Mediterranean Diet Score and Its Association with Age-Related Macular Degeneration

The European Eye Study

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Purpose: To examine associations between adherence to a Mediterranean diet and prevalence of age-related macular degeneration (AMD) in countries ranging from Southern to Northern Europe.

Design: Cross-sectional, population-based epidemiologic study.

Participants: Of 5060 randomly sampled people aged 65 years or older 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 years (standard deviation, 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 semiquantitative 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 the MDS score and AMD, taking account of potential confounders and the multicenter study design.

Main Outcome Measures: 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 (>125 μm). Age-related macular degeneration 4 included neovascular AMD (nvAMD) and 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 (odds ratio, 0.53; 0.27–1.04; P trend = 0.01). The association with MDS did not differ by Y204H risk allele (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 20% reduced odds compared with 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 in those with late AMD, although it 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 behavior change can be achieved and maintained investigated. Ophthalmology 2016; ■:1–8 © 2016 by the American Academy of Ophthalmology

Age-related macular degeneration (AMD) is the predominant cause of blindness in high-income countries.1 It is of growing importance in other settings, in association with increasing longevity.2 Age-related macular degeneration is considered to be a complex multifactorial disorder, involving an interplay among genetic, environmental, and lifestyle factors, such as smoking,3 obesity,4 cardiovascular disease,5 macular pigment,6 sunlight exposure,7 diet,8 high body mass index,9 and physical activity.10

It has been known for some time that dietary factors can modulate AMD risk.11,12 Epidemiologic 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 also have shown that a high dietary intake of trans fats is a risk factor for late AMD,13 whereas a higher intake of fish or omega-3 fatty acids is protective against AMD.14 However, the evidence from clinical trials is less consistent. Although high-dose multivitamin supplementation slowed the progression of AMD,15 a trial that added lutein and zeaxanthin supplements with or without omega-3 fatty acids to the Age-Related Eye Disease Study (AREDS) supplements showed no substantial added beneficial effect of the AREDS supplements on AMD progression (AREDS2).16 However, in the prespecified analyses of those subjects with the lowest dietary intake of lutein, there was a beneficial effect of lutein for retarding the progression to late

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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 behavior, with particular foods frequently consumed together, depending on the cultural, geographic, and economic context of the individual. Therefore, researchers are increasingly attempting to analyze relationships between dietary patterns or overall diet and disease, rather than specific foods or nutrients.17–19 To date, these analyses have been undertaken in populations living in defined geographic locations, such as Melbourne, Australia,17 and in US Carotenoids in Age-Related Eye Disease Study (CAREDS) participants20 or US AREDS participants.18,19 Consequently, there is a greater likelihood that the dietary patterns of the individuals in these studies were similar. This was particularly evident in the application of a Mediterranean Diet Score (MDS) to CAREDS participants20 in that high scores were uncommon in this sample (0.04%). Because 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. Of note, 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 7 European countries (Bergen, Norway; Tallinn, Estonia; Belfast, UK; Paris, France; Verona, Italy; Thessaloniki, Greece; and Alicante, Spain) using random sampling of the population older than 65 years of age. 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 have been published.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 next).

Dietary Assessment Methodology

During the interview, dietary intake during the previous 12 months was assessed by using a semiquantitative food-frequency questionnaire (FFQ). We used the UK European Prospective Investigation into Cancer and Nutrition (EPIC) Study FFQ, which was derived from the original FFQ devised by Willett.28 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, for example, for the United Kingdom.27 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, beta-carotene 0.19, lycopene 0.18, beta-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 Holland et al,25 and eicosapentaenoic acid and docosahexaenoic acid intake were estimated using US Department of Agriculture tables,31 because eicosapentaenoic acid and docosahexaenoic acid were not available in the tables of Holland et al.3 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

We used a previously published MDS to classify participants according to their responses on the FFQ.30 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 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° nonsimultaneous stereoscopic digitized color fundus images were obtained of each eye, centered on the fovea. The fundus images were sent to a single reading center (Erasmus University Rotterdam) and graded using the International Classification System for Age-Related Maculopathy33 and then categorized into 5 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 as follows: Grade 1 was 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 the presence of neovascular age-related macular degeneration (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 was validated within the Rotterdam Eye Study. Large drusen (≥125 μm) in any grade of early AMD also were categorized as a separate outcome.

Blood Samples

A nonfasting blood sample was collected. Participants were advised to consume a standard breakfast before attending the clinic, for example, not eating fruit and fruit juice before coming to the center. Samples were anticoagulated with ethylenediamine tetraacetic acid or allowed to clot. Samples were kept at room temperature for at least 1 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 −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 center (the biochemical analysis center), 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 Vuilleumier et al. Serum concentrations of retinol, -carotene, zeaxanthin, -carotene, β-carotene, and lycopene were measured by reverse-phase high-performance liquid chromatography. Assays were standardized against appropriate National Institute of Standards and Technology reference materials.

Statistical Analysis

Statistical analysis was carried out using Stata version 13 (StataCorp LP, College Station, TX). We categorized the MDS into 4 groups (MDS of ≤4, 5, 6, and >6) because 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); and comorbidity (diabetes and cardiovascular disease). We also investigated the relationship between MDS and dietary and serum biomarkers of nutritional status related to food groups that 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 the MDS with AMD and large drusen. We investigated whether rs1061170 modified any association with AMD and diet score, as has been reported. All analyses took account of the study design of the 7 study centers by use of survey estimators or robust errors.

Results

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

Mediterranean Diet Score and Participant Characteristics

In univariable analysis in people with no signs of AMD, significant trends were observed for the MDS 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, and having a history of diabetes or cardiovascular disease were not linearly associated with MDS category. There were highly significant differences in the MDS across the participating EUREYE countries, with the southern European countries having higher MDS compared with those in the Northern regions. Mean (standard deviation) values for the individual countries were as follows: Norway (Bergen) 4.89 (1.39), Estonia (Tallinn) 4.49 (1.11), United Kingdom (Belfast) 4.42 (1.30), France (Paris) 4.62 (1.50), Italy (Verona) 5.37 (1.18), Greece (Thessaloniki) 5.19 (1.09), and 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. Age-standardized mean (95% confidence interval) MDS 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).

Mediterranean Diet Score and Serum Levels of Antioxidants

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

Mediterranean Diet Score and Late Age-Related Macular Degeneration (Neovascular Age-Related Macular Degeneration and Geographic Atrophy)

Increasing MDS was significantly associated with reduced odds of nvAMD (Table 2) in both the unadjusted analysis and when adjusted for potential confounders such as age, sex, country, education, smoking, drinking, self-reported history of cardiovascular disease, aspirin consumption, and diabetes. A score of ≥6 was associated with an approximately 50% reduction in the adjusted odds ratio of developing nvAMD compared with those with a score of 4 or less. Preliminary analyses showed no association with GA, and we did not perform further analyses on GA with full data on diet score and confounders.

Age-Related Macular Degeneration and Early Age-Related Macular Degeneration (Grades 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 significant 20% reduced odds compared with those in the lowest category (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 the 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 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. The MDS has been inversely associated with cardiovascular disease, cancer, and mortality, and more recently progression to advanced AMD. 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 been identified as associated with a reduced prevalence of incidence of AMD, such as higher fruit and vegetable intake, reduced red meat intake, lower glycemic index, and higher fish intake. 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 nvAMD was not explained by lutein and zeaxanthin. This is not surprising because the MDS is based on hypothesized benefits of food patterns rather than single nutrients and therefore includes other important nutrients relevant to late AMD.

Dietary patterns rather than individual components increasingly are being studied in relation to AMD. Various methodological approaches have been taken but tend to fall into 2 broad categories. The first are a posteriori approaches, including the use of factor and principal component analysis to identify patterns that are then related to the disease under investigation. 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 diet.
or a set of dietary recommendations 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 as in the current study (odds ratio, 0.45; 95% confidence interval, 0.28–0.87). Conversely, a diet characterized by high intake of 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. Examination of dietary data from the baseline AREDS identified 2 major components that the investigators named “Oriental and Western patterns.” The Oriental pattern was associated with significantly reduced odds of both early and advanced AMD, and the Western pattern was associated with significantly increased odds of both early and advanced AMD. Although the associations were noted for both early and advanced AMD, the strength of the associations was greater for advanced AMD. A score-based approach has been applied within the Carotenoids in Age-Related Eye Disease Study (CAREDS). Responses to an FFQ were converted to a modified 2005 Healthy Eating Index, and those in the highest quintile compared with the lowest quintile of the index had 46% lower odds of developing early AMD. 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 seen only in subjects carrying no or 1 complement factor H Y402H risk allele and not observed in those at the highest risk with 2 alleles. In our analysis, we did not find that the association of diet score

### Table 2. Association of Mediterranean Diet Score and Neovascular Age-Related Macular Degeneration

<table>
<thead>
<tr>
<th>MDS</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4 (n = 808)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>5 (n = 774)</td>
<td>0.88 (0.55–1.39)</td>
<td>0.83 (0.55–1.26)</td>
</tr>
<tr>
<td>6 (n = 536)</td>
<td>0.62 (0.33–1.16)</td>
<td>0.62 (0.39–1.00)</td>
</tr>
<tr>
<td>&gt;6 (n = 201)</td>
<td>0.52 (0.29–0.93)</td>
<td>0.53 (0.27–1.04)</td>
</tr>
<tr>
<td>P trend</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CI = confidence interval; MDS = Mediterranean Diet Score; nvAMD = neovascular age-related macular degeneration; OR = odds ratio.

*Adjusted for age, sex, country, education, smoking, drinking, self-reported history of cardiovascular disease, aspirin consumption, and diabetes.
with AMD was modified by Y204H alleles. The small number of cases withnvAMD meant we lacked power to investigate significant gene/diet interactions (as found in that article), 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 performed before the widespread use of supplements for AMD and before 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 with serum antioxidants also provides us with helpful insight into what diet constituents the MDS is reflecting (Table 1). It also provides a degree of construct validity to the MDS.

This is a cross-sectional study; therefore, no causal inferences can be made regarding the associations noted. This study also was carried out before the routine use of optical coherence tomography in characterizing AMD features; therefore, some of the early features of AMD may have been misclassified and important phenotypes, such as reticular pseudodrusen, underascertained through the sole use of color fundus photographs. Dietary intake, including calculation of MDS, was based on a self-reported FFQ. Such a questionnaire, although commonly used in nutritional epidemiology, can be prone to bias, including overreporting of healthy foods, such as those contained within the MDS.

In conclusion, this study provides further evidence of the relationship between the Mediterranean diet and the prevalence of AMD. The MDS provides a useful method to characterize dietary patterns across geographically and culturally diverse populations. Interventions to encourage the adoption of the Mediterranean diet should be developed more widely, and methods by which such behavior change can be achieved and maintained over the long term should be investigated.

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15. Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular
Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; CAREDS = Carotenoids in Age-Related Eye Disease Study; EPIC = European Prospective Investigation into Cancer and Nutrition; EUREYE = European Eye; FFQ = food-frequency questionnaire; GA = geographic atrophy; MDS = Mediterranean Diet Score; nvAMD = neovascular age-related macular degeneration.

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