Sunlight Exposure, Antioxidants, and Age-Related Macular Degeneration

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Objective: To examine the association of sunlight exposure and antioxidant level with age-related macular degeneration (AMD).

Methods: Four thousand seven hundred fifty-three participants aged 65 years or older in the European Eye Study underwent fundus photography, were interviewed for adult lifetime sunlight exposure, and gave blood for antioxidant analysis. Blue light exposure was estimated by combining meteorologic and questionnaire data.

Results: Data on sunlight exposure and antioxidants were available in 101 individuals with neovascular AMD, 2182 with early AMD, and 2117 controls. No association was found between blue light exposure and neovascular or early AMD. Significant associations were found between blue light exposure and neovascular AMD in individuals in the quartile of lowest antioxidant level—vitamin C, zeaxanthin, vitamin E, and dietary zinc—with an odds ratio of about 1.4 for 1 standard deviation unit increase in blue light exposure. Higher odds ratios for blue light were observed with combined low antioxidant levels, especially vitamin C, zeaxanthin, and vitamin E (odds ratio, 3.7; 95% confidence interval, 1.6-8.9), which were also associated with early stages of AMD.

Conclusions: Although it is not possible to establish causality between sunlight exposure and neovascular AMD, our results suggest that people in the general population should use ocular protection and follow dietary recommendations for the key antioxidant nutrients.

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The retina is vulnerable to the damaging effects of light. While wavelengths in the UV radiation range are largely absorbed by the cornea and lens, the retina is exposed to visible light, including blue light. Animal and laboratory studies have shown that blue light damages the retinal pigment epithelium and choriocapillaris through generation of reactive oxygen species and may be a factor in the pathogenesis of age-related macular degeneration (AMD). Protection against the harmful effects of blue light is provided by the retinal antioxidant defense system, which includes antioxidant enzymes supported by vitamins C and E, the carotenoids (lutein and zeaxanthin), and zinc. The carotenoids are present in high concentrations in the macula, acting as scavengers of reactive oxygen species and filtering blue light.

Results have been inconsistent in the few studies that have investigated associations of sunlight exposure with AMD in human populations. Little attention has been paid to possible interactions between antioxidant levels and light exposure, though the adverse effects of sunlight may be attenuated by the protective effects of antioxidants. The European Eye (EUREYE) Study was specifically designed to maximize heterogeneity of sunlight exposure and antioxidant levels through selection of study centers throughout Europe.

Methods

Participants were recruited from random sampling of the population aged older than 65 years in Bergen, Norway; Tallinn, Estonia; Belfast, Northern Ireland; Paris, France; Verona, Italy; Thessaloniki, Greece; and Alicante, Spain. Participants were interviewed by fieldworkers, underwent fundus photography, and gave blood samples. Information collected at interviews included participants’ education, smoking and alcohol use, a brief medical history, and sunlight exposure. A dietary questionnaire was administered. All questionnaires, which were originally written in English, were translated into the local languages and back-translated by native speakers.
into the language of the participating center and then translated back to English. Written informed consent was obtained from all study participants. Ethical approval was obtained from the relevant ethics committee of each country.

MEASUREMENT OF SUNLIGHT EXPOSURE

Participants were sent a residence and job history to complete in advance to facilitate recall at the interview. We used a questionnaire that asked about time spent outdoors between the hours of 9 AM and 3 PM and between 11 AM and 3 PM daily throughout an individual’s working life for different occupational periods (including homecare) and in retirement up to one’s current age. For each period, we collected information on the use of hats and eyewear (glasses, contact lenses, and sunglasses). We modified the original questionnaire by extending the employment history to include after leaving school, by collecting information separately for summer and winter, and by asking about the terrain in which outdoor exposures took place and time spent outdoors on working and nonworking days. Information from the questionnaire, residence calendar, and geographical coordinates of residence were used to generate estimates of individual years of exposure to different wavelengths of light. We used a modified version of the model developed by Rosenthal et al. For all residences of 1 year or longer, ambient UVB (erythemally weighted) and UVA (J/cm²) light were estimated from published sources that take into account time was included in the model. Personal lifetime exposures were calculated as the sum of the time-weighted levels at each place of residence during different periods was used to estimate the proportion of maximum exposure actually experienced by an individual. Finally, the attenuation provided by ocular protection was included in the model. Personal lifetime exposures were estimated for the 3 wavelengths of UV radiation for a mean annual lifetime exposure at midday and all times of day.

DIETARY QUESTIONNAIRES

We used the United Kingdom version of the European Prospective Investigation Into Cancer and Nutrition Study Food Frequency Questionnaire. For each non–United Kingdom country in the study, we modified the Food Frequency Questionnaire for any food items that were redundant or relevant. Questionnaire data were converted to nutrients using food-composition tables. Adjustment for total energy intake was made using the residual model of Willett et al.

FUNDUS PHOTOGRAPHY AND GRADING

Fundus images were graded at a single reading center using the International Classification System for Age-Related Maculopathy, which identifies 5 mutually exclusive grades: grade 0 (no early or late AMD), grade 1 (soft distinct drusen ≤ 63 µm and < 125 µm) only or pigmentary irregularities only); grade 2 (soft indistinct ≥ 125 µm) or reticular drusen only or soft distinct drusen with pigmentary irregularities); grade 3 (soft indistinct or reticular drusen with pigmentary irregularities); and grade 4 (either neovascular AMD [presence of any of the following: serous or hemorrhagic retinal or retinal pigment epithelial detachment, subretinal neovascular membrane, periretinal fibrous scar] or geographic atrophy [well-demarcated area of retinal pigment atrophy with visible choroidal vessels]). This grading system had been validated within the Rotterdam Eye Study. Early AMD was defined as grades 1 to 3 and late AMD as grade 4.

BLOOD SAMPLES

Blood samples were sent to a single laboratory for analysis by reverse-phase high-performance liquid chromatography for lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, α-tocopherol, γ-tocopherol, lycopene, and retinol levels. Total vitamin C levels were measured using an enzyme-based assay in plasma stabilized with metaphosphoric acid. Cholesterol level was measured using an enzymatic assay (Randox, Crumlin, Ireland) on a Cobas FARA centrifugal analyzer (Roche Diagnostics, Burgess Hill, England).

STATISTICAL ANALYSIS

Statistical analysis was carried out using Stata, version 9 (Stata Corp, College Station, Texas). The dependent variable was neovascular AMD. We did not examine possible associations with geographic atrophy because there were only 49 cases of the disease. The primary exposure was blue light in the middle of the day, when light intensity is greatest. Blue light was z-transformed for investigation of a standard deviation change in exposure. Key antioxidants for hypothesis testing were vitamin C, zeaxanthin, lutein, α-tocopherol, and dietary zinc (blood measurements of zinc were not available). Potential confounders, including age, sex, smoking status, education, history of diabetes, cardiovascular disease, demiquet (weight in kilograms divided by the demispan in centimeters squared), operated cataract, iris color, aspirin consumption, and use of any vitamin or mineral supplements or other antioxidants (lycopene, alpha and beta carotene, gamma-tocopherol, beta-cryptoxanthin, and retinol) were entered in preliminary models and retained if associated with neovascular AMD. Cholesterol was retained in all models, as carotenoids and α-tocopherol are lipid soluble.

In all analyses, we used Stata survey methods that take account of the within- and between-center variation in the estimation of standard errors. We used survey logistic regression to investigate the association between blue light exposure and neovascular AMD. We also examined this association by quartiles of key antioxidant levels by including interaction terms in the models with design-adjusted Wald tests. We used multinomial survey logistic regression to investigate associations with blue light, key antioxidants, and early AMD. Since sex and smoking are known to affect plasma vitamin C levels (lower in men and in smokers) and possibly other antioxidant levels, we investigated whether associations of blue light exposure and key antioxidants with neovascular AMD differed by smoking status by including 3-way interactions among the lowest antioxidant level quartile, smoking (ever smoked vs never having smoked), and blue light exposure. We also investigated the association of blue light exposure at different ages in those with combined low antioxidant levels. In all analyses, the comparison group was grade 0 AMD.

RESULTS

The prevalence of AMD has been presented elsewhere. Of 5040 participants (45% response rate), 4753 had gradable fundus images. There were 109 cases of neovascular AMD, 49 of geographic atrophy, 2333 of early AMD (1734 grade 1, 482 grade 2, and 117 grade 3), and 2262

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controls with grade 0 AMD. Sunlight exposure data were not available in 21 people (12 control, 1 neovascular AMD, and 8 early AMD cases). Full data on all sunlight and antioxidant variables were available for 2117 controls and 101 neovascular AMD, 43 geographic atrophy, and 2182 early AMD cases. The mean age of participants was 73.2 years (standard deviation, 5.6) years and 59% were women. Blue light exposures tended to be higher in participants from northern centers in southern Europe, while participants in an exclusively urban center (Paris) had the lowest exposures (Table 1). Plasma antioxidant and zinc levels also varied across centers, but there was no clear pattern. There was considerable variation in the interquartile ranges of blue light exposure within centers. In multivariate regression models, women had lower levels of antioxidant variables—vitamin C, lutein, zeaxanthin, and dietary zinc—but in 2 of the 6 risk ratios, the 95% CIs crossed 1. There was no association of blue light exposure with neovascular AMD for those with combined high levels of antioxidants (P > .2 for all analyses).

The associations of blue light exposure in the lowest quartiles of plasma antioxidant level did not differ by smoking status for neovascular AMD (3-way test of interaction: vitamin C, P = .1; zeaxanthin, P = .6; lutein, P = .4; α-tocopherol, P = .2; and dietary zinc, P = .1). There was no association of blue light exposure at different ages during adult life with neovascular AMD (Figure 1). Odds ratios increased from early adult life to middle age and older for the associations of blue light in those with low antioxidant levels at the time of study, with the highest ORs at ages 50 to 59 (OR, 4.7; 95% CI, 2.16-10.30) (Figure 2).

We found no association between blue light exposure alone or of blue light exposure by quartiles of individual antioxidant levels and early AMD (Table 4). Relative risk ratios were between 1.5 and 2 for blue light and early AMD in those with low levels of several antioxidants—vitamin C, zeaxanthin, and either α-tocopherol or zinc—but in 2 of the 6 risk ratios, the 95% CIs crossed 1. There were no significant interactions of blue light and early AMD with high levels of antioxidants.

Laboratory and animal studies have shown adverse effects of blue light on the retina and a role of antioxidants, including the carotenoids lutein and zeaxanthin, in free-radical scavenging and singlet oxygen quenching.1,3 The

### Table 1. Annual Midday Blue Light Exposure, Plasma Antioxidants, and Dietary Zinc in EUREYE Study Centers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bergen, Norway (n=667)</th>
<th>Tallinn, Estonia (n=886)</th>
<th>Belfast, Northern Ireland (n=558)</th>
<th>Paris, France (n=659)</th>
<th>Verona, Italy (n=560)</th>
<th>Thessaloniki, Greece (n=570)</th>
<th>Alicante, Spain (n=543)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue light exposure, × 10^10 photons/s/cm²</td>
<td>1.12</td>
<td>1.01</td>
<td>0.79</td>
<td>0.25</td>
<td>1.20</td>
<td>2.07</td>
<td>1.11</td>
</tr>
<tr>
<td>Antioxidant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C, µmol/L</td>
<td>46.0</td>
<td>48.4</td>
<td>38.6</td>
<td>46.3</td>
<td>44.9</td>
<td>35.5</td>
<td>45.7</td>
</tr>
<tr>
<td>Zeaxanthin, µmol/L</td>
<td>0.0298</td>
<td>0.0147</td>
<td>0.0206</td>
<td>0.0355</td>
<td>0.0706</td>
<td>0.0291</td>
<td>0.0278</td>
</tr>
<tr>
<td>Lutein, µmol/L</td>
<td>0.128</td>
<td>0.054</td>
<td>0.089</td>
<td>0.138</td>
<td>0.435</td>
<td>0.117</td>
<td>0.082</td>
</tr>
<tr>
<td>α-Tocopherol, µmol/L</td>
<td>29.2</td>
<td>27.3</td>
<td>29.5</td>
<td>30.1</td>
<td>28.7</td>
<td>31.5</td>
<td>31.6</td>
</tr>
<tr>
<td>Dietary zinc, mg/d</td>
<td>10.3</td>
<td>10.0</td>
<td>8.6</td>
<td>10.6</td>
<td>8.5</td>
<td>8.5</td>
<td>10.4</td>
</tr>
</tbody>
</table>

Abbreviation: EUREYE, European Eye.

a Eleven AM to 3 PM.

b Adjusted for energy intake.
Table 2. Association of Midday Blue Light Exposure With Neovascular AMD by Antioxidant Quartile

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value (95% CI)</td>
<td>OR $^b$</td>
<td>P Value $^c$</td>
<td>Value (95% CI)</td>
</tr>
<tr>
<td>Vitamin C $^d$</td>
<td>1.40 (1.15-1.71)</td>
<td>&lt; .01</td>
<td>1.12 (0.57-2.20)</td>
<td>.7</td>
</tr>
<tr>
<td>Zeaxanthin $^e$</td>
<td>1.47 (1.00-2.16)</td>
<td>.04</td>
<td>0.54 (0.21-1.36)</td>
<td>.2</td>
</tr>
<tr>
<td>Lutein $^f$</td>
<td>1.37 (0.76-2.46)</td>
<td>.2</td>
<td>0.92 (0.52-1.61)</td>
<td>.7</td>
</tr>
<tr>
<td>α-Tocopherol $^g$</td>
<td>1.43 (1.06-1.94)</td>
<td>.03</td>
<td>0.96 (0.74-1.24)</td>
<td>.7</td>
</tr>
<tr>
<td>Zinc $^h$</td>
<td>1.33 (1.10-1.62)</td>
<td>.01</td>
<td>1.07 (0.79-1.46)</td>
<td>.6</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

* For blue light exposure. Adjusted for age, sex, smoking status, diabetes, cardiovascular disease, education, aspirin use, retinol, and cholesterol level.

* Effect of blue light exposure in each antioxidant level quartile.

* Interaction of quartiles of vitamin C with blue light exposure, $P=.04$.

* Interaction of quartiles of zeaxanthin with blue light exposure, $P=.01$.

* Interaction of quartiles of lutein with blue light exposure, $P=.64$.

* Interaction of quartiles of α-tocopherol with blue light exposure, $P=.25$.


EUREYE Study is the first to report an adverse association of blue light exposure with neovascular AMD in humans with low levels of antioxidants. It is likely that low levels of antioxidants in the blood will be reflected by low levels in the retina, as has been demonstrated for the macular carotenoids. In particular, the combination of blue light exposure in the presence of low levels of zeaxanthin, α-tocopherol, and vitamin C was associated with a nearly 4-fold OR of neovascular AMD. In vitro studies have reported synergistic protective associations of retinal antioxidants against photoinduced lipid peroxidation for the macular carotenoids with α-tocopherol and vitamin C, possibly by preventing zeaxanthin depletion. Low levels of zeaxanthin appeared more important than low levels of lutein, especially in the presence of low levels of vitamin C and α-tocopherol. The higher levels of zeaxanthin compared with lutein in the fovea may indicate that zeaxanthin plays a stronger protective role against light-induced damage than lutein. Smoking is a well-established risk factor for neovascular AMD in many studies, including the EUREYE Study. Smokers tend to have lower levels of antioxidants, especially vitamin C.
Dam Eye Study reported that leisure time outdoors in young adult life was associated with the incidence of early, but not late, AMD.32 In our study, the associations of blue light exposure in those with low antioxidant levels appeared stronger at older ages, reaching a peak at ages 50 to 59 years. Penetration of shorter wavelengths of blue light into the retina decreases with age, principally as a result of a yellowing of the lens and a decreasing pupil diameter.33 The aging eye also accumulates chromophores, indicating increased susceptibility to oxidative damage from blue light.34 It has been estimated that either the process of lens yellowing and chromophore accumulation are approximately balanced with no change in susceptibility with age35 or that susceptibility peaks in midlife followed by a decline from the age of 60 years. This latter estimate is broadly in line with our results.3 The methods used to estimate sunlight exposure have varied across studies. We used a detailed interview that required participants to recall lifetime location of residence and time spent outdoors throughout their adult lives. Our questionnaire was similar to that used in studies that have reported associations between sunlight exposure and melanoma (including ocular melanoma).36,37 Participants may have underestimated or overestimated their time spent outdoors, but we have no reason to believe that recall was biased, eg, by knowing their antioxidant levels or AMD stage. Participants had no knowledge of their antioxidant levels, and interviews took place before the eye examination. We tried to maximize recall of exposures by sending participants a residence and job calendar to complete before the interview. We included periods spent looking after the home (especially important in people who spend a lot of time at home), whereas some studies have asked only about professional exposure to sunlight.6,10,38,39 Other studies have not collected data on past exposures during adult life4 or have used summary categorical responses to type of work exposure.
Table 4. Association of Midday Blue Light Exposure With Grade of Early AMD by Lowest Quartile of Antioxidants

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Grade 1 vs Grade 0 ARM</th>
<th>Grade 2 vs Grade 0 ARM</th>
<th>Grade 3 vs Grade 0 ARM</th>
<th>Lowest Quartile of Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>1.13 (0.94-1.36)</td>
<td>0.2</td>
<td>1.12 (0.71-1.77)</td>
<td>0.6</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.97 (0.89-1.06)</td>
<td>0.6</td>
<td>0.98 (0.72-1.34)</td>
<td>0.9</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>1.14 (0.90-1.49)</td>
<td>0.05</td>
<td>0.96 (0.69-1.32)</td>
<td>0.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.93 (0.76-1.14)</td>
<td>0.4</td>
<td>0.98 (0.82-1.17)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Combined Lowest Quartiles of Antioxidants

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C, zeaxanthin, α-tocopherol</td>
<td>1.44 (1.05-1.97)</td>
<td>0.03</td>
<td>1.79 (0.93-3.44)</td>
<td>0.08</td>
<td>1.95 (1.06-3.58)</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin C, zeaxanthin, and zinc</td>
<td>1.58 (1.04-2.40)</td>
<td>0.04</td>
<td>2.34 (1.31-4.18)</td>
<td>0.01</td>
<td>2.18 (0.48-9.80)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

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Followings recommended dietary reference intakes should ensure that people have antioxidant levels above those associated with increased risk from blue light exposure, at least for vitamins C and zinc. Dietary reference intakes of 90 mg/d of vitamin C for older men and 75 mg/d for older women should lead to plasma vitamin C levels above 0.46 mg/dL (the cut-off point for the lowest quartile in our study, to convert to micromoles per liter, multiply by 56.78). Dietary reference intakes for zinc are 11 mg/d for men and 8 mg/d for women, reflecting sex differences in metabolism. Recommendations for dietary intakes of vitamin E to achieve specific plasma levels are problematic owing to the poor correlations observed between dietary and plasma measures. The dietary reference intake for vitamin E (as α-tocopherol) is 15 mg/d in older men and women. In the absence of dietary reference intakes for lutein or zeaxanthin, it is recommended to increase consumption of carotenoid-rich fruits and vegetables.

We found that the combination of blue light exposure and low plasma concentrations of antioxidants was also associated with the early stages of AMD, which are common in the population, and that blue light exposure in middle age might be more damaging than at younger ages. In the absence of cost-effective screening methods to identify people in the population with early AMD, we suggest that recommendations on ocular protection and diet target the general population, especially middle-aged people.

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References

18. Bingham SA, Welsh AA, McTaggart A, et al. Nutritional methods in the European Arterial Occlusive Disease Biomedical Research for Clinical and Population Sciences (Dr Young), and decision to submit the paper for publication.

**Correction**

Errors in Text. In the Clinical Sciences article titled “A Study of Histopathological Features of Latanoprost-Treated Irides With or Without Darkening Compared With Non–Latanoprost Treated Irides” by Albert et al, published in the May issue of the Archives (2008;126[5]: 626-631), there were errors in quoted data of the study by Arranz-Marquez et al. The number of iridectomy specimens from patients with latanoprost-induced darkening should have been listed as 14 instead of 22. No mitotic figures or prominent nucleoli were present in any specimens. Therefore, on page 626, the first 2 sentences of the Introduction’s second paragraph should have read as follows: “A recent study by Arranz-Marquez et al compared iridectomy specimens from patients with latanoprost-induced darkening (n=14) and those from untreated controls (n=8). The authors found that latanoprost-induced darkened irides had an increased number of melanocytes with intranuclear inclusions and nuclear invaginations suggestive of atypia.”