or retinal pigment epithelial detachment, subretinal neovascular membrane, periretinal fibrous scar] or geographic atrophy (GA) [well-demarcated area of retinal pigment atrophy with visible choroidal vessels]). This grading system had been validated within the Rotterdam Eye Study.³⁴ Large drusen (≥125 µm) in any grade of early AMD were additionally categorised as a separate outcome.

Blood Samples

A non-fasting blood sample was collected. Participants were advised to consume a standard breakfast before attending the clinic, e.g. not eating fruit and fruit juice prior to coming into the centre. Samples were anti-coagulated with EDTA or were allowed to clot. Samples were kept at room temperature for at least one hour (serum) or kept

at 4°C and centrifuged as soon as possible (plasma), but all samples were centrifuged a maximum of 2 hours after collection. Serum or plasma was aliquoted as required for biochemical analysis. A plasma sample was precipitated by adding 100 µl of plasma to 900 µl of 5% metaphosphoric acid for vitamin C analysis, and all samples were stored at either -20°C or less than -70°C in the short term (depending on what was locally available) and shipped at regular intervals to the Belfast centre (the biochemical analysis centre) where they were stored at -80°C. DNA was extracted from the stored blood sample and genotyped for Y204H (rs1061170). Plasma ascorbic acid concentrations were determined according to Vuillemier and Keck³⁵. Serum concentrations of retinol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene were measured by reverse phase high performance liquid chromatography (HPLC)³⁶. Assays were standardised against appropriate National Institute of Standards and Technology reference materials.

Statistical analysis

Statistical analysis was carried out using Stata version 13 (Stata Corp, College Station, Texas). We categorised the MDS score into 4 groups (MDS of ≤4; 5; 6; >6) as this provided a more even spread across groups. We used univariable regression in people without signs of AMD to investigate potential confounders expected to be associated with MDS and AMD, either negatively or positively: demographic (age, sex and education) lifestyle (smoking, alcohol, body mass index, and supplement use), comorbidity (diabetes, cardiovascular disease). We also investigated the relationship between MDS and dietary and serum biomarkers of nutritional status related to food groups which predominate within the MDS. Blood analyses were

seasonally adjusted and dietary nutrients were energy adjusted. We undertook multivariable logistic regression to investigate the association of MDS score with AMD and with large drusen. We investigated whether rs1061170 modified any association with AMD and diet score as has been previously reported.²⁰ All analyses took account of the study design of the seven study centers by use of survey estimators or robust errors.

Results

Of 5040 EUREYE participants, full dietary data was available in 4753 participants (109 nvAMD, 49 with GA, 2333 early AMD, 641 with large drusen and 2262 without signs of AMD). The mean age of participants was 73.2 (SD, 5.6) and 55% were women.

MDS and participant characteristics

In univariable analysis in people with no signs of AMD, significant trends were observed for MDS score with sex, education, and alcohol intake, but no association with age (Table 1). There were decreasing proportions of women in higher MDS categories, and conversely increasing proportions of those with higher education levels and weekly or more alcohol consumption. Smoking and being overweight or obese, history of diabetes or cardiovascular disease were not linearly associated with MDS category. There were highly significant differences in the MDS score across the participating EUREYE countries with the southern European countries having higher MDS compared to those in the Northern regions. Mean (SD) values for the individual countries were as follows: Norway (Bergen) 4.89 (1.39), Estonia (Tallinn) 4.49 (1.11), UK (Belfast) 4.42 (1.30), France (Paris) 4.62 (1.50), Italy

(Verona) 5.37 (1.18), Greece (Thessaloniki) 5.19 (1.09), Spain (Alicante) 5.32 (1.39), $p < 0.0001$. Figure 1 illustrates the proportion of participants in each country reporting a particular item of the MDS score. Age-standardised mean (95% CI) MDS scores by AMD grade showed a weak trend from no AMD to late AMD. AMD 0 5.03 (4.6-5.5), AMD 1 5.02 (4.6-5.4), AMD 2 5.08, 4.7-5.5) AMD 3 4.97 (4.5-5.5), AMD 4 4.76 (4.3-5.2), p for trend = 0.16.

MDS and serum levels of antioxidants

Table 1 also shows the relationship between MDS and serum antioxidant levels. The MDS diet score correlated well with serum levels of vitamin C, lutein, zeaxanthin and beta-cryptoxanthin, all biomarkers of fruit and vegetable intake. MDS was also negatively associated with saturated fatty acid and n-6 PUFA intake and positively associated with n-3 PUFA intake and dietary lutein plus zeaxanthin.

MDS and late AMD (nvAMD and GA)

Increasing MDS was significantly associated with reduced odds of nvAMD (Table 2), in both the unadjusted analysis and also when adjusted for potential confounders such as age, sex, country, education, smoking, drinking, self-reported history of CVD, aspirin consumption and diabetes. A score of ≥ 6 was associated with an approximately 50% reduction in the adjusted odds ratio of developing nvAMD compared to those with a score of 4 or less.

Preliminary analyses showed no association with GA and we did not run further analyses on GA with full data on diet score and confounders.

MDS and early AMD (grade 1-3)

For all early AMD participants (grades 1-3), there was no significant relationship with MDS (Table 3). There was a weak trend ($p=0.1$) between MDS and large drusen, those in the highest category of MDS had a significant 20% reduced odds compared to those in the lowest, $p=0.05$. Addition of plasma or dietary lutein and zeaxanthin did not affect the results for early AMD or large drusen.

We also examined whether the results for the diet score varied according to Y204H (rs1061170) genotype. We found no significant interactions between rs1061170 and the full diet score in the association with nvAMD ($p=0.89$), early AMD ($p=0.28$) or large drusen, $p=0.99$. We further explored whether the association with diet score was explained by the key macular carotenoids lutein and zeaxanthin. Addition of either dietary or plasma lutein and zeaxanthin made no material difference to the results for the diet score for any outcome.

Discussion

The current analysis has exploited the large regional differences in food consumption found in the EUREYE study to examine the relationship between diet and AMD in Europe. We have shown that increased adherence to a Mediterranean diet was associated with significantly reduced odds of having nvAMD. MDS has previously been inversely associated with cardiovascular disease, cancer and mortality³⁷⁻³⁹ and more recently progression to advanced AMD²⁰. Our current findings provide further evidence for the potential value of adhering to this diet on eye health.

We did not find a significant association between early AMD and MDS. However the classification of early AMD was based on color image grading which may not detect some features of early AMD. Many of the individual constituents of the MDS have previously been identified as associated with reduced prevalence of incidence of AMD, such as higher fruit and vegetable⁴⁰, reduced red meat⁴¹, lower glycaemic index⁴² and higher fish intake²³. It is also interesting that carotenoids such as lutein and zeaxanthin, which have well-established associations with AMD, but are also established biomarkers of fruit and vegetable intake, were significantly associated with MDS in those without signs of AMD. However the association we observed between MDS and neovascular AMD was not explained by lutein and zeaxanthin. This is not surprising because the MDS score is based on hypothesised benefits of food patterns rather than single nutrients and therefore includes other important nutrients relevant to late AMD.

Dietary patterns rather than individual components are increasingly being studied in relation to AMD. Various methodological approaches have been taken but tend to fall into two broad categories. The first are *a posteriori* approaches, including the use of factor and principal component analysis to identify patterns which are then related to the disease under investigation.^{17 18} This is a data driven approach, and requires no *a priori* hypothesis of what factors or food groups within the overall diet may be important. The second method uses *a priori* scores to assess an individual's adherence to a specific diet such as the Mediterranean diet²⁰ or a set of dietary recommendations^{41 43}, and therefore requires an *a priori* hypothesis of what may be significant. In this analysis, we have used the latter approach.

Several studies have evaluated dietary patterns and AMD. In the Melbourne Collaborative Cohort Study principal components analysis revealed 6 distinct dietary factors, the factor characterized by high fruit and nut intake demonstrated a similar risk reduction for advanced AMD to the current study (OR 0.45; 95% confidence interval, 0.28-0.87).¹⁷ Conversely a diet characterised by high cakes, sweet biscuits and desserts was associated with a higher prevalence of advanced AMD. No significant associations were reported between early AMD and dietary factors.

Evaluation of dietary data from the baseline Age-related eye disease study (AREDS) identified two major components which the investigators named Oriental and Western patterns²⁴. The oriental pattern was associated with significantly reduced odds of both early and advanced AMD while the western patterns was associated with significantly increased odds of both early and advanced AMD. While the associations were noted for both early and advanced AMD, the strength of the associations were much greater for advanced AMD. A score based approach has been applied within the Carotenoids in Age-related Eye Disease Study (CAREDS). Responses to a food frequency questionnaire were converted to a modified 2005 Healthy Eating Index and those in the highest quintile compared to the lowest quintile of the index had 46% lower odds of developing early AMD¹⁹. Most recently, in an analysis of the association of adherence to the Mediterranean diet and genetic susceptibility with progression to advanced AMD, higher adherence to the Mediterranean Diet was associated with reduced risk of progression to advanced AMD; the association was only seen in subjects carrying the carrying none or one CFH Y402H risk alleles and not observed in those at the highest risk with two alleles²⁰. In our analysis we did not find that the association of diet score with AMD was modified by Y204H alleles. The small number of cases with nvAMD meant we

lacked power to investigate significant gene/diet interactions (as found in that paper), but had adequate numbers for analyses in early AMD.

One of the strengths of our study in a European older population was that it was carried out prior to the widespread usage of supplements for AMD and also prior to public awareness of the relationship between dietary factors and AMD, thereby reducing the potential of recall bias by those with a diagnosis of AMD. In addition the grading of the fundus photographs was undertaken by independent graders with no knowledge of any characteristics of the participants including the nutrition data. Comparison of the MDS score with serum antioxidants also provides us with a helpful insight into what constituents of the diet that the MDS is reflecting (Table 1). It also provides a degree of construct validity to the MDS score.

This is a cross-sectional study therefore no causal inferences can be made regarding the associations noted. This study was also carried out prior to the routine use of Optical Coherence Tomography in characterising AMD features, therefore some of the early features of AMD may have been misclassified and important phenotypes such as reticular pseudo drusen under-ascertained through the sole use of colour fundus photographs. Dietary intake, including calculation of MDS, was based on a self-reported food frequency questionnaire. Such a questionnaire, although commonly used in nutritional epidemiology, can be prone to bias, including over-reporting of healthy foods, such as those contained within the MDS.

In conclusion, this study provides further evidence of the relationship between Mediterranean diet and prevalence of AMD. The MDS provides a useful method to

characterise dietary patterns across geographically and culturally diverse populations. Interventions to encourage the adoption of the Mediterranean diet more widely should be developed. While methods by which such behaviour change can be achieved and maintained over the long term should be investigated.

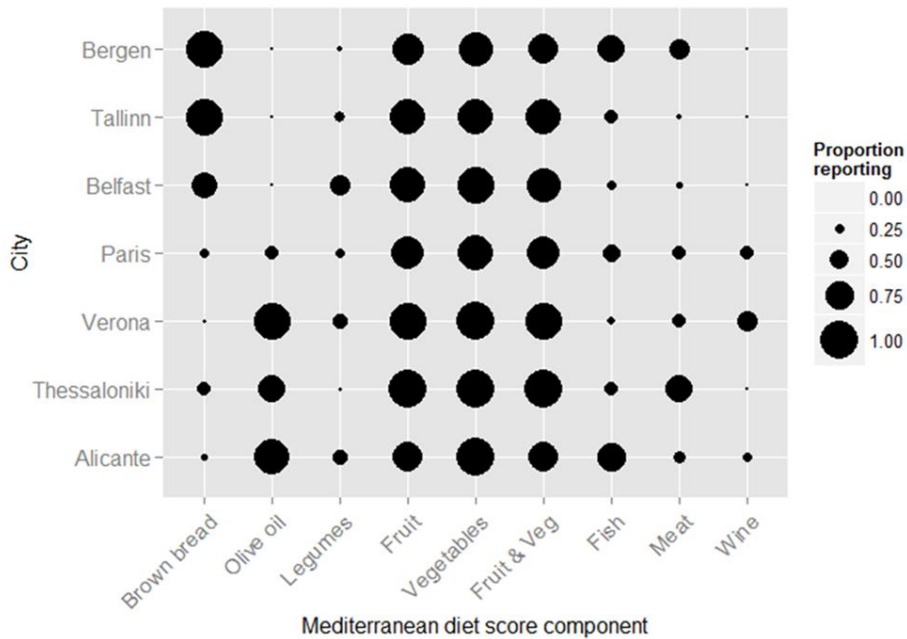
References

1. Evans JR, Fletcher AE, Wormald RP. Age-related macular degeneration causing visual impairment in people 75 years or older in Britain: an add-on study to the Medical Research Council Trial of Assessment and Management of Older People in the Community. *Ophthalmology* 2004;**111**(3):513-7.
2. 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. *Lancet Glob Health* 2014;**2**(2):e106-16.
3. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology* 2001;**108**(4):697-704.
4. Clemons TE, Milton RC, Klein R, et al. 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**(4):533-9.
5. Hogg RE, Woodside JV, Gilchrist SE, et al. Cardiovascular disease and hypertension are strong risk factors for choroidal neovascularization. *Ophthalmology* 2008;**115**(6):1046-52 e2.
6. Beatty S, Murray IJ, Henson DB, et al. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci* 2001;**42**(2):439-46.
7. Fletcher AE, Bentham GC, Agnew M, et al. Sunlight exposure, antioxidants, and age-related macular degeneration. *Arch Ophthalmol* 2008;**126**(10):1396-403.
8. VandenLangenberg GM, Mares-Perlman JA, Klein R, et al. Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol* 1998;**148**(2):204-14.
9. Seddon JM, Cote J, Davis N, et al. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. *Arch Ophthalmol* 2003;**121**(6):785-92.
10. Knudtson MD, Klein R, Klein BE. Physical activity and the 15-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Br J Ophthalmol* 2006;**90**(12):1461-3.
11. Broadhead GK, Grigg JR, Chang AA, et al. Dietary modification and supplementation for the treatment of age-related macular degeneration. *Nutr Rev* 2015;**73**(7):448-62.
12. Evans JR, Lawrenson JG. A review of the evidence for dietary interventions in preventing or slowing the progression of age-related macular degeneration. *Ophthalmic Physiol Opt* 2014;**34**(4):390-6.
13. Chong EW, Robman LD, Simpson JA, et al. Fat consumption and its association with age-related macular degeneration. *Arch Ophthalmol* 2009;**127**(5):674-80.
14. 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**(10):1417-36.
15. Age-Related Eye Disease Study 2 Research G. 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**(19):2005-15.
16. Age-Related Eye Disease Study 2 Research G, Chew EY, Clemons TE, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA ophthalmology* 2014;**132**(2):142-9.
17. Islam FMA, Chong EW, Hodge AM, et al. Dietary Patterns and Their Associations with Age-Related Macular Degeneration. *Ophthalmology* 2014;**121**(7):1428-U159.
18. Chiu CJ, Chang ML, Zhang FF, et al. The Relationship of Major American Dietary Patterns to Age-Related Macular Degeneration. *American Journal of Ophthalmology* 2014;**158**(1):118-27.
19. Mares JA, Voland RP, Sondel SA, et al. Healthy lifestyles related to subsequent prevalence of age-related macular degeneration. *Arch Ophthalmol* 2011;**129**(4):470-80.

20. Merle BM, Silver RE, Rosner B, et al. Adherence to a Mediterranean diet, genetic susceptibility, and progression to advanced macular degeneration: a prospective cohort study. *Am J Clin Nutr* 2015;**102**(5):1196-206.
21. Seland JH, Vingerling JR, Augood CA, et al. Visual impairment and quality of life in the older European population, the EUREYE study. *Acta Ophthalmol* 2011;**89**(7):608-13.
22. Topouzis F, Anastasopoulos E, Augood C, et al. Association of diabetes with age-related macular degeneration in the EUREYE study. *Br J Ophthalmol* 2009;**93**(8):1037-41.
23. Augood C, Chakravarthy U, Young I, et al. Oily fish consumption, dietary docosahexaenoic acid and eicosapentaenoic acid intakes, and associations with neovascular age-related macular degeneration. *Am J Clin Nutr* 2008;**88**(2):398-406.
24. Chakravarthy U, Augood C, Bentham GC, et al. Cigarette smoking and age-related macular degeneration in the EUREYE Study. *Ophthalmology* 2007;**114**(6):1157-63.
25. 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**(4):529-35.
26. Augood C, Fletcher A, Bentham G, et al. Methods for a population-based study of the prevalence of and risk factors for age-related maculopathy and macular degeneration in elderly European populations: the EUREYE study. *Ophthalmic Epidemiol* 2004;**11**(2):117-29.
27. Bingham SA, Welch AA, McTaggart A, et al. Nutritional methods in the European prospective investigation of cancer in Norfolk. *Public Health Nutr* 2001;**4**(3):847-58.
28. Willett W. *Nutritional epidemiology*. New York: Oxford University Press, 1990.
29. Vioque J, Weinbrenner T, Asensio L, et al. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Brit J Nutr* 2007;**97**(5):977-86.
30. Holland B, McCance RA, Widdowson EM. *McCance and Widdowson's the composition of foods*. 5th rev. and extended ed. / by B. Holland ... [et al.] ed: Cambridge : Royal Society of Chemistry, 1991-, 1991.
31. US Department of Agriculture ARS. *USDA National Nutrient Database for Standard Reference*. Washington, DC: USDA, 2005.
32. Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, et al. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *Eur J Clin Nutr* 2004;**58**(11):1550-2.
33. 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**(5):367-74.
34. van Leeuwen R, Klaver CC, Vingerling JR, et al. The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. *Arch Ophthalmol* 2003;**121**(4):519-26.
35. Vuilleumier JP, Keck E. Fluorometric Assay of Vitamin-C in Biological-Materials Using a Centrifugal Analyzer with Fluorescence Attachment. *J Micronutr Anal* 1989;**5**(1):25-34.
36. Craft NE, Wise SA, Soares JH. Optimization of an Isocratic High-Performance Liquid-Chromatographic Separation of Carotenoids. *J Chromatogr* 1992;**589**(1-2):171-76.
37. Lasheras C, Fernandez S, Patterson AM. Mediterranean diet and age with respect to overall survival in institutionalized, nonsmoking elderly people. *Am J Clin Nutr* 2000;**71**(4):987-92.
38. Schwingshackl L, Hoffmann G. Adherence to Mediterranean diet and risk of cancer: a systematic review and meta-analysis of observational studies. *Int J Cancer* 2014;**135**(8):1884-97.
39. Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;**368**(14):1279-90.
40. Cho E, Seddon JM, Rosner B, et al. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch Ophthalmol* 2004;**122**(6):883-92.
41. Chong EW, Simpson JA, Robman LD, et al. Red meat and chicken consumption and its association with age-related macular degeneration. *Am J Epidemiol* 2009;**169**(7):867-76.

42. Kaushik S, Wang JJ, Flood V, et al. Dietary glycemic index and the risk of age-related macular degeneration. *American Journal of Clinical Nutrition* 2008;**88**(4):1104-10.
43. Kaushik S, Wang JJ, Flood V, et al. Dietary glycemic index and the risk of age-related macular degeneration. *Am J Clin Nutr* 2008;**88**(4):1104-10.

Figure 1. Proportion of participants in each country within the EUREYE study reporting a particular item of the MDS.



The MDS score ranges from 0-9. The 9 points can be awarded according to consumption of the following dietary items: Olive oil (1 point for ≥ 1 spoon/day); wine (1 point for ≥ 1 glass/day); fruit (1 point for ≥ 1 serving/day); vegetables or salad (1 point for ≥ 1 serving/day); fish (1 point for ≥ 3 servings/week); legumes (1 point for ≥ 2 servings/week); meat or meat products (1 point for < 1 serving/day). A further point was awarded if a participants when ≥ 1 serving/day of both fruits and vegetables was consumed, and a final point was awarded for wholegrain consumption [when either consumption of both white bread (< 1 serving/day) and rice (< 1 serving/week) was low or when consumption of whole-grain bread was high (> 5 servings/week)].

Table 1 Characteristics by category of diet score in 2262 people with no signs of AMD

Characteristics	Mediterranean Diet Score				p trend*
	≤4 (n=787)	5 (n=749)	6 (n=527)	>6 (n=199)	
Age ¹	72.6 (5.5)	72.6 (5.3)	72.6 (5.3)	72.4 (5.2)	0.64
Women ²	58.3 (459)	56.3 (422)	50.1 (264)	44.7 (89)	0.10
Education (lowest tertile of years) ²	40.6 (317)	47.2 (352)	47.2 (248)	61.6 (122)	0.02
Ever smoker ²	48.3 (380)	44.2 (331)	49.1 (258)	58.3 (116)	0.05
Alcohol at least weekly ²	31.9 (249)	38.4 (287)	46.8 (246)	67.3 (134)	0.01
Overweight & Obese ²	73.5 (555)	83.2 (612)	81.1 (612)	81.1 (159)	0.01
Diabetes ²	11.6 (90)	13.8 (103)	14.3 (75)	12.1 (24)	0.48
Cardiovascular Disease ²	11.9 (101)	12.6 (97)	16.4 (85)	11.6 (24)	0.16
Aspirin use	36.9 (283)	39.2 (284)	41.9 (209)	43.4 (81)	0.27
Supplement use ²	33.0 (228)	30.2 (225)	35.5 (186)	26.9 (53)	0.44
<i>Blood antioxidants</i> <i>μmol/L</i>					
Vitamin C ¹	41.8 (26.4)	46.2 (24.1)	44.3 (21.4)	46.5 (22.3)	0.05
Retinol ¹	2.23 (0.80)	2.24 (0.77)	2.21 (0.77)	2.26 (0.75)	0.46
Lutein ¹	0.15 (0.15)	0.17 (0.17)	0.19 (0.18)	0.22 (0.20)	0.02
Zeaxanthin ¹	0.03 (0.03)	0.04 (0.03)	0.04 (0.03)	0.05 (0.03)	0.01
Lycopene ¹	0.74 (0.78)	0.73 (0.79)	0.84 (0.82)	0.92 (0.93)	0.02
Alpha –carotene ¹	0.10 (0.11)	0.097 (0.10)	0.11 (0.11)	0.11 (0.10)	0.16
Beta-carotene ¹	0.36 (0.36)	0.36 (0.35)	0.41 (0.39)	0.42 (0.39)	0.06
Beta-cryptoxanthin ¹	0.08 (0.10)	0.10 (0.11)	0.11 (0.13)	0.13 (0.14)	0.01
Alpha- tocopherol ¹	29.8 (7.16)	30.12 (7.03)	30.0 (7.0)	31.2 (7.10)	0.16
Gamma- tocopherol ¹	2.65 (1.36)	2.64 (1.36)	2.61 (1.39)	2.36 (1.12)	0.47
Total carotenoids ^{1,3} μmol/L	1.37 (1.23)	1.34 (1.16)	1.52 (1.29)	1.73 (1.53)	0.01
<i>Dietary Variables</i> <i>g/day</i>					
Saturated FAs	26.6 (6.5)	24.3 (6.2)	22.5 (6.4)	21.2 (5.7)	<0.0001
Total N-3PUFAs ¹	1.72 (0.45)	1.96 (0.57)	2.09 (0.60)	2.19 (0.63)	<0.001
Total N-6PUFAs ¹	9.14 (2.37)	8.61 (2.43)	8.36 (2.30)	8.11 (2.22)	0.01
Lutein/ zeaxanthin ⁴	1646 (996,3065)	2491 (1447,4513)	3043 (1821,5370)	4303 (2386,6989)	<0.0001

¹ Mean (SD)

² % (n)

³sum of lutein, zeaxanthin, lycopene, alpha carotene, beta carotene, beta cryptoxanthin

⁴ median energy adjusted dietary lutein/ zeaxanthin (interquartile range)

* Univariate analysis

⁵ p for between category differences in 2058 people with genotyping results for rs1061170

Table 2 Association of Mediterranean Diet Score (MDS) and nvAMD

	nvAMD	
	Unadjusted	Adjusted ¹
MDS score	OR ² (95% CI)	OR ² (95% CI)
≤4 (n=808)	1 (reference)	1 (reference)
5 (n=774)	0.88 (0.55-1.39)	0.83 (0.55-1.26)
6 (n=536)	0.62 (0.33– 1.16)	0.62 (0.39-1.00)
>6 (n=201)	0.52 (0.29 – 0.93)	0.53(0.27–1.04)
p trend	0.03	0.01

¹adjusted for age, sex, country, education, smoking, drinking, self-reported history of CVD, aspirin consumption, diabetes

² OR, Odds ratio. 95% CI, 95% Confidence Interval

Table 3. Association between Mediterranean Diet Score (MDS) and (i) early AMD (grade 1-3) (ii) large drusen ($\geq 125 \mu\text{m}$)

MDS score	All early AMD (grade 1-3)	
	Unadjusted OR (95% CI) ²	Adjusted ¹ OR (95% CI) ²
≤ 4 (n=1506)	1 (reference)	1 (reference)
5 (n=1481)	0.99 (0.92-1.07)	1.01 (0.91-1.12)
6 (n=1021)	0.98 (0.89– 1.08)	1.01 (0.90-1.14)
>6 (n=380)	0.94 (0.85 – 1.03)	0.96(0.83– 1.11)
p trend	0.4	0.9

MDS score	Large drusen	
	Unadjusted OR (95% CI) ²	Adjusted ¹ OR (95% CI) ²
≤ 4 (n=958)	1 (reference)	1 (reference)
5 (n=936)	0.96 (0.83-1.11)	0.99 (0.80-1.21)
6 (n=638)	0.89 (0.70-1.12)	0.90 (0.69-1.17)
≥ 6 (n=238)	0.79 (0.65-0.97)	0.80 (0.65-0.98)
p trend	0.05	0.1

¹ adjusted for age, sex, country, education, smoking, drinking, self-reported history of CVD, aspirin consumption, diabetes and body mass index

² OR, Odds ratio. 95% CI, 95% Confidence Interval