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The American Journal of Clinical Nutrition

Diet and risk factors for age-related maculopathy[1–3]

Eamonn D O'Connell, John M Nolan, Jim Stack, David Greenberg, Janet Kyle, LeighAnne Maddock, and Stephen Beatty

ABSTRACT

Background: Evidence continues to accumulate that oxidative stress is etiologically important in the pathogenesis of age-related maculopathy (ARM) and that appropriate antioxidants of dietary origin may protect against this condition.

Objective: Risk factors for ARM may be classed as established or putative. We report a study designed to investigate whether such risk factors are associated with a dietary lack of antioxidants relevant to retinal health.

Design: Dietary, anthropometric, and sociodemographic details relating to 828 healthy Irish subjects aged 20–60 y were recorded in a cross-sectional fashion and analyzed for associations between risk factors for ARM and dietary intake of relevant nutrients.

Results: Of the established risk factors for ARM, increasing age was associated with a relative lack of dietary zeaxanthin ($P < 0.05$) and tobacco use with a relative lack of dietary vitamin C ($P < 0.05$). Of the putative risk factors for ARM, alcohol consumption was associated with a relative lack of dietary α-linoleic acid ($P < 0.05$), and female sex was associated with a relative lack of dietary zinc ($P < 0.05$).

Conclusions: We showed that several variables related to risk for ARM are associated with a relative dietary lack of key nutrients. Our finding that age, the most important and universal risk factor for ARM, is associated with a relative lack of dietary zeaxanthin, is an important finding that warrants further investigation. *Am J Clin Nutr* 2008;87:712–22.

KEY WORDS Age-related macular degeneration, age-related maculopathy, antioxidants, lutein, n−3 fatty acids, zeaxanthin

INTRODUCTION

Age-related maculopathy (ARM) is a degenerative condition of the macula, the advanced form of which results in the loss of central vision (1). At present, late ARM (advanced ARM/age-related macular degeneration) is the leading cause of blindness in the developed world (2). Although the etiopathogenesis of ARM remains uncertain, a growing body of evidence indicates that oxidative stress may play a central role (3, 4). It follows that the risk of ARM is dependent on factors that provoke oxidative stress (or tissue susceptibility to oxidative stress) or that influence the retina's antioxidant defenses.

Numerous variables are associated with an increased risk of ARM. For some of these variables (risk factors), a rationale whereby they contribute to ARM pathogenesis can be readily expounded, eg, cigarette smoking is believed to increase oxidant load (5). For others, however, a causal link consistent with an etiological role for reactive oxygen intermediates is less obvious. It is possible that some risk factors for ARM are associated with poor dietary intake of key nutrients, which are an essential component of the retinal antioxidant defense system, and accounting for, at least in part, the risk that the variable in question represents for this condition.

Defense mechanisms against retinal oxidative stress include endogenous antioxidant enzymes (glutathione, superoxide dismutase, and catalase) and exogenous antioxidants (carotenoids, bioflavonoids, selenium, zinc, and vitamins A, C, and E). Of note, the exogenous antioxidants are entirely of dietary origin. Indeed, the Age-Related Eye Disease Study (AREDS) has shown that fortification of retinal antioxidant defenses with vitamins C and E, β-carotene, and zinc reduces the risk of visual loss among individuals with ARM (6). The AREDS did not include the dietary carotenoids that make up the macular pigments lutein and zeaxanthin, because these compounds were unavailable in supplement form at the inception of that study. Macular pigment is entirely of dietary origin, is ideally located, and exhibits important optical and antioxidant properties (3), which suggest that this pigment protects against ARM.

In this study we tested whether risk factors for ARM are associated with the dietary intake of nutrients, which are important to retinal antioxidant defenses, in subjects aged 20–60 y. The importance of investigating the dietary intake of key retinal antioxidants in this age group rests on the fact that a protective effect against chronic and cumulative oxidative stress, if any, will need to be exerted in young to middle age.

SUBJECTS AND METHODS

Subjects

Eight hundred twenty-eight healthy subjects volunteered to participate in this study, which was endorsed by the Research

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Ethics Committee of Waterford Regional Hospital and the Ethics Committee of the Waterford Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki. The first subject (001) was recruited on the 25 November 2002, and the last subject (828) was recruited on 16 March 2005.

Subjects were recruited to this single-visit study at the Waterford Institute of Technology by 1 of 2 means: *1*) a self-selected sample population volunteered in response to posters, newsletters, and word of mouth in the local community (group 1; n = 647), and *2*) patients attending the Department of Ophthalmology at Waterford Regional Hospital with ARM (early, late, or both) were encouraged to invite their offspring to participate (group 2; n = 181). All subjects in group 2 had documented evidence of clinically confirmed ARM (early, late, or both) in the relevant parent, provided by the consulting ophthalmologist who made the diagnosis. Early ARM was defined as the presence of characteristic macular changes (drusen, pigment clumping, and retinal pigment epithelial atrophy) in the absence of visual symptoms. Late ARM was defined as the presence of characteristic macular changes with related visual symptoms. Late ARM was further divided into 2 subgroups: wet ARM (characterized by the presence of choroidal neovascularization and its sequelae in the absence of other contributory retinal pathology) and atrophic ARM (characterized by the presence of features of late ARM in the absence of choroidal neovascularization or its sequelae). Inclusion criteria included white race and age between 20 and 65 y, and exclusion criteria were any ocular pathology, visual acuity <6/18 in both eyes, and pregnancy.

Fundus and iris photography

Photographic documentation of fundus and iris were obtained from each subject with the use of a NIDEK (Gamagori, Japan) handheld fundus camera. Fundus photography was conducted to ensure that none of the subjects included in the study had any form of ocular pathology, in keeping with the study's exclusion criteria. An experienced ophthalmologist inspected all images, and no subject was excluded on the basis of fundus findings (possibly because of our age inclusion criteria).

Iris photography was conducted for the purpose of documentation of the color of the subjects' irides. The color of the subjects' irides was then graded by matching each subject's iris color (obtained from the photograph) to standard iris colors on a Cooper Vision iris color chart. In addition, iris color was graded based on self-report. In 96% of cases, the subject's self-reported iris color matched the iris color as assessed by the photographic grading system we used. For the 4% of cases that did not match, we used the results of the photographic documentation. Of note, these are the most common methods used to grade iris color in epidemiologic studies (7–10).

Visual acuity testing

In a parallel arm of this study reported elsewhere (11), the subjects' macular pigment optical densities were measured with equipment (Maculometer device developed by Professor Mellerio, School of Biosciences, University of Westminster, London, United Kingdom) (12) that required a best-corrected visual acuity of no worse than 6/18 in the study eye. Consistent with this requirement, the subjects' best-corrected visual acuities (Snellen and LogMAR) were measured for each eye with a Lighthouse Distance Visual Acuity Test (2nd ed; Lighthouse International, New York, NY). No subjects were excluded from the study on the basis of level of best-corrected visual acuity.

Body mass index

Body mass index (BMI), a weight-to-height ratio, was obtained by recording each subject's height (m) and weight (kg). Height was measured with a Leicester height measure (SECA, Hamburg, Germany) portable stadiometer. Subjects were asked to stand with their backs to the wall with footwear removed. The measurement was taken with the head level, and readings were rounded to the nearest centimeter. Weight was measured with a heavy-duty digital balance (SECA Alpha). Subjects were asked to stand on the balance with footwear removed, and weight was recorded in kilograms. BMI was calculated in kg/m^2.

Personal details questionnaire and risk factor index

Each subject completed a personal details questionnaire to record demographic, lifestyle, and personal medical details, with emphasis on characteristics related, putatively or otherwise, to risk of ARM based on a review of the literature.(13, 14)

Lifestyle details recorded included alcohol consumption and tobacco use. Alcohol consumption was recorded, semiquantitatively, as the estimated average units of alcohol consumed per week as follows: never drinks alcohol, drinks 0–1 unit of alcohol/wk, drinks 2–5 units of alcohol/wk, drinks 6–10 units of alcohol/wk, and drinks >10 units of alcohol/wk. One unit of alcohol was defined as 10 g ethanol, in accordance with Irish standards (15). Tobacco use was recorded as follows: never smoker of cigarettes, current cigarette smoker (including the number of cigarettes smoked per day), past cigarette smoker (including the number of cigarettes smoked per day and date of cessation of cigarette smoking), and other tobacco use (including type and quantity of use). For the purpose of analyzing any association between cigarette smoking and dietary intake of key micronutrients, past cigarette smokers and subjects who had never smoked cigarettes were considered together as nonsmokers. Current cigarette smokers were subdivided into those who smoked <20 cigarettes/d and those who smoked >20 cigarettes/d. Three persons who reported smoking cigars and 2 persons who reported smoking a pipe were considered as nonsmokers.

Personal medical details recorded included a known history of: cardiovascular disease, including high blood pressure; high serum cholesterol concentration; diabetes; and eye disease. The subjects' refractive status and use of medications and dietary supplements was also recorded. Questionnaires were reviewed immediately after completion, and subjects were asked to complete any missing items to prevent blank responses.

Food-frequency questionnaire

A self-administered, semiquantitative food-frequency questionnaire (FFQ) developed by the Scottish Collaborative Group was used to assess the dietary intake of relevant nutrients. The semiquantitative FFQ is the primary dietary assessment method used in epidemiologic studies (16). This FFQ was developed based on FFQs used in the Scottish Heart Health Study (17) and was previously validated against weighed food records and biomarkers (18–21).

The questionnaire used is a research instrument and was developed to estimate daily intake of a wide range of nutrients in large-scale epidemiologic studies in the adult population. The questionnaire is designed to estimate habitual diet over the previous 2–3 mo, which includes 166 commonly eaten types of food or drink grouped into 19 selections. A portion or measure for each food was specified, and subjects were asked to record how many measures per day and how many days per week they consumed the food, ranging from "rarely or never" to "7 days per week." A "measure" was designed to be a small portion so that a single standard portion of a food would often be composed of 2 measures. The questionnaire included an example of how to fill in the questionnaire and a color photograph depicting examples of food measures. In addition to assessment of a subject's normal dietary intake of various foodstuffs, this FFQ was specifically designed to assess and control for supplement use. The questionnaire was completed by subjects under supervision and took between 25 and 35 min to complete. The questionnaire was reviewed immediately after completion, and subjects were asked to complete any missing items to prevent blank responses.

To assess dietary intakes of lutein and zeaxanthin, separately, the MRC Human Nutrition Research (Cambridge, United Kingdom) conducted an extensive search of the literature and databases designed to identify reports of estimation of lutein and zeaxanthin contents of the 569 individual foods, which were combined to make up the 166 commonly eaten food types in the questionnaire. Sources of data included, but were not limited to, The UK Ministry of Agriculture, Fisheries and Food (analytic report no: AS50/97/05), which reports the individual lutein and zeaxanthin contents of 50 foods; the US Department of Agriculture National Nutrient database, which also reports the zeaxanthin (alone) content of 22 foods; Kellogg Co, Manchester, United Kingdom, who kindly provided an in-house analysis of the individual lutein and zeaxanthin contents of the breakfast cereal cornflakes; and Sommerburg et al (22) and other investigators (23–25), who contributed to a large body of literature on the specific proportions of carotenoids and xanthophylls in a wide range of foods, and these proportions were used to calculate the individual lutein and zeaxanthin contents of foods based on the reported contents of other carotenoids. Furthermore, 2 of these studies (24, 25) have shown trends of decreasing lutein and increasing zeaxanthin during fruit ripening, and this phenomenon was taken into account when estimating the nutrient content of these foods.

Calculations based on the above data were used to estimate the lutein and zeaxanthin contents of the 569 foods included in the questionnaire. Using this approach, 141 foods (combined into 56 commonly eaten food types) were deemed to be sources of lutein, and 82 foods (combined into 33 commonly eaten food types) were deemed to be sources of zeaxanthin.

Estimations of the dietary intake of lutein, so calculated, were shown to be positively and significantly correlated with serum concentrations of lutein ($r = 0.28$) and zeaxanthin ($r = 0.237$), respectively (11). Both of these correlations are significantly different from zero ($P < 0.001$); however, the only significant partial correlation ($P < 0.001$) was that between dietary lutein and serum lutein, when serum zeaxanthin was controlled for. From this, it seems reasonable to conclude that the real relation is that between dietary lutein and serum lutein, which in turn may be interpreted as suggesting that the dietary questionnaire was indeed estimating dietary intake of lutein. Indeed, such a correlation of dietary estimates with serum biomarkers is a recognized method of validation of FFQs (26). Also, the strength of these correlations is in keeping with the strength of correlations between dietary intake and serum concentrations of carotenoids reported by previous authors using other methods of dietary estimation (27).

Similarly, the positive relation between dietary intake of zeaxanthin and serum zeaxanthin was stronger than that between dietary intake of zeaxanthin and serum lutein ($r = 0.237$ and $r = 0.16$, respectively), and only the partial correlation between dietary intake of zeaxanthin and serum zeaxanthin was significant when serum lutein was controlled for ($P < 0.001$). Analogous conclusions to those in the preceding paragraph may be drawn, in that dietary zeaxanthin is significantly related to serum zeaxanthin, which suggests that the dietary intake of zeaxanthin has been successfully and independently estimated by using our questionnaire.

The FFQs were scanned and verified by a trained dietary data coder using optical recognition software (Teleform, version 7; Cardiff Software, Vista, CA) at the Medical Research Council, Human Nutrition Research, Cambridge. Nutrient analysis was conducted by using Oracle Relational Database Management System (version 7) by the University of Aberdeen. Analyses of the FFQ generated continuous semiquantitative data regarding subjects' daily dietary intake of 45 nutrients. After a review of the literature, 11 of these nutrients (lutein, zeaxanthin, vitamin A, vitamin C, vitamin E, selenium, zinc, α-linolenic acid, eicosapentaenoic acid, decosahexaenoic acid, and total n−3 fatty acids) were deemed particularly relevant to retinal health.

Statistical analysis

Data entry

The statistical software package SPSS (version 12.0.1; SPSS Inc, Chicago, IL) was used for analysis. Initially, all data from the "personal details questionnaire/risk factor index" was entered into an Excel (Microsoft, Redmond, WA) spreadsheet and then transferred to the SPSS file. Dietary data, processed by the University of Aberdeen, was received in the form of an Excel spreadsheet and transferred to the SPSS file. Care was taken to match each subject's dietary data with his or her "personal details questionnaire/risk factor index" data, before analysis. Random checks were made by another researcher to ensure that all data were entered correctly. Bias in data entry and measurement was avoided, where possible, as detailed standard operating procedures (SOPs) were constructed for each measurement (eg, height and weight for the calculation of BMI) before this research began. These SOPs were adhered to at all times during data collection.

Data cleaning

Before the analysis, criteria for acceptable data quality were carefully considered. A common issue with dietary data is the treatment of questionnaires in which some food items have been left blank. In this study, reviewing forms immediately after completion and asking participants to complete any missing items prevented blank responses. Therefore, no participants were excluded from the analysis for this reason.

After the analysis of the FFQs, the total energy intake of some subjects was implausibly high or low. Extreme values for dietary

intake of nutrients, particularly at the higher end of the distribution, may represent improper completion of the questionnaire (requiring exclusion) or may simply reflect unusual patterns of food intake (and merit inclusion). However, because total energy intake is physiologically fixed within a fairly narrow range, exclusion of subjects with implausibly high and low energy intakes may be justified. Therefore, we excluded subjects with total energy intakes outside the range of 500 to 3500 kcal/d for women and of 800 to 4000 kcal/d for men, as recommended by Willett (28), to produce a new set of dietary data. Both clean (after exclusion of subjects with improbable total energy intake) and unclean (before exclusion of subjects with improbable total energy intake) data sets were analyzed.

Energy adjustment

Total energy intake deserved further consideration in our study for 3 reasons: *1*) the level of energy intake may be a primary determinant of ARM (29); 2) individual differences in total energy intake produce variation in intake of specific nutrients unrelated to dietary composition, because the consumption of most nutrients is positively correlated with total energy intake; and *3*) if energy intake is associated with ARM, but not causally so, the effects of specific nutrients may be distorted or confounded by total energy intake. Therefore, when the relation between micronutrient intake and risk factors for ARM is analyzed, it must be decided whether to analyze micronutrient intakes in terms of absolute intake or relative to total caloric intake (energy-adjusted intake).

In this study we analyzed the relation between both absolute and energy-adjusted micronutrient intake and risk factors for ARM. Energy adjustment of nutrient intake was performed by using both nutrient densities and the residuals method. Both techniques were performed in accordance with previously published descriptions (30). Nutrient densities are measures of dietary composition computed by dividing absolute nutrient intake by total caloric intake. In our study we divided daily nutrient intake (in g) by daily energy consumption (in kcal) and multiplied this value by 1000 to calculate nutrient density in mg/kcal. Energy adjustment with the use of the residuals method was performed as follows: for each nutrient, linear regression analysis was performed with antioxidant intake as the dependent variable and total energy intake as the independent variable. This regression equation was used to calculate the expected mean antioxidant intake of the study population for the mean total energy intake of the study population. Next, for each individual, the energy-adjusted intake was calculated by adding the expected mean antioxidant intake of the study population to the residual derived from the regression analysis.

Seasonal variation in dietary intake of micronutrients

To assess whether seasonality was associated with dietary intake of key antioxidant nutrients, one-factor analysis of variance was used to test for a statistically significant variation in the absolute and energy-adjusted dietary intake of each antioxidant nutrient according to the month of completion of the FFQ and according to the season of its completion (spring: March, April, and May; summer: June, July, and August; autumn: September, October, and November; and winter: December, January, and February).

Data analysis

Descriptive statistics were used to summarize the population's demographics and its dietary intake of nutrients deemed relevant to retinal health.

Multiple linear regression analysis

Multiple linear regression analysis was used to determine associations between dietary intakes of micronutrients deemed relevant to retinal health (dependent variable) and risk factors (both known and putative) for ARM (independent variables). Qualitative independent variables (such as sex and family history of ARM) were incorporated into the regression equation by using indicator variables. For each nutrient, all risk factors were entered into the initial model. Subsequent stepwise removal of the least-significant risk factor from the model and refitting of the equation with the remaining risk factors was continued until all remaining risk factors were found to be associated with the nutrient in question in a statistically meaningful way.

Broad-spectrum antioxidant index

Because some risk factors were found to have significant associations with the dietary intake of a number of micronutrients that might be expected to have opposing effects on retinal health (eg, we found that cigarette smoking is associated with a decreased energy-adjusted dietary intake of vitamin C but with an increased energy-adjusted dietary intake of vitamin A; *see* Results and **Table 1**), and because the relevant importance of these micronutrients is unknown, a broad-spectrum antioxidant index was constructed to compare the incidence of risk factors for ARM among those with the highest dietary intakes of the 5 antioxidants (lutein, zeaxanthin, vitamin C, vitamin E, and zinc) believed most likely to be protective against ARM, with the prevalence of the same risk factors among those with the lowest dietary intake of these 5 antioxidants.

Tabulation of results

Each row of Table 1 presents the results of a linear regression model. Each linear regression model explains the variation in the dietary intake of the micronutrient (dependent variable) named in the leftmost column in terms of its association with the risk factors for ARM (independent variables). The P values in each row represent the probability that the true regression coefficient for the corresponding risk factor could be zero; P values are provided only for those risk factors included in the final model (ie, where $P < 0.05$).

P values in bold and italic font indicate that the risk factor is associated with a decreased dietary intake of the antioxidant in the model (ie, where the regression coefficient is negative). P values in normal font indicate that the risk factor is associated with an increased dietary intake of the antioxidant in the model (ie, where the regression coefficient is positive).

For example, in Table 1 it can be seen that the nutrient density of zeaxanthin is related to age and sex in a statistically significant way, but that no other risk factors for ARM were found to significantly affect the nutrient density of this carotenoid when these 2 variables were included in the model. The nutrient density of zeaxanthin was significantly lower in older individuals than in younger individuals ($P = 0.025$) and is shown in bold and italic font. Conversely, the nutrient density of zeaxanthin was significantly higher in female subjects than in their male counterparts

TABLE 1
Relation between risk factors for age-related maculopathy (ARM) and nutrient intake by multiple linear regression analysis[1]

Nutrient (dependent variable)	R^2	P for independent variable[2]					
		Age	Female sex	Cigarette smoking	Alcohol intake	Iris color	High cholesterol
Absolute intake							
Lutein	0.018	—	0.008	—	—	***0.008***	—
Zeaxanthin	0.024	***<0.001***	—	—	—	—	—
Vitamin A	0.05	0.004	***<0.001***	0.007	—	—	***0.002***
Vitamin C	0.018	—	0.002	***0.033***	—	—	—
Vitamin E	0.02	***<0.001***		—	—	—	—
Selenium	0.022	—	***<0.001***	—	—	—	—
Zinc	0.128	—	***<0.001***	—	—	—	***0.023***
ALA	0.053	—	***<0.001***	—	***0.036***	—	—
EPA	0.015	0.001	—	—	—	—	—
DHA	0.012	0.003	—	—	—	—	—
n−3 FAs	0.029	—	***<0.001***	—	—	—	—
Nutrient density							
Lutein	0.061	—	<0.001	—	—	***<0.001***	—
Zeaxanthin	0.04	***0.025***	<0.001	—	—	—	—
Vitamin A	0.037	<0.001	—	0.004	—	—	***0.004***
Vitamin C	0.095	0.012	<0.001	***0.03***	—	—	—
Vitamin E	0.046	—	<0.001	—	***0.024***	—	—
Selenium	0.009	0.011	—	—	—	—	—
Zinc	0.022	<0.001	—	—	—	—	***0.045***
ALA	0.026	0.001	—	—	***0.008***	—	—
EPA	0.03	<0.001	—	—	—	—	—
DHA	0.026	<0.001	—	—	—	—	—
n−3 FAs	0.041	<0.001	—	—	***0.039***	—	—
Energy-adjusted intake							
Lutein	0.041	—	<0.001	—	—	***0.004***	—
Zeaxanthin	0.038	***0.002***	<0.001	—	—	—	—
Vitamin A	0.038	<0.001	—	0.007	—	—	***0.003***
Vitamin C	0.055	—	<0.001	***0.014***	—	—	—
Vitamin E	0.04	—	<0.001	—	***0.014***	—	—
Selenium	0.006	0.028	—	—	—	—	—
Zinc	0.016	0.004	***0.021***	—	—	—	—
ALA	0.021	0.014	—	—	***0.004***	—	—
EPA	0.027	<0.001	—	—	—	—	—
DHA	0.022	<0.001	—	—	—	—	—
n−3 FAs	0.033	<0.001	—	—	***0.046***	—	—

[1] ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAs, fatty acids; R^2, coefficient of determination. Cardiovascular disease (including high blood pressure), BMI, and family history of ARM were not found to be significantly associated with variation in the dietary intake of any of the nutrients examined, regardless of adjustment for energy intake.

[2] P values (probability that true regression coefficient = 0) represent the significance of the relation between micronutrient intake and risk factors for ARM for the 754 subjects analyzed after data cleaning. The standard t test for each regression coefficient was carried out in all cases. Each row presents the results of a linear regression model for variation in the dietary intake of the nutrient (dependent variable) named in the leftmost column. P values are shown only for the risk factors (independent variables) included in the final model. P values in bold and italic font indicate that the risk factor is associated with a decreased dietary intake of the antioxidant in the model (ie, where the regression coefficient is negative). P values in normal font indicate that the risk factor is associated with an increased dietary intake of the antioxidant in the model (ie, where the regression coefficient is positive).

($P < 0.001$) and is shown in normal font. The coefficient of determination (R^2) for this model is 0.04, which indicates that the variation in the independent variables included in the model (in this case age and sex) accounts for 4% of the total variation in the dependent variable being tested (in this case the nutrient density of zeaxanthin).

Throughout this manuscript we use the term *relative lack* (of dietary intake of a variable) when the concentration of the variable in the diet is significantly lower ($P < 0.05$) in one group than in another. The term *relative lack* is the most scientifically appropriate and accurate means of describing any comparative deficiency of a variable. We believe that it is preferable to terms such as *deficiency*, because the concentrations in question do not necessarily represent a dietary deficiency in absolute terms.

RESULTS

The results of analyses for clean and unclean data sets were broadly similar; therefore, the results of the analyses for the unclean data sets were omitted from the manuscript but are

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TABLE 2
Demographic and lifestyle characteristics of the subjects[1]

	Total	Sex		p^2
		Male	Female	
	n (% of total)	*n (% of total)*		
Age group				0.002
20–29 y	151 (18)	63 (24.1)	88 (17.8)	
30–39 y	155 (18.5)	65 (24.9)	90 (18.3)	
40–49 y	187 (22.3)	47 (18)	140 (28.4)	
50–59 y	234 (27.9)	81 (31)	153 (31)	
>60 y	27 (3.2)	5 (1.9)	22 (4.5)	
BMI				<0.001
≤18.5 (underweight)	9 (1.1)	0 (0)	9 (1.8)	
18.5–24.9 (normal)	428 (51)	117 (44.8)	311 (63.5)	
25–29.9 (overweight)	225 (26.8)	109 (41.8)	116 (23.7)	
30–34.9 (obese)	67 (8)	31 (11.9)	36 (7.3)	
35–39.9 (obese)	17 (2)	3 (1.1)	14 (2.9)	
≥40 (extremely obese)	5 (0.6)	1 (0.4)	4 (0.8)	
Family history of ARM				0.256
None	588 (78)	206 (78.9)	382 (77.5)	
Early	92 (12.2)	28 (10.7)	64 (13)	
Late (dry ARM)	26 (3.4)	6 (2.3)	20 (4.1)	
Late (wet ARM)	48 (6.2)	21 (8)	27 (5.5)	
Smoking habits				0.338
Nonsmoker	604 (80.2)	214 (82.3)	390 (79.1)	
Smoker, <20 cigarettes/d	106 (14.1)	30 (11.5)	76 (15.4)	
Smoker, >20 cigarettes/d	43 (5.7)	16 (6.2)	27 (5.5)	
Alcohol consumption				<0.001
<10 units/wk	625 (83.2)	178 (68.7)	447 (90.9)	
>10 units/wk	126 (16.8)	81 (31.3)	45 (9.1)	
Iris color				0.093
Dark brown, chestnut	52 (6.9)	19 (7.3)	33 (6.7)	
Light brown hazel	56 (7.5)	12 (4.6)	44 (9)	
Green, blue grey	643 (85.6)	230 (88.1)	413 (84.3)	
Cardiovascular disease (self reported), including high blood pressure				0.506
Known personal history	68 (9)	26 (10)	42 (8.5)	
No known personal history	684 (91)	234 (90)	450 (91.5)	
High cholesterol (self reported)				0.98
Known history	95 (12.6)	33 (12.7)	62 (12.6)	
No known history	656 (87.4)	227 (87.3)	429 (87.4)	

[1] ARM, age-related maculopathy.
[2] For significant difference between descriptive statistics of male and female subgroups for each risk factor. The standard chi-square test for contingency tables was used in all cases.

available on request from the corresponding author. Throughout this section, we report on clean data sets only.

Demographics

The FFQs of 70 individuals were considered unreliable because they suggested energy intakes that were deemed physiologically unlikely. After the exclusion of data relating to these individuals, data from the remaining 754 individuals were analyzed (**Table 2**).

The sample consisted of 261 (34.6%) men and 493 (65.4%) women. The age range of the sample was 20–64 y ($\bar{x}$ ± SD: 42.45 ± 11.64 y). The mean (±SD) age for men and women was 41.03 ± 12.01 and 43.19 ± 11.39 y, respectively.

The mean (±SD) BMI (kg/m^2) was 25.1 ± 4.28 and was significantly greater ($P < 0.001$) for the men (26.03 ± 3.74) than for the women (24.6 ± 4.46). The subjects' estimated average weekly alcohol consumption (n; %) was as follows: none (128; 16.9%), <2 units (111; 15.3%), 2–5 units (239; 31%), 5–10 units (147; 19%), and >10 units (126; 16.8%).

Dietary intake of micronutrients

The dietary intake of all micronutrients was analyzed in terms of absolute intake, energy-adjusted intake, and energy-adjusted (with the residuals method) intake. The mean (±SD) absolute, energy-adjusted (nutrient density), and energy-adjusted (with the residuals method) dietary intakes of micronutrients were analyzed and are presented in **Table 3**.

Seasonal variation in dietary intake of micronutrients

There was no statistically significant variation in the subjects' absolute or energy-adjusted nutrient intake with respect to either the month or the season in which the FFQ was completed for any

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TABLE 3
Descriptive statistics relating to the daily absolute, nutrient-density, and energy-adjusted (by the residuals method) dietary intakes of antioxidants and n−3 fatty acids (FAs) in 754 subjects, analyzed after data cleaning, and Dietary Reference Intakes (31, 32)[1]

					Dietary Reference Intake			
					Males aged 20–60 y		Females aged 20–60 y	
Nutrient	Range	Mean	SD	SE	RDA	UL	RDA	UL
Absolute intake								
Zeaxanthin (mg/d)	0.13 to 0.97	0.19	0.11	0.004	ND	ND	ND	ND
Lutein (mg/d)	0.7 to 8.5	1.3	0.9	0.033	ND	ND	1.3[2]	ND
Vitamin C (mg/d)	20 to 448	138	68.2	2.484	90	2000	75	2000
Vitamin E (mg/d)	2.5 to 27.6	8.9	3.4	0.124	15	1000	15	1000
Vitamin A (μg/d)	40 to 3433	581	414	15.077	900	3000	700	3000
Selenium (μg/d)	14 to 391	82.7	37.3	1.358	55	400	55	400
Zinc (mg/d)	3.9 to 23.5	11.9	3.5	0.127	11	40	8	40
n−3 FAs (mg/d)	517 to 7195	2118	836	30.445	ND	ND	ND	ND
ALA (mg/d)	373 to 4508	1519	550	20.030	1.6	ND	1.1	ND
EPA (mg/d)	1 to 2307	260	212	7.721	ND	ND	ND	ND
DHA (mg/d)	15 to 3054	339	276	10.051	ND	ND	ND	ND
Nutrient density								
Zeaxanthin (mg/1000 kcal/d)	0.007 to 0.5	0.077	0.043	0.002	ND	ND	ND	ND
Lutein (mg/1000 kcal/d)	0.04 to 4.4	0.56	0.4	0.015	ND	ND	ND	ND
Vitamin C (mg/1000 kcal/d)	8.3 to 217	57.3	28.8	1.049	ND	ND	ND	ND
Vitamin E (mg/1000 kcal/d)	1.4 to 12.7	3.6	1.1	0.040	ND	ND	ND	ND
Vitamin A (μg/1000 kcal/d)	29.6 to 1527	233	159	5.790	ND	ND	ND	ND
Selenium (μg/1000 kcal/d)	10 to 123	33.6	12.9	0.470	ND	ND	ND	ND
Zinc (mg/1000 kcal/d)	2.6 to 9.6	4.82	0.81	0.029	ND	ND	ND	ND
n−3 FAs (mg/1000 kcal/d)	332 to 3606	867	313	11.399	ND	ND	ND	ND
ALA (mg/1000 kcal/d)	256 to 2920	621	201	7.320	ND	ND	ND	ND
EPA (mg/1000 kcal/d)	0.44 to 787	107	85	3.096	ND	ND	ND	ND
DHA (mg/1000 kcal/d)	7.2 to 1041	140	110	4.006	ND	ND	ND	ND
Energy-adjusted intake								
Zeaxanthin	−0.01 to 1.0	0.19	0.1	0.004	ND	ND	ND	ND
Lutein	−0.07 to 8.5	1.34	0.9	0.033	ND	ND	ND	ND
Vitamin C	14.5 to 452	138	65.8	2.396	ND	ND	ND	ND
Vitamin E	2.2 to 26.8	8.9	2.7	0.098	ND	ND	ND	ND
Vitamin A	−6.9 to 3490	581	384	13.984	ND	ND	ND	ND
Selenium	4.6 to 370	83	32	1.165	ND	ND	ND	ND
Zinc	5.3 to 21	12	2	0.073	ND	ND	ND	ND
n−3 FAs	789 to 6887	2118	728	26.512	ND	ND	ND	ND
ALA	553 to 4422	1519	450	16.388	ND	ND	ND	ND
EPA	−38 to 2276	260	208	7.575	ND	ND	ND	ND
DHA	−15.9 to 3014	339	271	9.869	ND	ND	ND	ND

[1] ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAs, fatty acids; RDA, Recommended Dietary Allowance; UL, upper limit; ND, not determined.

[2] No established Dietary Reference Intake; 1.3 mg/d is the mean intake from dietary recall data for 1102 adult women participating in the 1986 Continuing Survey of Food Intake by Individuals Study.

of the nutrients studied, including the macular carotenoids ($P >$ 0.05 for all).

Dietary micronutrient intake with respect to risk factors for ARM

The relations between dietary intake of micronutrients and risk factors for ARM were analyzed by using multiple linear regression (Table 1) and were repeated using binary logistic regression. (The dietary intake variables were dichotomized by using the median in each case.) The results of both analyses were similar; therefore, the results of the logistic regression analysis were excluded from the text, but are available to interested readers on request from the author.

Broad-spectrum antioxidant index

Absolute dietary intakes of the antioxidants lutein, zeaxanthin, vitamin C, vitamin E, and zinc were divided into quintiles. The cutoffs for the upper and lower quintiles are presented in **Table 4**. Twenty-one subjects whose dietary intakes were in the upper quintile for all 5 antioxidants were considered to have a high broad-spectrum antioxidant index and were compared with 21 subjects whose dietary intakes were in the lower quintile for all 5 of these antioxidants.

There was no statistically significant difference between these 2 groups (high broad-spectrum antioxidant index compared with low broad-spectrum antioxidant index) in terms of their associations with risk factors for ARM.

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TABLE 4
Definitions of upper and lower tertiles and quintiles for the dietary intake of select antioxidants[1]

	Lutein	Zeaxanthin	Vitamin C	Vitamin E	Zinc
Absolute intake (mg)					
Upper quintile (n = 21)	>1.863	>0.255	>190	>11.15	>14.8
Lower quintile (n = 21)	<0.667	<0.105	<81	<6.17	<8.8
Nutrient density (g/kcal)					
Upper tertile (n = 29)	>0.5905	>0.08696	>63.828	>3.809	>5.152
Lower tertile (n = 29)	<0.3545	<0.05606	<41.25	<3.115	<4.449
Energy-adjusted intake (mg)					
Upper tertile (n = 29)	>1.4164	>0.2062	>155.481	>9.4363	>12.7023
Lower tertile (n = 35)	<0.8742	<0.1388	<102.1909	<7.7309	<11.0373

[1] The subjects were ranked according to their dietary intake of lutein, zeaxanthin, vitamin C, vitamin E, and zinc; those subjects with a high dietary intake of all 5 nutrients (high broad-spectrum antioxidant index) were compared with those subjects with a low dietary intake of all 5 nutrients (low broad-spectrum antioxidant index). For this purpose, the absolute dietary intake of each micronutrient was divided into quintiles, whereas nutrient-density and energy-adjusted dietary intakes of each micronutrient were divided into tertiles.

Energy-adjusted dietary intake (nutrient density) of the antioxidants lutein, zeaxanthin, vitamin C, vitamin E, and zinc were divided into tertiles. Tertiles were chosen to ensure an acceptable number of subjects in the high (n = 29) and low (n = 29) broad-spectrum energy-adjusted antioxidant index groups. The cutoffs for the upper and lower tertiles are presented in Table 4. The ratio of men to women [n (%):n (%)] in the high broad-spectrum, energy-adjusted antioxidant index group [3 (10.3%):26 (89.7%)] was significantly different (P = 0.011) from the ratio of men to women [n (%): n (%)] in the low broad-spectrum, energy-adjusted antioxidant index group [13 (44.8%):16 (55.2%)]. Furthermore, the mean (±SD) BMI of subjects in the high broad-spectrum, energy-adjusted antioxidant index group (24.35 ± 3.05) was significantly lower (P = 0.039) than the mean (±SD) BMI of subjects in the low broad-spectrum, energy-adjusted antioxidant index group (26.76 ± 5.28). Neither the frequency nor the distribution of all other risk factors was significantly different between the high and low broad-spectrum, energy-adjusted antioxidant index groups ($P > 0.05$ for all).

Finally, energy-adjusted (by the residuals method) dietary intakes of the antioxidants lutein, zeaxanthin, vitamin C, vitamin E, and zinc were divided into tertiles. Tertiles were chosen to ensure an acceptable number of subjects in the high (n = 29) and low (n = 35) broad-spectrum, energy-adjusted antioxidant index groups. The cutoffs for the upper and lower tertiles are presented in Table 4. The ratio of men to women [n (%):n (%)] in the high broad-spectrum, energy-adjusted antioxidant index group [5 (17.2%):24 (82.8%)] was significantly different (P = 0.032) from the ratio of men to women [n (%):n (%)] in the low broad-spectrum, energy-adjusted antioxidant index group [17 (48.6%):18 (51.4%)]. Neither the frequency nor the distribution of all other risk factors was significantly different between the high and low broad-spectrum, energy-adjusted antioxidant index groups ($P > 0.05$ for all).

DISCUSSION

This study was designed to test the hypothesis that independent risk factors for ARM represent risk for this condition, wholly or partly, because of an association with a relative lack of protective dietary nutrients.

Data relating to the dietary intake of nutrients was manipulated to yield energy-adjusted values for the dietary intake of the compound in question, because energy-adjusted values for nutrient intake are considered a more relevant measure of nutrients metabolized in approximate proportion to total caloric intake. In contrast, for nutrients for which metabolism is unaffected by energy requirements, absolute intake may be the most relevant measure (30). In the case of dietary intake with respect to ARM, it remains unclear whether absolute nutrient intake or energy-adjusted nutrient intake is more appropriate for analysis. Previous investigators have been inconsistent in their approach to this question, with some studying absolute nutrient intake (33) and others energy-adjusted nutrient intake (34–36), either in the form of nutrient densities (36) or by means of the residuals method (34–36).

In our study, associations between dietary intakes of nutrients and risk factors for ARM were investigated both for absolute and for energy-adjusted (nutrient densities and residuals method) nutrient intakes. We restricted our discussion to associations that were found to be significant for both absolute and energy-adjusted values.

We found no statistically significant variation in the dietary intake of antioxidant nutrients in relation to the month or season of FFQ completion. This is consistent with reports of previous investigators who found that serum concentrations of lutein and zeaxanthin do not vary with season (37, 38), but is at variance with one study that reports a seasonal variation in dietary intake of carotenoids (39). Our findings may reflect the increased availability of a wide variety of foodstuffs throughout the year, which tends to attenuate seasonal variations in dietary antioxidant intake. Regional variation in dietary habits may also explain, to some extent, the conflicting findings of different authors with respect to the seasonality of dietary antioxidant intake. For example, Zeigler et al (39) noted that fresh corn, an important source of lutein, was eaten predominantly in season by 42% of the respondents. However, fresh corn is not commonly eaten in Ireland, which possibly explained the absence of seasonal variation in the dietary intake of lutein in our study. Because no relation between the month or season of FFQ completion and dietary intake of micronutrients was identified in our study, the month or season of FFQ completion was not included as an

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independent variable in the regression models to explain the variation in dietary micronutrient intake.

No association was found between family history of ARM and dietary micronutrients. The incidence and prevalence of ARM has been repeatedly shown to increase with increasing age (14, 40-46). We found that increasing age was associated with a relative lack of dietary zeaxanthin. To our knowledge, this association has not been previously reported. Conversely, we found that increasing age was associated with a relative dietary abundance of vitamin A, eicosapentaenoic acid, and docosahexaenoic acid.

Zeaxanthin is a powerful antioxidant that is concentrated, together with its constitutional isomer lutein, at the macula. Together, these antioxidants are believed to play a key role in protecting against oxidative stress and, by extension, against ARM (47). Several studies have reported an inverse association between risk of ARM and serum concentrations (2, 48, 49) and dietary levels (33, 34, 48) of lutein and zeaxanthin.

Caloric intake is known to decrease with increasing age (50, 51); however, the observed reduction in energy-adjusted intake of zeaxanthin with increasing age suggests a qualitative change in diet that is unrelated to an age-related decline in total caloric intake.

Cigarette smoking is associated with an increased incidence (52, 53) and prevalence (14, 41, 53–56) of ARM. Cigarette smoke contains free radicals, which increase the oxidant load in smokers (57). We found that cigarette smoking was associated with a relative lack of dietary vitamin C, which is consistent with previous studies (56, 58–63). Vitamin C is found in high concentrations in all ocular tissues (63) and is a highly effective antioxidant. Furthermore, vitamin C is involved in the regeneration of the active form of vitamin E (63).

Evidence that sex represents risk of ARM is equivocal. Studies in Japan have found that the prevalence of early (65) and late (65, 66) ARM and the incidence of late ARM (64) are higher in men than in women. Conversely, studies in Western countries have found the opposite to be the case (43, 46, 67, 68).

We found that the women had a lower dietary intake of zinc than did their male counterparts. Zinc is fundamentally involved in many facets of antioxidant activity within cells. Newsome et al (69) have shown an age-related decline in zinc in human retinal pigment epithelium. This decline has been shown to result in a reduction in metallothionein and catalase antioxidant activity. It is conceivable that this decline might be more profound in subjects with a lower dietary intake of zinc, and that this might contribute to the increased risk of ARM noted in females by some observers.

The protection that retinal antioxidants confer against ARM must be exercised over many years, and decades before the onset of disease, because the oxidative basis of ARM pathogenesis is a chronic and cumulative process. In other words, the appropriate age profile of subjects to be investigated for any protective effect that diet confers against ARM is the 20–60-y age group and to relate findings to established and putative risk factors for this condition.

It is for this reason that we investigated the dietary intake of key nutrients in this age group, a design feature that distinguishes this study from previous studies and that, we believe, represents a unique strength of this article. Although the authors acknowledge that the homogeneity of the subject population in this study may limit its applicability to other races and ethnicities, we do not necessarily perceive this as a weakness of the current study. In multifactorial diseases, demonstrating potentially causal associations is often hindered by the presence of multiple potential confounders, which must be controlled for and which effectively decreases the power of the study.

We showed that several variables related to risk of ARM are associated with a relative dietary lack of key nutrients, which may protect against this condition. Indeed, it is biologically plausible that the risk that these variables represent for ARM may reflect, at least in part, an associated and parallel dietary lack of key nutrients (eg, age and dietary intake of zeaxanthin). It is important to note, however, that no risk factor was associated with a pervasive relative lack of dietary intake of all nutrients believed to protect against ARM. Because the relative contribution of individual nutrients to the overall retinal antioxidant defense system remains unclear, it is difficult to draw firm conclusions with respect to the relation between risk factors for ARM and the dietary intake of key nutrients. Nevertheless, our finding that the most important and universal risk factor for ARM, age, is associated with a relative lack of dietary zeaxanthin is an important and novel finding, which warrants further investigation.

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