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EXTENDED ABSTRACT

P22

Nutritional Patterns using Principal Component Analysis and Their Associations with Age-Related Macular Degeneration. The Coimbra Eye Study – Report 7

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Introduction

Age-related macular degeneration (AMD) is a chronic disease of the central retina that represents the leading cause of irreversible blindness in the elderly population in developed countries [1,2]. Therefore, there is a great need to identify preventive measures to delay or halt AMD progression, particularly in light of the current lack of effective pharmacological options for the dry form of the disease, present in 0.61% of the patients [3]. Nutritional intervention seems to hold some promise toward these ends and this was recently corroborated by trials from the Age-Related Eye Disease Study (AREDS) [4] and AREDS2 [5] that show that use of supplements containing vitamins C and E, lutein/zeaxanthin, and zinc delays progression of advanced AMD in subjects with intermediate AMD. Previous studies have suggested that certain micro- and macro-nutrients [4–9], namely lutein/ zeaxanthin [10,11] and long-chain Ω-3 fatty acids [12,13] are beneficial. However, the association of single nutrients with AMD have often been inconsistent across studies, and they are impossible to totally disentangle from other aspects of diet as they do not account for the synergistic relationship of food components [14].

Several studies have evaluated dietary combinations and AMD using predefined food groups or dietary patterns derived from principal components analysis (PCA) for each food item collected [15–20]. However, to the best of our knowledge, no study has evaluated the associations of nutrient consumption (obtained by conversion of the individual food intakes) and AMD using PCA. Preventive strategies through dietary modulation or supplements intake are attractive because they are easy to implement and relatively cheap. The aim of this study is to identify major nutrient intake patterns using PCA. Factor analysis is a statistical method which can summarize several nutrient items into a single factor which represents a major nutrient pattern [21]. Thus, by identifying such factors we can identify nutrient patterns that may be associated with AMD.

Methods

Study Design and Study Population - This is a case-control study (NCT01715870) nested in the "Epidemiological Study of the Prevalence of Age-Related Macular Degeneration in Portugal: The Coimbra Eye Study" (NCT01298674). This study was approved by the Association for Innovation and Biomedical Research on Light and Image (AIBILI) Ethics Committee and all subjects provided written informed consent (NCT01715870) [3,22]. For this study, a selected sample of subjects with AMD (Rotterdam classification [23] 1 to 4) and an age- and gender-matched control group (Rotterdam classification 0 – no AMD features or only drusen <63 μm) were invited to answer a validated lifestyle and food habits questionnaire. A total of 2007 subjects were included in this study but only 1992 could be considered for analysis due to AMD grading issues, 768 (38.6%) with AMD and 1224 (61.4%) without AMD. Details of this study have been previously reported [24].

Keywords:
AMD, Principal Component Analysis, nutrients, nutrition.

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AMD grading and staging - Under the Coimbra Eye Study, all subjects were subjected to a complete bilateral ophthalmological examination. Ocular images were graded for the presence of AMD or no AMD in a centralized reading center (Coimbra Ophthalmology Reading Center, CORC – AIBILI). Fifteen (15) subjects were excluded from analysis due to image quality issues. Details of AMD grading and staging have been previously reported [3].

Demographic and lifestyle data - The questionnaire include data on education, smoking habits and regular physical activity (any kind of exercise reported by the subject, such as walking, cycling, fitness, swimming, etc., at least once a week), and medical history, as well as a food frequency. The food frequency questionnaire was adapted from food frequency questionnaire of Willett et al. (1998) [25] and validated for the Portuguese population by the Faculty of Medicine of the University of Porto [26]. This questionnaire includes 86 types of food that are structured in nine major food groups: vegetables, legumes, fruits and nuts, cereals, fish, meat, dairy products, alcohol and a ratio of monounsaturated lipids (mainly olive oil) to saturated lipids. For each food, nine categories of frequencies are included, ranging from "never or less to once per month", to "six or more times per day". Food groups were obtained by merging food items according to their nutritional composition similarity. The questionnaire was completed during a single visit by specially trained interviewers and all questionnaires were checked for completeness. For each of the items, subjects were asked to report the frequency of their consumption in the past year, the portion size and whether or not this consumption was seasonal. The interviewer also measured each subject's weight, height (for BMI calculation) and abdominal perimeter.

Processing nutritional data - Food frequency was calculated for average daily consumption values, adjusted for the size of the portion to yield a value in grams (g) or millilitres (ml) per type of food. A factor of seasonal variation consumption was also included if indicated by the subject (0.25 for a period of 3 months). The average daily consumption values of 38 nutrients was obtained: water, protein, total fat, total available carbohydrates, total carbohydrates expressed monosaccharides, monosaccharides disaccharide, organic acids, alcohol, starch, oligosaccharide, fiber, saturated fatty acids, monounsaturated fatty acid, polyunsaturated fatty acid, trans fats, linoleic acid, cholesterol, total vitamin A, carotene, vitamin D, α-tocopherol, thiamine, riboflavin, niacin equivalent, niacin, tryptophan, vitamin B6, vitamin B12, vitamin C, folate, ashes, sodium, potassium, calcium, phosphorus, magnesium, iron and zinc. The adherence to the Mediterranean diet was also assessed using the mediSCORE. Details on how to obtain the macro- and micro-nutrient and how to assess the mediSCORE are published elsewhere [24].

Statistical Analysis - The PCA [27,28] of the 38 nutrients was performed to extract nutritional patterns. PCA is a mathematical algorithm that reduces the dimensionality of the data while retaining most of the variation in the data set [27]. PCA computes new variables, called principal components (PC), which are obtained as linear combinations of the original variables (i.e. the nutrients). The first PC is required to have the largest possible variance. The second PC is computed under the constraint of being orthogonal to the first PC and to have the largest possible variance, and so on. To measure the sampling adequacy of the data for factor analysis the Kaiser–Meyer–Olkin (KMO) index was used [29]. To determine the number of factors to retain, the eigenvalues of the correlation matrix, represented in the scree plot, and the interpretability of the factors were considered. Afterwards, for each subject, each factor score was estimated as the sum of products of the standardized nutrient daily consumption value multiplied by their loading. The loadings are the correlation coefficients for each variable on that factor, so that variables with higher loadings are those that tend to define the corresponding factor.

To evaluate the associations between the nutritional patterns and AMD a logistic regression model was used to estimate the odds ratio (OR) and 95% confidence intervals (CIs), adjusting for covariates. Covariates were based on the modifiable risk factors previously proposed, smoking [30–32], body mass index (BMI) [33] outside the normal range and physical activity [14,17]. Moreover, since adherence to the Mediterranean diet, assessed by the mediSCORE, was found to be protective of AMD [24], this score was included as a covariate to analyze its influence in the final model. The following characteristics were also considered as covariates: age, sex, abdominal perimeter, diabetes and hypertension history.

Categorical variables were summarized with frequencies and percentages and numerical variables with mean and standard deviations (SD). All statistical analyses were performed with Stata version 12.1 (StataCorp LP, College Station, TX, USA), and adjusted p-values less or equal than 0.05 were considered statistically significant. A Bonferroni correction was performed to address the multiple comparisons problem.

Results

The KMO measure of sampling adequacy obtained was equal to 0.83 being considered meritorious. First four (4) eigenvalues for the correlation matrix ranging from 19.7 to 2.2 were retained, accounting for 74% of the total variance that could be explained by the 38 nutrients (Figure 1). Factor loadings for the nutrients are shown in Table 1.

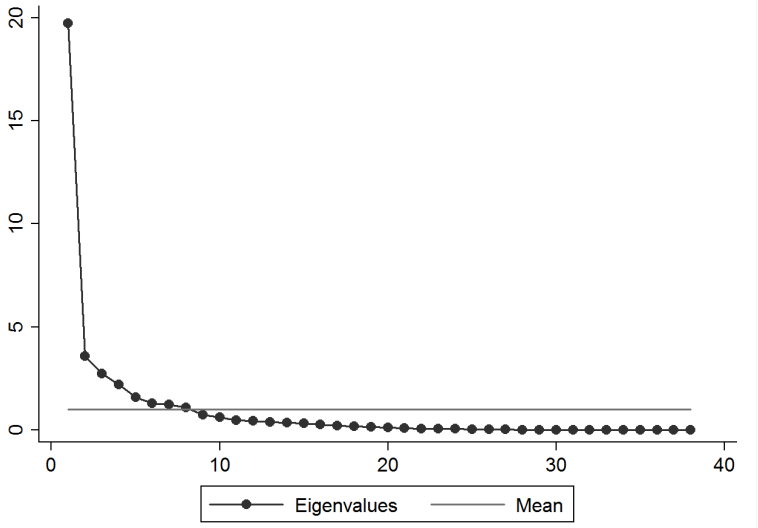

Figure 1 - Scree plot of eigenvalues after PCA.

Factor 1 is characterized by high and positive loadings for all the nutrients corresponding to a balanced intake of all nutrients. Factor 2 is characterized by the negative loadings for protein, total fat, monounsaturated and polyunsaturated fatty acids, linoleic acid, cholesterol, vitamin D, tryptophan and vitamin B12 corresponding to a low intake or deficiency in the intake of these nutrients. Factor 3 is characterized by the positive loadings for α-Tocopherol, carotene, vitamins A and C and folate corresponding to a high intake of these nutrients, and by the negative loadings for niacin and niacin equivalent, ashes, potassium and magnesium corresponding to a low intake or deficiency in the intake of these nutrients. Finally, factor 4 is characterized by negative loadings for total fat, saturated and monounsaturated fatty acids and trans fats corresponding to a deficiency in the intake of these nutrients.

Univariate logistic regression identified that AMD was statistically significantly associated with factor 1 (OR=0.96, 95% CI: 0.94-0.98; p=0.001) and factor 3 (OR=0.91, 95% CI: 0.86-0.96; p=0.001), having these two factors a protective role. For factors 2 and 4 there was no statistically significantly association with AMD (OR=0.97, 95% CI: 0.93-1.02; p=0.259; OR=0.97, 95% CI: 0.91-1.03; p=0.352, respectively). Considering the multivariate analysis, adjusted for the different covariates, the protective role of the two factors, 1 and 3, remained statistically significant (Table 2). Of the covariates included in the model only age, physical exercise, diabetes and hypertension history were statistically significant, being that older subjects were associated with higher odds for AMD (OR=1.02, 95% CI: 1.01-1.03, p=0.005) as well as subjects with a history of hypertension (OR=1.32, 95% CI: 1.10-1.60, p=0.004); oppositely, regular practice of physical exercise and the presence of diabetes were associated with lower odds for AMD (OR=0.79, 95% CI: 0.65-0.97, p=0.024; OR=0.74, 95% CI: 0.59-0.93, p=0.010, respectively).

Considering the adherence to the Mediterranean diet in the multivariate model, we obtained an OR=0.85 (95% CI: 0.66-1.10) without reaching statistical significance. In this study, subjects with a high adherence to the Mediterranean diet present a positive averaged score for the four factors while the subjects with a low adherence present an average score negative (Table 3).

Discussion and Conclusions

This study allows to study the relationship between nutrients intake and AMD. Using PCA, we identified 4 factors (nutrient patterns). We observed that a high score for factor 1, characterized by the balanced intake of all nutrients, and a high score for factor 3, characterized by a high intake of α-Tocopherol, carotene, vitamins A and C and folate and by the negative loadings for niacin and niacin equivalent, ashes, potassium and magnesium corresponding to a deficiency in the intake of these nutrients, were associated with a lower prevalence of AMD, adjusted for covariates.

These findings are consistent with the indications from the World Health Organization (WHO) that refers in the European Food and Nutrition Action Plan 2015–2020 [34] that promotion and accessibility of a healthy and varied diet (that is both available and affordable) is thus a key lever to improve the health, well-being and quality of life of the population, promoting healthy ageing and reducing health inequalities.

Regarding the Mediterranean diet, characterized as being rich in vitamins and minerals, derived from vegetables and fruits, whole-meal cereals, nuts, virgin olive oil and fish, which made the risk of deficient micronutrient intakes quite infrequent [35], previously our group found a significant association between a high adherence (mediSCORE ≥6) with a decreased risk for AMD [24]. The present analysis showed that

Table 1 - Factor loadings for the first 4 principal components. For simplicity, only nutrients with loadings (correlation between nutrients and factors) with absolute values of 0.3 or greater are shown Alcohol was removed due to the low loading values).

	Factor 1	Factor 2	Factor 3	Factor 4
Water	0.804	0.334		
Protein	0.880	-0.341		
Total fat	0.747	-0.406		-0.376
Total available carbohydrates	0.786	0.389		
Total carbohydrates expressed monosaccharides	0.785	0.380		
Monosaccharides disaccharide	0.656	0.525		
Organic acids		0.343		
Starch	0.659			
Oligosaccharide	0.461			
Fiber	0.771	0.480		
Saturated fatty acids	0.721			-0.545
Monounsaturated fatty acid	0.560	-0.409		-0.356
Polyunsaturated fatty acid	0.759	-0.432		
Trans fats	0.473			-0.610
Linoleic acid	0.743	-0.408		
Cholesterol	0.693	-0.466		
Total vitamin A	0.528		0.363	
Carotene	0.460	0.396	0.404	0.384
Vitamin D	0.403	-0.456		0.468
α-Tocopherol	0.653		0.453	
Thiamine	0.884			
Riboflavin	0.815			
Niacin equivalent	0.800		-0.481	
Niacin	0.680		-0.583	
Tryptophan	0.884	-0.332		
Vitamin B6	0.849			
Vitamin B12	0.593	-0.500		0.381
Vitamin C	0.547	0.504	0.378	
Folate	0.789	0.310	0.349	
Ashes	0.896		-0.382	
Sodium	0.830			
Potassium	0.766		-0.500	
Calcium	0.702			
Phosphorus	0.937			
Magnesium	0.821		-0.452	
Iron	0.885			
Zinc	0.865			
% of variance explained	**51.9**	**9.4**	**7.2**	**5.8**

Table 2 - Associations between Nutritional Factors and Age-related Macular Degeneration.

	OR	95% CI	P-value
Factor 1	**0.97**	**0.95 – 0.99**	**0.020**
Factor 2	0.96	0.91 – 1.01	0.087
Factor 3	**0.92**	**0.86 – 0.97**	**0.003**
Factor 4	1.01	0.94 – 1.08	0.821
Age (yrs)	**1.02**	**1.01 – 1.03**	**0.005**
Sex (M/F)	0.87	0.68 – 1.11	0.258
BMI (kg/m2)	1.00	0.97 – 1.04	0.801
Abdominal Perimeter (cm)	0.99	0.98 – 1.00	0.227
Physical Activity (Y/N)	**0.79**	**0.65 – 0.97**	**0.024**
Smoking Status:			
Non Smoker	-	-	-
Former Smoker	1.23	0.93 – 1.64	0.147
Smoker	1.21	0.70 – 2.10	0.490
Diabetes (Y/N)	**0.74**	**0.59 – 0.93**	**0.010**
Hypertension History (Y/N)	**1.32**	**1.10 – 1.60**	**0.004**
Supplement Intake (Y/N)	1.31	0.86 – 1.98	0.204
mediSCORE (n/a)	0.85	0.66 – 1.10	0.218

AMD = age-related macular degeneration; CI = confidence interval; OR=odds ratio. Statistically significance at p<0.05 are shown in bold.

Table 3 - Associations between Nutritional Factors* and adherence to the Mediterranean diet.

Nutritional factors	MediSCORE <6 (n=1596) Mean±SD	MediSCORE ≥6 (n=396) Mean±SD	Adjusted p-value
Factor 1	-0.47±4.33	1.88±4.39	<0.001
Factor 2	-0.06±1.80	0.25±2.22	0.003
Factor 3	-0.13±1.61	0.52±1.71	<0.001
Factor 4	-0.20±1.41	0.81±1.50	<0.001

*For each subject, each factor score was computed as the sum of products of the standardized nutrient daily consumption value multiplied by their loading.

subjects with a high adherence to the Mediterranean diet presented an average score for factor 1 five times higher than the group of subjects with a low adherence to the Mediterranean diet. Regarding factor 3, subjects with a high adherence to the Mediterranean diet presented an average score also significantly higher than the group of subjects with a low adherence. This reinforce the idea that the Mediterranean diet is well balanced, characterized by a high intake of α-Tocopherol, carotene, vitamins A and C and folate and by a low intake of niacin and niacin equivalent, ashes, potassium and magnesium, and that a high adherence to this diet has a protective effect on the development of AMD.

Several limitations can be identified in this study, being common to most of the studies published. The limitations of this study include the "healthy user bias", which is consistent across most epidemiologic studies. This reflects a tendency for healthier individuals to be more likely to adhere to a preventive strategy or healthy behaviors [36]. In this case, it is possible that "healthy users" have less severe disease because they have more frequent routine eye care, greater adherence to screening and prevention strategies, and more motivation and health consciousness, which is also reflected in their levels of physical activity. In addition, lifestyle and dietary data were based on self-reported information. Another limitation of this study is its cross-sectional nature, which does not allow us to analyze the development of AMD. In fact, due to the type of the study no causal inferences can be made regarding the associations found. We also did not take into account the influence of genetic factors and their potential interaction with the remaining risk parameters and dietary interactions in AMD. Regarding supplement intake (defined as vitamins and/or minerals supplements available on the market) the only collected information was if the subject take any supplement. The lack of detail of these supplements did not allow us to include this pharmacological variables in the model. Lastly, our measurement of diet was based on a single questionnaire administered in a single visit, and this may not have been representative of lifelong consumption. Random error in measuring dietary intake also is likely to have attenuated our associations. However, we adjusted the analysis for the presence of diseases such as diabetes that may be associated with dietary change.

In conclusion, our results show that a frequent consumption of balanced intake of all nutrients, resulting from a balanced diet, but with a low intake of niacin and niacin equivalent, ashes, potassium and magnesium was associated with a lower prevalence of AMD.

Thus, our results evidence that the choices we make about foods consumption and consequently of the nutrients intake may play a role in contributing to the risk of developing AMD. More than the selection of some foods, the intake of different and varied foods, resulting in a balanced diet, shows to be protective for the development of AMD.

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