The association between myopia, ultraviolet B exposure, serum vitamin D and genetic polymorphisms in vitamin D metabolic pathways in a multi country European study (EUREYE)

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Abstract

Importance: Myopia is becoming increasingly common globally and is associated with potentially sight-threatening complications. Spending time outdoors is protective but the mechanism underlying this association is poorly understood.

Objective: To examine the association of myopia with ultraviolet B (UVB) radiation (directly related to time outdoors and sunlight exposure), serum vitamin D, and Vitamin D pathway genetic variants, taking account of years in education.

Design, Setting and Participants: Cross-sectional, population-based, random sample aged ≥65 from six study centers from north to south Europe (EUREYE) between November 6th 2000 to November 15th 2002. Of 4187 participants, 4166 attended an eye examination including refraction, gave a blood sample and were interviewed by trained fieldworkers using a structured questionnaire. Myopia was defined as mean spherical equivalent ≤-0.75 diopters. Exclusions included aphakia, pseudophakia, late age related macular degeneration and vision impairment due to cataract, resulting in 371 myopes and 2797 non-myopes (overall mean age 72.4 years (SD=5) and 46% male).

Exposures: UVB exposure estimated by combining meteorological and questionnaire data at different ages, single nucleotide polymorphisms (SNPs) in Vitamin D metabolic pathway genes, serum vitamin D$_3$ (25(OH)D$_3$), and years of education.

Main outcome measure: Odds Ratios (OR) of UVB, 25(OH)D$_3$, vitamin D SNPs and myopia estimated from logistic regression.
Results: A standard deviation increase in UVB exposure at ages 14-19 (0.81, 95% confidence interval (CI) 0.71-0.92) and 20-39 (0.7, 95% CI 0.62-0.93) was associated with reduced adjusted OR of myopia. Those in the highest tertile of years of education had twice the OR of myopia (2.08, 95% CI 1.41-3.06). No independent association between myopia and 25(OH)D₃ nor variants in genes associated with vitamin D metabolism was found. An unexpected finding was that the highest quintile of plasma lutein was associated with a reduced OR of myopia (0.57, 95% CI 0.46-0.72).

Conclusion & relevance: Increased UVB exposure was associated with reduced myopia, particularly in adolescence and young adulthood. The association was not altered by adjustment for education. We found no convincing evidence for a direct role of vitamin D in myopia risk. The relationship between high plasma lutein and less myopia requires replication.
Myopia, or short-sightedness, is a complex trait influenced by numerous environmental and genetic factors. Myopia is becoming more common worldwide, most dramatically in urban Asia, but prevalence rises have also been identified in the US and Europe. This has major implications, both visually and financially, for the global burden from this potentially sight-threatening condition.

An increased risk of myopia has been associated with urbanization, higher socio-economic status, prenatal factors, near work, and education. The protective effect of time outdoors on myopia has been identified in studies of school age children and young adults, with replication in different climates. A meta-analysis of seven cross-sectional studies concluded a 2% reduced odds of myopia per additional hour of time spent outdoors per week. The recommendation for children to spend time outdoors provides an attractive option, and intervention studies are in progress. However, it remains unclear which of the numerous elements associated with time spent outdoors, such as light intensity, ultraviolet radiation (UVR) or distant focus, confers the reduced risk of myopia. Vitamin D status has been inversely associated with myopia in several studies but not all, whilst genetic polymorphisms in vitamin D pathway genes have been associated in one study but not in another.

We exploited the availability of relevant existing information (refractive status, UVR, education, serum vitamin D and genetic polymorphisms in Vitamin D pathway genes) in the European Eye Study (EUREYE) with the objective of investigating their association with myopia.
Methods

Study population

EUREYE was designed to maximize heterogeneity of UVR exposure and diet by selection of study centers from northern to southern Europe. Participants were recruited from November 6th 2000 to November 15th 2002 from random sampling of the population aged over 65 years in the following centers: Bergen (Norway), Tallinn (Estonia), Belfast (UK), Paris-Creteil (France), Verona (Italy), Thessaloniki (Greece), and Alicante (Spain). Over 11000 people were invited of whom 5040 participated (45% response rate). Written informed consent was obtained from all study participants. Details of study design are described elsewhere. Ethical approval was obtained for each center from the relevant ethics committee and the research adhered to the tenets of the Declaration of Helsinki. Participants attended the examination center where they were interviewed by trained fieldworkers, underwent an ophthalmological examination and gave a blood sample for blood measurements and genotyping. Information collected by the interviewers included years of education, smoking, alcohol use, a brief medical history, a semi-quantitative food frequency questionnaire, and a detailed questionnaire on outdoor exposure.

Measurement of UV exposure

Full details of the methods have been published previously. Participants were sent a residence and employment history to complete in advance to facilitate recall at the interview. We used a questionnaire which asks about time spent outdoors between the hours of 9 a.m. and 5 p.m. and between 11 a.m. and 3 p.m. daily (from the age of 14), for different
occupational and leisure time periods (including homecare), and in retirement up to current age. Information from the questionnaire and residence calendar and geographical co-ordinates for residence were sent to the University of East Anglia to generate estimates of individual years of all day (9 a.m. to 5 p.m.) or middle of the day (11 a.m. and 3 p.m.) exposure for different wavelengths of light (UVA, UVB, blue light). For all residences of one year or more, ambient UVB (minimal erythema dose $^{21}$) and UVA ($J/cm^2$) were estimated from published sources that take into account time of day, month, and latitudinal variations $^{22}$. We used published coefficients to adjust ambient clear-sky UV for cloud cover $^{23}$ and terrain $^{24}$. For each wavelength of light, maximum potential lifetime dose was calculated as the sum of the time-weighted levels at each of the places of residence of the individual. Personal adult lifetime (from age 14) UV exposure was estimated for each of the three wavelengths and summed for a mean annual lifetime dose at different ages, for all day and middle of the day exposure.

Visual acuity and refraction

The protocol for testing visual acuity (VA) was different in one of the EUREYE centers (Alicante) and therefore data from this center was not included in the present analysis. All other centers followed the procedures described below. Presenting distance VA (i.e. with spectacles if worn) was tested separately in each eye using the 4-meter EDTRS logMAR chart. Any participant who was unable to achieve 0.3 logMAR (20/40 Snellen) in either eye underwent automated refraction or manual retinoscopy and recording of best-corrected VA. For persons who achieved 0.3 logMAR or better the spectacle correction (if any) worn by the participant for each eye was measured by neutralization using a focimeter or by hand-held lenses. The spherical
equivalent (SE) was obtained by adding half of the cylindrical value to the spherical value and the mean of the two eyes was calculated, commonly used in epidemiological studies. Myopia was defined as $SE \leq -0.75$ diopters (D) (categorized as: low $SE \leq -0.75$ to $>-3$, moderate $\leq -3$ to $>-6$, severe $\leq -6$). Non-myopes were defined as those with a $SE > -0.75$, and additionally those with an unaided VA better than 0.3 logMAR where refraction was not measured. Participants with late age-related macular degeneration (AMD), aphakia or pseudophakia in either eye or visual impairment (< 0.5 logMAR or 20/60 Snellen) due to cataract were excluded.

Blood measurement

Blood samples were sent to a single laboratory (Queens University Belfast) for analysis. Serum 25-hydroxy vitamin D$_2$ and D$_3$ (25(OH)D$_2$ and 25(OH)D$_3$) were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In all analyses vitamin D levels were adjusted for season of measurement. Plasma lutein, zeaxanthin, beta cryptoxanthin, alpha and beta-carotene, alpha and gamma tocopherol, lycopene and retinol were measured by reverse phase high performance liquid chromatography. Total ascorbate was measured using an enzyme-based assay in plasma stabilized with metaphosphoric acid. All assays were standardized against appropriate National Institute of Standards and Technology standard reference materials. Cholesterol was measured using an enzymatic assay (Randox, Crumlin, UK) on a Cobas FARA centrifugal analyzer (Roche Diagnostics, UK).

Statistical analysis
Statistical analysis was carried out using Stata software version 13 (Stata Corp., College Station, TX). All analyses took account of the study design of the six centers by use of robust errors. All day (9 a.m. to 5 p.m.) adult lifetime UVB and 25(OH)D$_3$ were the primary exposures of interest as vitamin D$_3$ is produced in the skin following exposure to UVB, whereas 25(OH)D$_2$ is mainly derived from fortified foods and vitamin supplements $^{26}$. Following the exclusion of 67 participants with very high levels, the distribution of 25(OH)D$_3$ was normal. We investigated 25(OH)D$_3$ both as a continuous variable and categorized by quintiles. Dietary vitamin D was estimated using food composition tables $^{27}$ and energy adjusted. UVB was normalized using a square root transformation and then Z transformed for investigation of a one standard deviation (SD) increase in exposure. We calculated years of education from the difference between the start and leaving dates, and categorized into tertiles in order to reflect the common tiers of education (primary, secondary and higher) for inclusion as independent myopia risk factor.

We ran preliminary regression analyses to identify factors associated with 25(OH)D$_3$ and with UVB as possible confounders of any association with myopia. A large number of variables were independently associated with 25(OH)D$_3$: age, sex, season, center, current smoking, diabetes, obesity, dietary vitamin D intake, fish intake and fish oil supplements, antioxidants including vitamin C, lutein (or zeaxanthin), retinol, alpha tocopherol, and cholesterol. Of these only lutein (or zeaxanthin) were (inversely) associated with myopia and entered the models as potential confounders. The factors independently associated with UVB were 25(OH)D$_3$, center, sex and
Only education was (positively) associated with myopia. Therefore, in our final logistic regression models for myopia we retained age, sex, center and season, together with our primary exposure variables (UVB, 25(OH)D$_3$ and education) and identified confounders, namely lutein (or zeaxanthin). Lutein and zeaxanthin were highly correlated ($r=0.85$) and results were almost identical when separately introduced into the models. Our outcome measure was the confounder-adjusted association between myopia and our key exposures expressed as the adjusted odds ratio (OR) in logistic regression.

SNP selection, genotyping and genetic analyses

For reason of costs, genotyping was undertaken in a sub-sample of the main study. Data on vitamin D pathway SNPs were available for a subset of 109 myopes and 782 non-myopes. Ninety-three common SNPs located across 7 genes involved in the vitamin D metabolism (GC (10), RXRA (14), CYP2R1 (7), DHCR7 (5), VDR (29), CYP27B1 (7), CYP24A1 (21)) were selected from Phase III, release 2 HapMap (http://www.hapmap.org) CEPH data (Utah residents with ancestry in northern and western Europe; CEU) using Haploview to determine linkage disequilibrium. Tag SNPs were selected using multimarker tagging where $r^2>0.8$ for all downloaded SNPs with a minor allele frequency $\geq$5%, genotype call rate $\geq$95%, and no significant deviation from Hardy Weinberg equilibrium (HWE). Genotyping was performed by KBiosciences (Hoddesdon, United Kingdom) and associations between genotypes and myopia status were investigated. Quality filters for exclusion of SNPs included call rates below 95% and deviation from HWE ($P<0.001$).
DNA samples were excluded if missing genotypes exceeded 10%. Other quality control measures included duplicates on plates, random sample allocation to plates, independent scoring of problematic genotypes by two individuals and re-sequencing of selected DNAs to validate genotypes. KBiosciences quality control also included validation of all SNP assays on a panel of 44 random Caucasian-derived samples plus 4 non-template (negative) controls. Statistical genetic tests were performed using PLINK (version 1.07) under an additive genotypic model. Logistic regression adjusted for age, sex, season and study center was used to examine association with individual SNPs.
Results

The flow of participants in the study design is illustrated in Figure 1. We excluded 515 for aphakia or pseudophakia, 116 for late AMD and 36 for vision impairment due to cataract. Relevant exposure data (mainly serum 25(OH)D$_3$) was missing in 297 participants (32 myopes, 265 non-myopes). Our final analysis was based on 371 myopes and 2797 non-myopes with complete data on all relevant exposures, of which 6% (n=24) had high myopia. Included participants had a mean age of 72.4 years (SD 5) and 46% were male.

In univariable analyses, there was no difference in the age or sex of people with myopia compared to those without [Table 1], nor in smoking habit, alcohol use or obesity. Differences were observed between myopes and non-myopes in years of education, UVB exposure and serum 25(OH)D$_3$, but there was no difference in dietary vitamin D intake.

In analyses adjusted for age, sex and center, a one SD increase in personal lifetime UVB exposure was associated with reduced odds of myopia: OR = 0.72 (95% confidence interval (CI) 0.56-0.93), p=0.001 [Table 2]. Those in the highest tertile of years of education (median 14 years) had twice the odds of myopia (OR = 2.08, 95% CI 1.41-3.06, p=0.001) compared to those in the lowest tertile (median 7 years). In age, sex, center and season adjusted analyses there was no clear evidence for an association of 25(OH)D$_3$ (either continuous or by quintiles) with myopia. In contrast, those in the highest quintile of plasma lutein had nearly half the risk of myopia (adjusted OR = 0.57, 95% CI 0.46-0.72) compared to the lowest quintile. In a further
model incorporating 25(OH)D$_3$, lutein, education and UVB, adjusted for age, sex, center and season, the estimates for each exposure were virtually unchanged. There was evidence for a stronger inverse association of UVB with increasing myopia severity: low myopia OR=0.87 (95% CI 0.75-1.01, p=0.06), moderate myopia OR=0.59 (95% CI 0.36-0.97, p=0.04), severe myopia OR=0.39 (95% CI 0.25-0.63, p=0.001).

We investigated whether the association with myopia and UVB exposure varied by the personal UVB exposure experienced at different ages. Significant ORs for less myopia with increased UVB exposure were observed in adolescence and early adulthood, between the ages 14-19 and 20-29 [Figure 2], but not for other age groups.

The subset with genetic data were similar in age (73 years), sex (49% male) and myopia severity (59% low, 34% moderate and 7% high myopia) to those without genetic data. Of the 93 genetic variants associated with Vitamin D metabolism, one SNP in GC was excluded for deviation from HWE. Of the remaining SNPs four were nominally associated with myopia, three in CYP2RI and one in CYP24A1, but none withstood correction for multiple testing [eTable 1].
Discussion

We found that higher annual lifetime UVB exposure, directly related to time outdoors and sunlight exposure, was associated with reduced odds of myopia. UVB exposure between the ages of 14-29 was associated with the highest reduction in odds of adult myopia. Myopia was more than twice as common in participants in the highest tertile of education. The association between UVB, education and myopia remained even after respective adjustment. This suggests that the high rate of myopia associated with educational attainment is not solely mediated by lack of time outdoors.

The protective effect of time outdoors on myopia is well-established. Time outdoors reflects various physiological effects that have been associated with or hypothesized to influence myopia - brighter light levels, a different spectrum of wavelengths compared to artificial lighting with reduced UVR, and an extended focal distance with less hyperopic peripheral defocus. UV conjunctival autofluorescence, an indirect marker of ocular sun exposure (in particular UVR), is inversely associated with myopia, and has a stronger effect than time outdoors assessed using questionnaires. One small study measuring UVR using dosimeters found differing exposure between emmetropes, stable myopes and progressing myopes.

Proposed mediating mechanisms include activation of dopaminergic retinal amacrine cells which are stimulated by light and influence ocular axial growth, and higher serum vitamin D induced by sunlight. We, like others, did not find evidence to support the association
between myopia and serum vitamin D, or genes involved in vitamin D metabolism. A previous publication examined 12 SNPS from two vitamin D pathway genes (VDR and GC); reporting a significant association between rs2853559 in VDR in the overall sample of 289 cases and 81 controls and a further three variants in VDR with a low/moderate myopic subset. In a more recent publication 33 SNPs across six genes associated with vitamin D metabolism were examined in over 2000 individuals in relation to both refractive error and axial length. Nominal significance was identified for variants in CYP24A1 and VDR but none withstood correction for multiple testing. We investigated the association between myopia and 92 variants in vitamin D metabolism genes, identifying nominal significance in three SNPs in CYP2R1 and one SNP in CYP24A1 (not the same variant as the aforementioned study). None withstood correction for multiple testing. We acknowledge low power for this type of analysis but notably studied more variants and previously unexamined genes (CYP2R1, RXRA) in a substantial cohort.

Those in the highest fifth of plasma lutein had approximately 40% reduced odds of myopia. Results were similar for zeaxanthin but as the two are highly correlated we presented lutein only for simplicity. We excluded cases of late AMD because we have previously shown an increased risk for late AMD with blue light exposure in those with low levels of key antioxidants, including lutein. Sensitivity analyses made no appreciable difference; when seventy-two individuals with late AMD were included myopia OR = 0.56 (95% CI 0.46 - 0.70) with the highest quintile of lutein. Lutein is a retinal carotenoid, responsible for much of the macular pigment optical density (MPOD) and has anti-oxidative, anti-inflammatory and structural effects in neural tissue. Lutein has been associated with a reduced risk of AMD, improved contrast
sensitivity in healthy subjects\textsuperscript{37}, and inversely related to axial length (and thus axial myopia)\textsuperscript{38}.

Although limited evidence for an association between lutein and myopia is gained from this analysis and importantly no causative role can be inferred, it does raise interesting hypotheses for a potential role.

Study Limitations

This study utilizes retrospectively calculated UVB exposure through highly detailed questionnaires over the life course, together with geographical-specific, historical data on UVR. UVB was selected for analysis given its role in stimulating vitamin D\textsubscript{3} production in the skin. Our measure is subject to recall error and lacks the heightened accuracy of UV exposure achieved with light meters. However we do not have any reason to believe that the UVB association would be biased since myopia was identified after the interview. A weakness of our study was that we did not collect any data on UVB exposure during childhood, which could be argued to be more relevant in myopia development. However, a significant proportion of refractive error develops in adolescence and early adulthood\textsuperscript{39} and our results showed the greatest effects for these age groups. Non-myopes were defined either by refraction or good unaided VA where refraction was unknown. This definition was used in attempt to minimize bias but to ensure this was appropriate we performed sensitivity analyses where non-myopes were only classified on the basis of measured refractive error; analysis using this definition produced very similar results. A limitation is also that vitamin D and lutein levels were measured in later life. The association between myopia development and these factors may be more relevant in younger ages. However there is evidence, albeit limited, that an individual’s 25(OH)D levels are
reproducible over time. Variants in vitamin D pathway genes are not subject to these concerns of temporality and confounding (Mendelian randomization), and hence any association with myopia would strengthen a causal relationship with vitamin D. We therefore consider it unlikely that vitamin D plays a role in myopia.

In conclusion this study suggests lifetime exposure of UVB is associated with reduced myopia in adulthood. The protective association is strongest with exposure in adolescence and younger adult life and with increasing severity of myopia. As the protective effect of time spent outdoors is increasingly used in clinical interventions, a greater understanding of the mechanisms and life stages at which benefit is conferred is warranted.
Acknowledgements

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Conflicts of Interest

No conflicting relationship exists for any author.

Contributorship Statement
The authors’ responsibilities were as follows—KW: had the idea for the analysis and wrote the paper; GB: responsible for the estimation of light exposure; IY: responsible for the antioxidant assays; A McG: responsible for the vitamin D assays; GMcK: responsible for statistical genetic analyses; RH: responsible for processing of refractive error data; CH: provided assistance on data interpretation and drafting of the manuscript. UC, JS, MR, GS, LT, FT: responsible for the acquisition of data in each of the local study centers; AEF: had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors commented on the drafts, were responsible for the decision to submit for publication, and contributed to the study design.
References


Legends for figures

Figure 1 Flow chart of inclusion of study participants

Figure 2 Association of all-day UVB exposure at different ages with myopia, adjusted for age at study examination, sex, center and years of education
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Myopia</th>
<th>Non-myopia</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>72.9 (5.5)</td>
<td>72.4 (5.0)</td>
<td>0.58</td>
</tr>
<tr>
<td>Men (%)</td>
<td>174 (46.9)</td>
<td>1282 (45.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>Years of education</td>
<td>11 (7,14)</td>
<td>9 (7,12)</td>
<td>0.01</td>
</tr>
<tr>
<td>UVB (Minimal Erythema Dose)</td>
<td>314 (140,566)</td>
<td>358 (224,585)</td>
<td>0.01</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>45.3 (20.8)</td>
<td>47.5 (20.9)</td>
<td>0.01</td>
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<tr>
<td>Dietary vitamin D mcg/day</td>
<td>1.86 (1.32, 2.62)</td>
<td>1.89 (1.35, 2.56)</td>
<td>0.62</td>
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<td>Ever smoked (%)</td>
<td>179 (48.2)</td>
<td>1350 (48.3)</td>
<td>0.98</td>
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<tr>
<td>Alcohol at least weekly (n,%):</td>
<td>134 (36.1)</td>
<td>1106 (39.5)</td>
<td>0.49</td>
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<tr>
<td>Obesity (BMI &gt; 30) (n,%):</td>
<td>138 (37.2)</td>
<td>1001 (35.8)</td>
<td>0.82</td>
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<tr>
<td>Lutein (µmol/l):</td>
<td>0.087 (0.04, 0.24)</td>
<td>0.130 (0.05, 0.39)</td>
<td>&lt;0.01</td>
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Table 1: Characteristics of participants with and without myopia.

Univariable analyses.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UVB exposure (one SD increase)</th>
<th>25(OH)D</th>
<th>p trend</th>
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<tr>
<td></td>
<td></td>
<td>Quintiles (Q) of plasma lutein</td>
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<td></td>
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<td>p trend</td>
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<td></td>
<td></td>
<td>Median (nmol/L)</td>
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<td>p trend</td>
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<tr>
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<td></td>
<td>Quintiles (Q) of 25(OH)D³</td>
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<td></td>
<td></td>
<td>p trend</td>
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<td></td>
<td></td>
<td>Median (continous)</td>
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<table>
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<tr>
<th>Characteristic</th>
<th>95% CI</th>
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<tr>
<td></td>
<td>Adjusted OR</td>
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<td>UVB exposure (one SD increase)</td>
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<td>0.01</td>
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</table>
Table 2

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>Education</td>
<td>0.84 (0.69-1.02)</td>
<td>0.22</td>
</tr>
<tr>
<td>Serum 25(OH)D3</td>
<td>1.00 (1.00-1.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma Lutein</td>
<td>0.97 (0.87-1.09)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, center, season, and all variables in the model (name ly UVB exposure, education, 25(OH)D3, etc.)
Attended eye examination \((n = 4187)\)

Distance visual acuity measured \((n = 4166)\)

Exclusions:
- Aphakia or pseudophakia \((n = 515)\)
- Late age-related macular degeneration \((n = 116)\)
- Visual impairment due to cataract \((n = 36)\)

Spherical equivalent measurement \((n = 2748)\) in those eligible \((n = 2788)\)

- Myopic \((\leq -0.75 \text{ D})\) \(n = 403\)
- Non-myopic \((> -0.75 \text{ D})\) \(n = 2345\)

No spherical equivalent measurement \((n = 1418)\)

Unaided visual acuity better than 0.3 logMAR \((n = 717)\) - classified as non-myopic

3465 Individuals
Myopic = 403
Non-myopic = 3062

Missing exposure data \((n = 297)\);
myopic = 32, non-myopic = 265

3168 Individuals
Myopic = 371
Non-myopic = 2797