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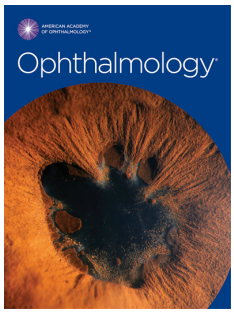

Mediterranean diet and incidence of advanced AMD: The EYE-RISK CONSORTIUM

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ABSTRACT

Objective

To investigate associations of adherence to the Mediterranean diet (MeDi) with incidence of advanced AMD (the symptomatic form of AMD) in two European population-based prospective cohorts.

Design

Prospective cohorts: the Rotterdam Study I (RS-I) and the Alienor Study.

Participants:

4 446 participants aged ≥55 years from RS-I (The Netherlands) and 550 French adults aged 73 years or older from Alienor Study with complete ophthalmologic and dietary data were included in the present study.

Methods

Examinations were performed approximately every 5 years over a 21-year period (1990 to 2011) in RS-I and every 2 years over a 4-year period (2006 to 2012) in Alienor Study. Adherence to the MeDi was evaluated using a 9 component score based on intake of vegetables, fruits, legumes, cereals, fish, meat, dairy products, alcohol and the monounsaturated-to-saturated fatty acids ratio. Associations of incidence of AMD with MeDi were estimated using multivariate Cox proportional Hazard models.

Main outcomes measures

Incidence of advanced AMD based on retinal fundus photographs.

Results

Among the 4 996 included participants, 155 developed advanced incident AMD (117 from RS-I and 38 from Alienor Study). The mean follow-up time was 9.9 years (range 0.6 to 21.7) in RS-I and 4.1 years (range 2.5 to 5.0) in Alienor Study.

Pooling data for both RS-I and Alienor study, participants with a high (6-9) MeDi score had a significantly reduced risk for incident advanced AMD compared to participants with a low (0-3) MeDi score in the fully-adjusted Cox model (HR, 0.59 [95% CI, 0.37-0.95], p for trend=0.04).

Conclusion

Pooling data from RS-I and Alienor, higher adherence to the MeDi was associated with a 41% reduced risk of incident advanced AMD. These findings support the role of a diet rich in healthful nutrient-rich foods such as fruits, vegetables, legumes and fish in the prevention of AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blindness in industrialized countries[1]. This degenerative disease affects the central part of the retina, which is crucial for daily living tasks such as reading, driving and recognition of faces. Worldwide, 196 million people will be affected by AMD in 2020, increasing to 288 million in 2040[2]. Advanced forms of the disease (neovascular or atrophic AMD) associated with a deep visual impairment, are generally preceded by asymptomatic early stages. While no treatment is currently available for atrophic AMD, effective treatments are available for the neovascular form[3, 4]. These treatments also incur major costs to society, with an estimated 2.3 billion dollars of Medicare claims in 2013[5]. The risk of developing AMD is jointly determined by age, individual genetic background and lifestyle[1, 6]. Prevention strategies based on the modifiable risk factors of AMD may help decrease the major medical and social burden associated with AMD.

Epidemiological studies have observed a reduced risk of AMD associated with high consumption of antioxidants (lutein and zeaxanthin[7-12], fruits and vegetables rich in these nutrients), and omega-3 polyunsaturated fatty acids[8,9, 13-15], provided by fish and nuts[13, 14, 16, 17]. However a single nutrient/food approach cannot capture the synergistic effects of food and nutrients consumed in combination in the diet. The Mediterranean diet (MeDi) is characterized by high consumption of plant foods and fish, low consumption of meat and dairy products, olive oil as the primary fat source and a moderate consumption of wine[18]. Adherence to the MeDi has been linked to lower rates of mortality[19], chronic diseases, stroke[20], cognitive decline[21] and recently to diabetic retinopathy[22]. Regarding AMD, very few studies are available to date[23-27]. In three

population-based studies, it was associated with a lower prevalence of early AMD[23], neovascular AMD[25] and any AMD[26, 27], although dietary modifications due to AMD cannot be excluded in these cross-sectional studies. In a post-hoc analysis of a randomized clinical trial, the MeDi was associated with a lower incidence of advanced AMD[24], but the selected nature of the sample limits its generalizability. We therefore investigated the associations between MeDi and incidence of advanced AMD in a large sample from two population-based prospective studies.

METHODS

Study population

The EYE-RISK project aims at identifying risk factors, molecular mechanisms and therapeutic approaches for AMD (http://www.eyerisk.eu/). It uses epidemiological data describing clinical phenotype, molecular genetics, lifestyle, nutrition and in-depth retinal imaging derived from existing European epidemiological cohorts to provide major insights needed for prevention and therapy of AMD. Within the EYE-RISK consortium, a unique harmonized database of individual data from 16 European epidemiological studies was constructed[28]. Two prospective studies with appropriate data for the present analyses were available: the Rotterdam Study I[29] (RS-I) and the Alienor Study[30].

Rotterdam Study I

At baseline 7 983 eligible persons aged 55 years or older were interviewed and examined. Ophthalmological examinations and fundus photography were taken at each round starting in 1990-1993 (RS-I-1). Follow-up rounds were completed in 1993-1995 (RS-I-2), 1997-1999 (RS-I-3), 2002-2004 (RS-I-4) and 2009-2011 (RS-I-5).

The RS has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The RS has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831.

After pharmacologic mydriasis, 35° stereoscopic color fundus photos of the macula (Topcon TRV-50VT; Topcon Optical Co., Tokyo, Japan) were taken in each of the first 3 visits, and 35° digital images (Topcon TRC 50EX) were taken for the fourth and fifth visits[31].

Alienor Study

At baseline (2006-2008), 963 participants aged 73 years or more were interviewed and had an ophthalmological examination[30]. Of these, 624 and 614 were reexamined at the second (2009-2010) and third (2011-2012) visits, respectively. The design has been approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006 (http://www.alienor-study.com/langue-english-1.html).

The eye examinations took place in the Department of Ophthalmology of the University Hospital of Bordeaux. Two 45° non-mydriatic color retinal photographs were taken using a high-resolution digital non-mydriatic retinograph (Topcon TRC-NW6S)[30]. At the third visit (2011-2012), for participants who were not able to come to the hospital, the eye examination took place at home and 40° retinal photographs were taken using a digital non-mydriatic portable retinograph (Optomed Smartscope M5).

For both studies, all participants provided written informed consent in accordance with the Declaration of Helsinki to participate in the study.

AMD classification

Retinal photographs of both eyes were graded by trained graders of each study and were interpreted according to a modification of the Wisconsin Age-Related System[32] for RS-I and according to the International Classification[33] for Alienor. All advanced AMD cases were adjudicated and confirmed by retina specialists of the corresponding study. Phenotype harmonization was performed within the EYE-RISK Consortium[28].

Incidence

At each visit, each subject was classified according to the worst eye into one of the following exclusive groups: no AMD, early AMD, advanced AMD. Advanced AMD was defined by the presence of neovascular or atrophic AMD.

Neovascular AMD included serous or hemorrhagic detachment of the retinal pigment epithelium (RPE) or sensory retina, subretinal or sub-RPE hemorrhages, and fibrous scar tissue. Geographic atrophy was defined as a discrete area of retinal depigmentation, 175 µm in diameter or larger, characterized by a sharp border and the presence of visible choroidal vessels. Early AMD (in the absence of advanced AMD) was defined by the presence of (1) soft indistinct (≥125 µm, decreasing density from the center outward and fuzzy edges) / reticular drusen only or soft distinct drusen (≥63 µm, with uniform density and sharp edges) and pigmentary abnormalities or by (2) soft indistinct large drusen (≥125 µm, decreasing density from the center outward and fuzzy edges) / reticular drusen and pigmentary abnormalities (corresponding to grades 2 and 3

of the Rotterdam Classification). No AMD was defined by the absence of early AMD and advanced AMD.

Incidence of advanced AMD was defined as the subject progressing from no or early AMD at baseline to advanced AMD (either neovascular or atrophic AMD) at any time-point during the study period. The date of occurrence of advanced AMD was calculated as the midpoint of the interval between the last visit without advanced AMD and the first visit with advanced AMD. Follow-up ended at the date of occurrence of advanced AMD, or the date of the last gradable photograph. Subjects with advanced AMD or no gradable eyes at baseline were excluded from the analysis.

For the purpose of AMD subtype analysis, neovascular AMD comprised all subjects presenting some neovascular lesions, with or without coexisting atrophy. Atrophic AMD was defined as pure geographic atrophy (in the absence of neovascular AMD).

Dietary assessment

In RS-I, participants completed a checklist at home and had a face-to-face interview conducted by a trained dietitian at the research center using a 170-items validated semi-quantitative food frequency questionnaire (FFQ)[34]. The food items were converted into quantities consumed per day (g/day). By using the computerized Dutch Food Composition Table, these dietary data were converted to total energy intake (TEI) (kcal/day) and nutrient intakes (g/day)[34].

In Alienor, participants were visited at home by a specifically trained dietician who administered a 40-items validated FFQ and a 24-hour dietary recall[35, 36]. The food items

were converted into number of servings per day. The 24-hour recall was used to estimate nutrient intake (g/day) and TEI (kcal/day) and to compute the monounsaturated fatty acids (MUFAs) to saturated fatty acids (SFAs) ratio.

Adherence to the MeDi was assessed using the MeDi score developed by Trichopoulou et al[37]. This score including 9 components: vegetables, fruits, legumes, cereals, fish, meat, dairy products, alcohol and the MUFAs-to-SFAs ratio was applied to both studies. The daily intake of each food/beverage group was calculated as quantity in g/day in RS-I and as the number of servings/day in Alienor. Participants with unreliable TEI were excluded (valid TEI range: women: 600–3200; men: 600–4200 kcal). For each component hypothesized to benefit health (vegetables, fruits, legumes, cereals and fish, MUFAs-to-SFAs ratio), 1 point was given if intake was above the sex-specific median values and zero otherwise. For components presumed to be detrimental to health (meat and dairy products), 1 point was given if intake was below the sex-specific median values and zero otherwise. For alcohol, 1 point was given for moderate consumption and zero otherwise (moderate consumption: women: 1-10; men: 5-15 g/day). Sex-specific median were calculated separately for each study. The total MeDi score was computed by adding the scores (0 or 1 point) for each component for each participant. Scores ranged from 0 (non-adherence) to 9 (perfect adherence). Subjects were classified according to 3 categories of the MeDi score: low (0–3), medium (4–5), or high (6–9).

Covariates

Age (years), sex, education (primary, secondary, higher), smoking (never smoker, smoker <20 pack-years (PY), smoker ≥20 PY, PY=packs (20 cigarettes) smoked per day X years of smoking), multivitamins/minerals supplement use (Yes/No) were measured using self-reported questionnaires at baseline[30 38] for each study. Vascular risk factors included body mass index (BMI: weight (kg)/height (m^2)), diabetes (treated or self-reported), hypertension (blood diastolic blood pressure ≥90mmHg or systolic blood pressure ≥140mmHg or treated or self-reported), and hypercholesterolemia (treated or self-reported). *Complement Factor H* (*CFH*) Y402H (rs1061170) and *Age-Related Maculopathy Susceptibly 2* (*ARMS2)* A69S (rs10490924), the two main AMD-related SNPs were assessed in each study[39, 40].

Statistical analysis

Subjects excluded from analyses were compared to those included using logistic regression model adjusted for age and sex for each characteristic separately. The same method was used to compare characteristics of subjects included between the two cohorts.

The associations of MeDi score with incidence of advanced AMD were analyzed using Cox proportional hazards models with delayed entry and age as a time scale, which allow for a better adjustment for age than the classical Cox models based on time from entry in the study[41]. Model 1 was unadjusted and model 2 was adjusted for sex, AMD grade at baseline (no or early AMD), TEI (continuous), education, BMI, smoking, multivitamins/minerals supplement use, diabetes and hypercholesterolemia. Variables

retained in model 2 were factors associated with incidence of AMD and/or with MeDi score, after adjustment for age and sex ($p<0.10$). For the pooled analysis, including data from both studies, models were further adjusted for the study (fixed study effect). Low MeDi score was designated as the reference group. P-trend was calculated by using the median value of the MeDi score for each category. In all Cox models, the proportional hazard assumptions were tested.

Participants from RS-I and Alienor were different regarding some characteristics. To estimate the potential effect of these differences, interactions between study and each covariate were assessed and none was significant. Thus, as the proportional hazard assumptions were satisfied and there were no interactions, to account for differences between the two studies, all models combining both studies were adjusted for a fixed study effect.

Secondary analyses

We also assessed whether associations of MeDi score with incident advanced AMD may be due to individual dietary components by examining associations between the individual components of the MeDi score and advanced AMD. Each component was introduced independently into model 2. In secondary analyses, *CFH* Y402H and *ARMS2* A69S polymorphisms were added to model 2. Interactions between *CFH* Y402H and *ARMS2* A69S polymorphisms and MeDi score were also analyzed. Interaction terms for the number of risk alleles and MeDi score were assessed separately for each genetic variant using model 2. All *p*-values representing a 2-tailed test of significance with

235 α=0.05 and SAS version 9.4 (SAS Institute Inc. Cary, NC, USA) was used for all
236 analyses.

RESULTS

Of the 7 146 participants at risk of developing advanced AMD, 1 337 had no follow-up data for both eyes. In addition, 813 were excluded due to missing/unreliable dietary data (**Figure 1**). Overall 4 996 (4 446 from RS-I and 550 from Alienor) participants free from advanced AMD at baseline with complete and reliable dietary data together with follow-up information were included in our analyses.

Among the 4 996 included participants, 155 developed advanced incident AMD (117 from RS-I and 38 from Alienor). The mean follow-up time was 9.9 years (range 0.6 to 21.7) in RS-I and 4.1 years (range 2.5 to 5.0) in Alienor.

In both studies, participants included in the analyses tended to be younger than those excluded (**Table 1**). In RS-I, after adjustment for age and sex, included participants tended to be more women, to have a higher education, to have a history of smoking and diabetes, a lower TEI, a higher MeDi score and to carry less often a *CFH* Y402H CC genotype than excluded participants. In Alienor, included participants were more likely to have a higher education, than excluded participants.

Participants from RS-I, tended to be younger, to have a lower education, to have a history of smoking, to have less hypertension and less hypercholesterolemia as well as a higher TEI, a lower adherence to the MeDi score and to have less early AMD than participants from Alienor. Also *CFH* Y402H polymorphism was slightly different between the two studies.

Participants from Alienor tented to have a higher median of consumption of vegetables, cereals and fish, whereas subjects from RS-I tented to have a higher median of

consumption of dairy products. Consumption of fruits, legumes, meat and the MUFAs-to-SFAs ratio were similar (**eTable 1**).

For both studies, the incidence of advanced AMD was lower among subjects who had a high adherence to the MeDi score (**Figure 2**). The effect of MeDi is more noticeable among older people (85+), at higher risk of AMD, but as the proportional hazard assumption is respected, associations are considered similar among the different age groups.

In the unadjusted model, similar estimations were obtained in both studies, with a HR of 0.56 [95% CI 0.33 to 0.96] in RS-I and 0.48 [95% CI 0.18 to 1.26] in Alienor for participants with a high MeDi score, by comparison with a low MeDi score (**Table 2**). When pooling both studies, a high MeDi score was significantly associated with a lower risk for incident advanced AMD (HR, 0.53 [95% CI, 0.33-0.84], p-trend=0.009). These associations remained similar and significant after further adjustment for sex, TEI, AMD grade at baseline, education, BMI, smoking, supplement use of multivitamins/minerals, diabetes and hypercholesterolemia, (HR, 0.59 [95%CI, 0.37-0.95], p-trend=0.04).

In secondary analyses, we further adjusted for *CFH* Y402H and *ARMS2* genes and the HR remained unchanged (data not shown).

Interactions terms between MeDi and *CFH* Y402H and *ARMS2* genes were not statistically significant (p for interaction=0.89 and 0.18, respectively, data not shown).

Adherence to MeDi score was not significantly associated with the risk for incident neovascular AMD neither in RS-I nor in Alienor or in the pooled analysis (**Table 3**). It was significantly associated with the risk for incident atrophic AMD in RS-I (HR=0.41, p-

trend=0.046) but the association did not reach significance in Alienor (HR=0.52, p-trend=0.52). In the pooled data analysis, a higher MeDi score was significantly associated with a reduced risk for incident atrophic AMD (HR, 0.42 [95%CI, 0.20-0.90], p-trend=0.04).

We assessed whether the benefit of high adherence to the MeDi score was due to a specific component. Using the sex-specific median as cutoffs, no component was significantly associated with incidence of advanced AMD (**eTable 2**).

DISCUSSION

High adherence to the MeDi was associated with a 41% reduced risk of incident advanced AMD in the pooled analysis. None of the nine components, including vegetables, fruits, legumes, cereals, fish, the MUFAs-to-SFAs ratio, meat, dairy products and alcohol consumption, was significantly associated with incidence of advanced AMD, highlighting the importance of assessing dietary patterns rather than single components. In our studies, a high adherence to the MeDi was significantly associated with a reduced risk of incident atrophic AMD. A similar association was observed for neovascular AMD but did not reach statistical significance.

By evaluating the individual and the pooled associations of the adherence to the MeDi and incidence of advanced AMD in two well established and harmonized European population-based prospective cohorts, this study expands on prior studies, mainly cross-sectional, case-control, and clinical trials on this topic. Visual impairment due to AMD could influence dietary practices; prospective studies, by assessing diet prior the onset of the disease, limits reverse causation. Thus, prospective design is more accurate and less biased than a cross-sectional or case-control design to evaluate the association between diet and AMD. In addition, although using a prospective design, clinical trials are limited by the selected nature of the sample. Results from population-based studies are more generalizable.

Our results are partially consistent with previous cross-sectional studies: the CAREDS study reported a lower prevalence of early AMD in American women with high adherence to the MeDi[23], the Coimbra Study demonstrated a lower prevalence of any

AMD in Portuguese participants who were having a high adherence to the MeDi[26, 27] and the European Eye Study (Eureye) showed a lower prevalence of neovascular AMD in subjects with a high MeDi score while atrophic AMD was not associated with MeDi score[25]. Our findings confirm the post-hoc analyses of the AREDS clinical trial. In this sample of American participants aged 55 to 80 years, a high MeDi score was associated with a 26% lower risk of progression to advanced AMD[24]. The AREDS study also showed that fish and vegetable components were associated with a lower risk of progression to advanced AMD[24]. Our results were in the same direction but did not reach the statistical significance when sex-specific median cutoffs were used. No significant interactions were observed between MeDi score and *CFH* Y402H and *ARMS2* genes. Our findings report a significant association with advance AMD. Regarding subtypes, only atrophic AMD was significantly associated with MeDi score. For neovascular AMD even if the association was not statistically significant, the HRs were similar to those for atrophic AMD. These differences could be explained by a low number of incident cases. In the Eureye Study, the only study to show separate results for the two advanced forms of AMD, association was significant with neovascular AMD. While in our studies this association with neovascular AMD was not statistically significant, HRs were similar to those for Eureye study.

Our results thus support public health efforts to emphasize adherence to the MeDi for everyone. The biological basis for the potential benefits of the MeDi is associated with a decrease in oxidative stress and inflammation, which are also involved in the pathophysiology of AMD[42, 43].

The PREDIMED study, a clinical trial among persons at high cardiovascular risk, showed that adhering to a MeDi reduced the incidence of major cardiovascular events[20]. Median consumptions were similar to the goals suggested by PREDIMED for vegetables (≥2 serving/d), fish (≥3 serving/w) and meat (<1 serving/d) in Alienor and for meat in RS--I. For both studies, median of fruits and legumes were below the goals of PREDIMED (≤3 serving/d) as well as median of vegetables, and fish in RS-I. Even though the medians in our study were lower for vegetables and fruits, the association with the MeDi score was significant, suggesting the importance of a global approach to prevent the development of AMD.

By showing a prospective association between AMD and MeDi, an energy-unrestricted diet mainly composed of nutrient-rich food, our study confirms the importance of dietary quality focused on healthful foods and dietary patterns rather than single nutrients or low-energy diet for AMD.

In observational studies, residual confounding is always a concern. In the present study, results were similar in the basic model (unadjusted model) and the fully-adjusted model (adjusted for sex, TEI, AMD grade at baseline, cardiovascular risk factors, educational level and dietary supplement use), suggesting that our results are not highly confounded. In the fully-adjusted model, association between MeDi and incidence of AMD seems to be weaker in RS-I. This could be explained by a lower statistical power due to a low incidence of participants developing advanced AMD combined to the increasing number of covariates compared to the unadjusted model. In addition, our findings are based on prospective follow-up, thereby limiting reverse causation. However, only randomized clinical trials can prove the causal nature of the associations.

Such randomized clinical trials testing dietary interventions have proven to be efficient in the prevention of stroke[20] or diabetes[44], for instance, but none are available in the field of AMD.

Selection bias cannot be completely dismissed, as participants included in this analysis were different from non-participants in both RS-I and Alienor. Moreover, participants included from RS-I were different from those from Alienor regarding some sociodemographic and medical characteristics as well as follow-up time duration and frequency. Incidence rates of AMD were also higher in Alienor than in RS-I. These differences might be explained by the older age at baseline and a closer follow-up (every 2 years instead of 5 years in RS-I, with home examinations for participants unable to come to the hospital in Alienor but not in every RS-I follow-up visit), or by different incidence rates in France and the Netherlands.

The MeDi score uses cutoffs based on each study population and results can only be generalizable to similar populations. To calculate the MeDi score, we used validated FFQs for both studies, adapted to the specific dietary habits of each population (France and the Netherlands). As the FFQ in Alienor was a 40-items FFQ, we used the 24h recall to calculate the MUFAs-to-SFAs ratio and the TEI to increase the exactitude of their ascertainment, as previously published[21]. The distribution of the MeDi score was different between the two studies, participants from RS-I were less adherent. This result was expected in a North European population.

Despite these major differences in populations (different countries, different time periods, different generations and different diet habits) and methods (different follow-up

time and frequency, different dietary assessment methods), the association between MeDi and incidence of advanced AMD was similar in both cohorts. This association thus appears to be robust.

To strengthen our analyses, we excluded subjects with unusually high or low TEI and adjusted for several factors know to be related to MeDi and AMD. We used a well-known and validated score to assess diet and probable synergistic effects between nutrients and food groups. Our MeDi score was developed by using sex-specific thresholds according to each study to better account for differences between men and women and studies. Other strengths include a large sample from two well documented and data-harmonized population-based prospective cohorts in the framework of the European EYE-RISK project.

In conclusion, combined results from our two observational studies suggest that adopting an energy-unrestricted diet rich in healthful nutrient-rich foods such as fruits, vegetables, legumes and fish, and, reducing the unhealthful foods such as red and processed meats, savory and salty industrialized products may contribute to the prevention of AMD.

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Competing financial interest of members of the EYE-RISK consortium:

Verena Arndt, Sebastian Bühren, Tanja Endermann and Markus Zumbansen are employees of AYOXXA. Marc Biarnés received travel fees from Bayer and is a consultant for Roche. Cécile Delcourt is a consultant for Allergan, Bausch+Lomb, Laboratoires Théa, Novartis, Roche. Roberto Iacone, Hanno Langen, Cyrille Maugeais and Everson Nogoceke are employees of F. Hoffmann-La Roche Ltd. Jordi Monés received financial support from Bayer, Alcon, Ophthotech, Notal Vision, Novartis, Roche and Ophthotech. Imre Lengyel receives unrestricted research support from OPTOS Plc. Bénédicte Merle is consultant for Bausch+Lomb and received travel fees from Laboratoires Théa. Audrey Cougnard-Grégoire received travel fees from Laboratoires Théa. The other authors do not have any competing financial interest.

REFERENCES

1. Lim LS, Mitchell P, Seddon JM, et al. Age-related macular degeneration. Lancet 2012;379(9827):1728-38.
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. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006;355(14):1419-31.
4. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology 2012;119(12):2537-48.
5. Erie JC, Barkmeier AJ, Hodge DO, Mahr MA. High Variation of Intravitreal Injection Rates and Medicare Anti-Vascular Endothelial Growth Factor Payments per Injection in the United States. Ophthalmology 2016;123(6):1257-62.
6. Sobrin L, Seddon JM. Nature and nurture- genes and environment- predict onset and progression of macular degeneration. Prog Retin Eye Res 2014;40:1-15.
7. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 1994;272(18):1413-20.
8. Mares-Perlman JA, Fisher AI, Klein R, et al. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. Am J Epidemiol 2001;153(5):424-32.
9. Delcourt C, Carriere I, Delage M, et al. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. Invest Ophthalmol Vis Sci 2006;47(6):2329-35.

10. Cho E, Hankinson SE, Rosner B, et al. Prospective study of lutein/zeaxanthin intake and risk of age-related macular degeneration. Am J Clin Nutr 2008;87(6):1837-43.

11. Ma L, Dou HL, Wu YQ, et al. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. Br J Nutr 2012;107(3):350-9.

12. van Leeuwen R, Boekhoorn S, Vingerling JR, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. JAMA 2005;294(24):3101-7.

13. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. Arch Ophthalmol 2003;121(12):1728-37.

14. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. Arch Ophthalmol 2006;124(7):995-1001.

15. Merle BM, Benlian P, Puche N, et al. Circulating omega-3 Fatty acids and neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci 2014;55(3):2010-9.

16. 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.

17. Chong EW, Kreis AJ, Wong TY, et al. Dietary omega-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: a systematic review and meta-analysis. Arch Ophthalmol 2008;126(6):826-33.

18. Willett WC, Sacks F, Trichopoulou A, et al. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr 1995;61(6 Suppl):1402S-6S.

19. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, et al. Diet and overall survival in elderly people. BMJ 1995;311(7018):1457-60.

20. 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.

21. Feart C, Samieri C, Rondeau V, et al. Adherence to a Mediterranean diet, cognitive decline, and risk of dementia. JAMA 2009;302(6):638-48.

22. Diaz-Lopez A, Babio N, Martinez-Gonzalez MA, et al. Mediterranean Diet, Retinopathy, Nephropathy, and Microvascular Diabetes Complications: A Post Hoc Analysis of a Randomized Trial. Diabetes Care 2015;38(11):2134-41.

23. 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.

24. Merle BM, Silver RE, Rosner B, Seddon JM. 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.

25. Hogg RE, Woodside JV, McGrath A, et al. Mediterranean Diet Score and Its Association with Age-Related Macular Degeneration: The European Eye Study. Ophthalmology 2017;124(1):82-9.

26. Nunes S, Alves D, Barreto P, et al. Adherence to a mediterranean diet and its association with age-related macular degeneration. The Coimbra Eye Study-Report 4. Nutrition 2018;51-52:6-12.

27. Raimundo M, Mira F, Cachulo MdL, et al. Adherence to a Mediterranean diet, lifestyle and age-related macular degeneration: The Coimbra Eye Study – Report 3. Acta Ophthalmol 2018.

28. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. Ophthalmology 2017;124(12):1753-63.

29. Ikram MA, Brusselle GGO, Murad SD, et al. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol 2017;32(9):807-50.

30. Delcourt C, Korobelnik JF, Barberger-Gateau P, et al. Nutrition and age-related eye diseases: the Alienor (Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes) Study. J Nutr Health Aging 2010;14(10):854-61.

506 31. van Leeuwen R, Klaver CC, Vingerling JR, et al. The risk and natural course of age-
507 related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. Arch Ophthalmol
508 2003;121(4):519-26.
509 32. Klein R, Davis MD, Magli YL, et al. The Wisconsin age-related maculopathy grading
510 system. Ophthalmology 1991;98(7):1128-34.
511 33. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading
512 system for age-related maculopathy and age-related macular degeneration. The International
513 ARM Epidemiological Study Group. Surv Ophthalmol 1995;39(5):367-74.
514 34. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, et al. Dietary assessment in the
515 elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr
516 1998;52(8):588-96.
517 35. Feart C, Jutand MA, Larrieu S, et al. Energy, macronutrient and fatty acid intake of
518 French elderly community dwellers and association with socio-demographic characteristics: data
519 from the Bordeaux sample of the Three-City Study. Br J Nutr 2007;98(5):1046-57.
520 36. Samieri C, Jutand MA, Feart C, et al. Dietary patterns derived by hybrid clustering
521 method in older people: association with cognition, mood, and self-rated health. J Am Diet Assoc
522 2008;108(9):1461-71.
523 37. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean
524 diet and survival in a Greek population. N Engl J Med 2003;348(26):2599-608.
525 38. Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA. Determinants of disease
526 and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7(4):403-22.
527 39. Ho L, van Leeuwen R, Witteman JC, et al. Reducing the genetic risk of age-related
528 macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam
529 study. Arch Ophthalmol 2011;129(6):758-66.
530 40. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at
531 CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41(10):1094-9.

41. Lamarca R, Alonso J, Gomez G, Munoz A. Left-truncated data with age as time scale: an alternative for survival analysis in the elderly population. J Gerontol A Biol Sci Med Sci 1998;53(5):M337-43.

42. Fung TT, McCullough ML, Newby PK, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82(1):163-73.

43. Dai J, Jones DP, Goldberg J, et al. Association between adherence to the Mediterranean diet and oxidative stress. Am J Clin Nutr 2008;88(5):1364-70.

44. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346(6):393-403.

542 **Figure 1.** Selection of participants for analyses.

543 **Figure 2.** Incidence of advanced AMD according to adherence to Mediterranean Diet

544 (MeDi) score.

Table 1. Baseline characteristics of the Rotterdam Study I (RS-I) and the Alienor Study, according to participants included and excluded from analyses.

Characteristics	Rotterdam Study I			Alienor Study			
	Included[a] (n=4 446)	Excluded[b] (n=1 867)	P value[c]	Included[a] (n=550)	Excluded[b] (n=283)	P value[c]	P value[c] RS-I vs Alienor for included subjects
	No. (%)			No. (%)			
Age, mean (SD), y	66.9 (7.3)	73.4 (10.0)	<0.0001	79.2 (4.2)	81.1 (4.3)	<0.0001	<0.0001
Sex			0.006			0.61	0.08
Men	1813 (40.8)	766 (41.0)		209 (38.0)	108 (38.2)		
Women	2633 (59.2)	1101 (59.0)		341 (62.0)	175 (61.8)		
Education	N=4426	N=1808	<0.0001		N=283	0.03	<0.0001
Primary	2200 (49.7)	1151 (63.6)		301 (54.7)	180 (63.6)		
Secondary	1823 (41.2)	529 (29.3)		130 (23.7)	56 (19.8)		
Higher	403 (9.1)	128 (7.1)		119 (21.6)	47 (16.6)		
Smoking, pack-years	N=4131	N=1644	0.009	N=547	N=278	0.77	<0.0001
Never smoker	1501 (36.3)	667 (40.6)		356 (65.1)	179 (64.4)		
<20	1173 (28.4)	413 (25.1)		95 (17.4)	51 (18.3)		
≥20	1457 (35.3)	564 (34.3)		96 (17.5)	48 (17.3)		
Multivitamin/mineral supplement use	N=4442	N=1867	0.11		N=281	0.32	0.05
No	4070 (91.7)	1660 (88.9)		475 (86.4)	237 (84.3)		
Yes	370 (8.3)	207 (11.1)		75 (13.6)	44 (16.7)		
Body mass index, mean (SD), kg/m²	N=4426	N=1775	0.26	N=542	N=274	0.87	0.17
	26.3 (3.6)	26.2 (4.0)		26.0 (4.0)	25.9 (4.1)		
Diabetes	N=4444		0.003		N=281	0.33	0.63
No	3941 (88.7)	1693 (90.7)		489 (88.9)	243 (86.5)		
Yes	503 (11.3)	174 (9.3)		61 (11.1)	38 (13.5)		
Hypertension			0.28		N=281	0.48	<0.0001
No	1883 (42.3)	593 (31.8)		86 (15.6)	46 (16.4)		
Yes	2563 (57.7)	1274 (68.2)		464 (84.4)	235 (83.6)		
Hypercholesterolemia	N=4442		0.18		N=281	0.76	<0.0001
No	4317 (97.2)	1837 (98.4)		275 (50.0)	146 (52.0)		
Yes	125 (2.8)	30 (1.6)		275 (50.0)	135 (48.0)		
CFH (rs1061170)	N=3972	N=1581	0.02	N=450	N=235	0.81	0.03
TT	1649 (41.5)	632 (40.0)		212 (47.1)	101 (43.0)		
CT	1801 (45.3)	709 (44.8)		181 (40.2)	110 (46.8)		
CC	522 (13.2)	240 (15.2)		57 (12.7)	24 (10.2)		
ARMS2 (rs10490924)	N=3971	N=1582	0.16	N=450	N=235	0.12	0.11
GG	2490 (62.7)	1028 (65.0)		309 (68.7)	145 (61.7)		
GT	1339 (33.7)	500 (31.6)		126 (28.0)	85 (36.2)		

TT	142 (3.6)	54 (3.4)		15 (3.3)	5 (2.1)		
Total energy intake, mean (SD), kcal		N=687	0.0002		N=242	0.90	<0.0001
	1968 (484)	2016 (609)		1719 (530)	1704 (549)		
Mediterranean Diet score		N=398	0.04[d]		N=209	0.70[d]	<0.0001[d]
Low 0-3	1376 (31.0)	153 (38.4)		171 (31.1)	58 (27.8)		
Medium 4-5	2123 (47.7)	181 (45.5)		236 (42.9)	100 (47.8)		
High 6-9	947 (21.3)	64 (16.1)		143 (26.0)	51 (24.4)		
AMD grade at baseline			0.11			0.05	0.001
No AMD	4179 (94.0)	1654 (88.6)		444 (80.7)	241 (85.2)		
Early AMD	267 (6.0)	213 (11.4)		106 (19.3)	42 (14.8)		

[a] Participants included in one or more analyses for incidence of advanced AMD

[b] Participants excluded from all analyses

[c] p value from logistic regression adjusted for age and sex

[d] p value from logistic regression adjusted for age, sex and total energy intake

Table 2. Association between Mediterranean Diet (MeDi) score and incidence of advanced age-related macular degeneration (AMD).

	No. at risk for advanced AMD	No. incident cases	Mediterranean Diet Score			P for trend[a]
			Low 0-3	Medium 4-5	High 6-9	
Model 1[b]						
Rotterdam I	4446	117				
HR (95% CI)[c]			Reference	0.69 (0.46-1.03)	0.56 (0.33-0.96)	*0.036*
Alienor	550	38				
HR (95% CI)[c]			Reference	0.80 (0.39-1.63)	0.48 (0.18-1.26)	*0.16*
Overall	4996	155				
HR (95% CI)[d]			Reference	0.71 (0.50-1.00)	0.53 (0.33-0.84)	*0.009*
Model 2[e]						
Rotterdam I	4104	108				
HR (95% CI)[c]			Reference	0.70 (0.46-1.06)	0.69 (0.40-1.20)	*0.19*
Alienor	539	38				
HR (95% CI)[c]			Reference	0.83 (0.38-1.80)	0.52 (0.19-1.40)	*0.23*
Overall	4643	146				
HR (95% CI)[d]			Reference	0.70 (0.49-1.01)	0.59 (0.37-0.95)	*0.04*

[a] p for trend is calculated using the median value for each Mediterranean Diet score category.

[b] Model 1, unadjusted model.

[c] estimated using Cox proportional hazard model.

[d] estimated using Cox proportional hazard model with additional adjustment for study.

[e] Model 2, adjusted for sex, total energy intake, AMD grade at baseline, education, body mass index, smoking, supplement use of multivitamins/minerals, presence of diabetes and hypercholesterolemia.

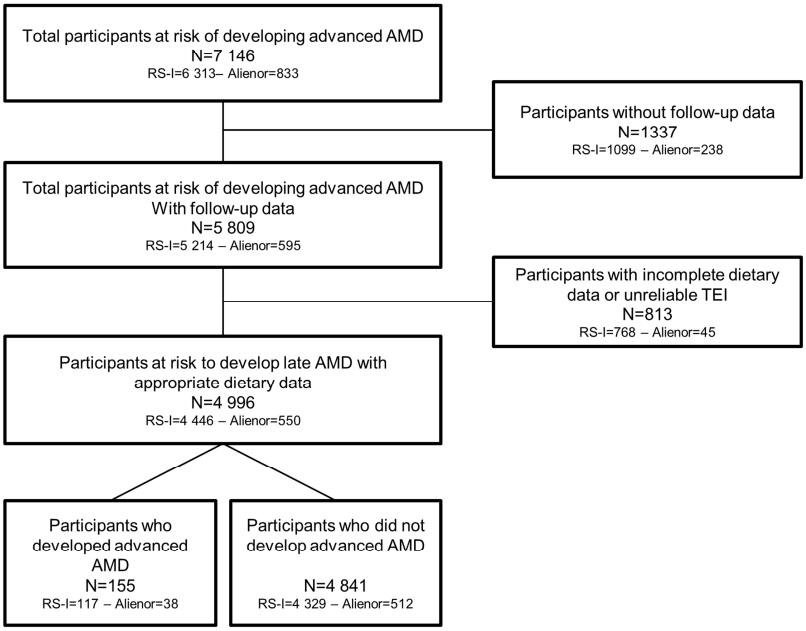
Table 3. Association between Mediterranean Diet score (MeDi) and incidence of advanced neovascular and atrophic age-related macular degeneration (AMD).

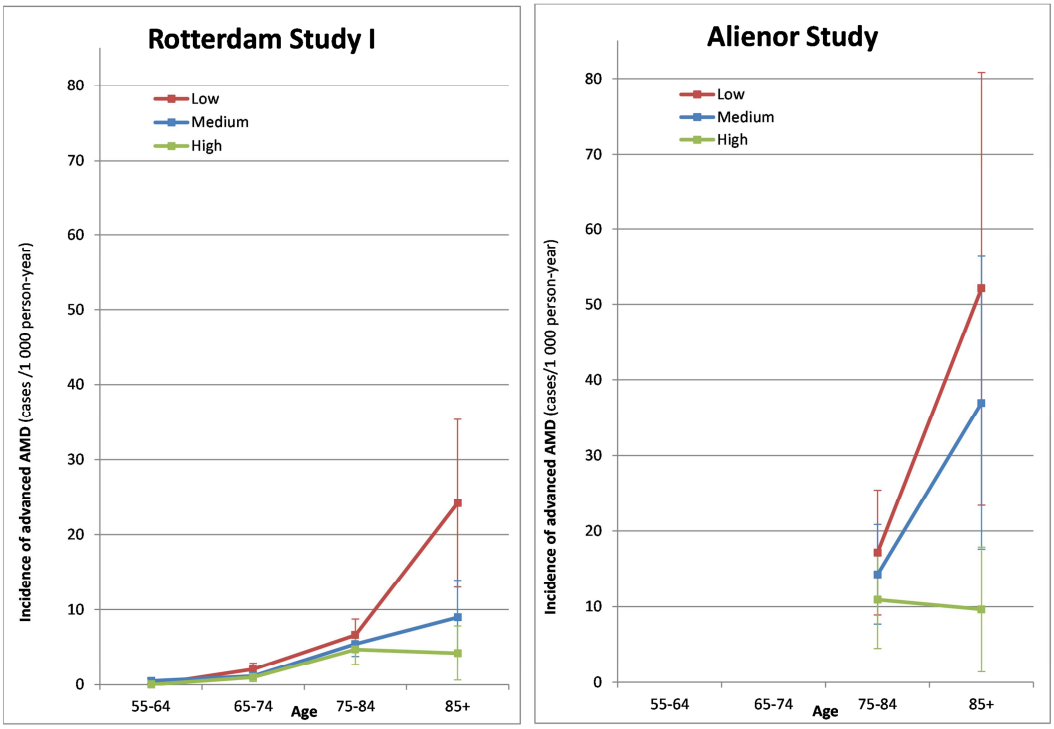
	No. at risk for advanced AMD	No. incident cases	Mediterranean Diet Score categories			P for trend[a]
			Low 0-3	Medium 4-5	High 6-9	
Neovascular AMD						
Rotterdam I	4104	68				
HR (95% CI)[b]			Reference	0.87 (0.51-1.51)	1.03 (0.53-1.99)	*0.91*
Alienor	538	18				
HR (95% CI)[b]			Reference	0.80 (0.25-2.63)	0.75 (0.20-2.91)	*0.65*
Overall	4642	86				
HR (95% CI)[c]			Reference	0.78 (0.48-1.27)	0.88 (0.49-1.57)	*0.64*
Atrophic AMD						
Rotterdam I	4104	52				
HR (95% CI)[b]			Reference	0.61 (0.34-1.10)	0.41 (0.16-1.03)	*0.046*
Alienor	538	21				
HR (95% CI)[b]			Reference	1.08 (0.38-3.06)	0.52 (0.13-2.12)	*0.52*
Overall	4642	73				
HR (95% CI)[c]			Reference	0.70 (0.42-1.15)	0.42 (0.20-0.90)	*0.04*

[a] p for trend is calculated using the median value for each MeDi score category.

[b] Cox proportional hazard model adjusted for sex, total energy intake, AMD grade at baseline, education, body mass index, smoking, supplement use of multivitamins/minerals, diabetes and hypercholesterolemia.

[c] Cox proportional hazard adjusted for sex, total energy intake, AMD grade at baseline, study, education, body mass index, smoking, supplement use of multivitamins/minerals, diabetes and hypercholesterolemia.





Highlights

We examined the association of the Mediterranean diet with incident AMD in two European population-based prospective cohorts. A higher adherence to the Mediterranean diet was associated with a reduced risk of developing advanced AMD.