

Myopia Is Associated With Lower Vitamin D Status in Young Adults

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PURPOSE. To investigate the association between serum vitamin D levels and myopia in young adults.

METHODS. A total of 946 individuals participating in the 20-year follow-up of the Western Australian Pregnancy Cohort (Raine) Study were included in this study. Ethnicity, parental myopia, and education status were ascertained by self-reported questionnaire. A comprehensive ophthalmic examination was performed, including postcycloplegic autorefractometry and conjunctival UV autofluorescence photography. Serum 25-hydroxyvitamin D₃ (25(OH)D₃) concentrations were determined using mass spectrometry. The association between serum 25(OH)D₃ concentrations and prevalent myopia was determined using multivariable logistic regression. Myopia was defined as mean spherical equivalent ≤ -0.5 diopters.

RESULTS. Of the 946 participants, 221 (23.4%) had myopia ($n = 725$ nonmyopic). Myopic subjects had lower serum 25(OH)D₃ concentrations compared to nonmyopic participants (median 67.6 vs. 72.5 nmol, $P = 0.003$). In univariable analysis, lower serum 25(OH)D₃ concentration was associated with higher risk of having myopia (odds ratio [OR] for <50 vs. ≥ 50 nmol/L: 2.63; confidence interval [95% CI] 1.71–4.05; $P < 0.001$). This association persisted after adjustment for potential confounders, including age, sex, ethnicity, parental myopia, education status, and ocular sun-exposure biomarker score (adjusted OR 2.07; 95% CI 1.29–3.32; $P = 0.002$).

CONCLUSIONS. Myopic participants had significantly lower 25(OH)D₃ concentrations. The prevalence of myopia was significantly higher in individuals with vitamin D deficiency compared to the individuals with sufficient levels. Longitudinal studies are warranted to investigate whether higher serum 25(OH)D₃ concentration is protective against myopia or whether it is acting as a proxy for some other biologically effective consequence of sun exposure.

Keywords: myopia, vitamin D, Raine Study, ocular sun exposure, young adults

Worldwide, including in Australia, the prevalence of myopia has been increasing.¹ Myopia prevalence varies across populations of different regions, ethnicities, and age groups. In some East Asian countries, myopia is an epidemic, with as many as 80% of children estimated to be myopic.² In two Australian schoolchildren cohorts who were aged 6 and 12 at the baseline examination, the incidence of myopia was reported to be 2.2% and 4.1% over a 5- to 6-year period.³ Mild myopia is a relatively benign disorder, and blurred vision due to elongation of the eye can be corrected with spectacles, contact lenses, or laser refractive surgery. However, individuals with severe myopia are at increased risk of visual impairment and blindness due to associated conditions such as retinal detachment, retinal degeneration, and choroidal neovascular-

ization.^{4,5} Myopia is also associated with increased risk of age-related eye diseases including cataract and glaucoma.^{6,7}

Several possible mechanisms have been proposed for the development of refractive error. One of the earliest of these hypothesized that vitamin D may have a role in the development of myopia. In the 1930s and 1940s, several researchers investigated the association of myopia with vitamin D status both experimentally and clinically.⁸ Recently, Mutti and Marks⁹ proposed that decreasing population-level vitamin D status (measured by the concentration in blood of 25-hydroxyvitamin D [25(OH)D]) may be associated with the rising prevalence of myopia. In a small cohort of 22 participants, after adjustment for age and dietary intake, myopes had lower 25(OH)D concentrations than nonmyopes.⁹ More recently, low serum 25(OH)D levels were found to be

associated with higher myopia prevalence in a large Korean population.¹⁰

Epidemiological studies have identified a range of potential environmental risk factors for the development of myopia.¹¹ The rapid increase in myopia prevalence in East Asian populations points to environmental or lifestyle factors sufficient to exert an effect in a short time period. Within these factors, decreasing time spent outdoors has been identified as a potential explanatory lifestyle behavior. In the last decade, a number of observational studies have investigated the hypothesis that greater time spent outdoors is protective against myopia.^{12–15} This is supported by findings from a recent meta-analysis of cross-sectional studies that demonstrated an inverse association between time spent outdoors and myopia prevalence.¹⁶ These findings have been substantiated in prospective population-based studies and randomized controlled trials.^{17,18}

In addition to the evidence of a well-grounded environmental contribution to risk, some variation in myopia and refractive error is accounted for by genetic factors. Interestingly, one of the candidate genes identified in family-based studies is the vitamin D receptor (*VDR*). Polymorphisms within this gene were associated with low-to-moderate myopia in Caucasians,¹⁹ and a polymorphism in the *VDR* gene start codon (Fok1) was associated with high myopia in Indians.²⁰ However, these studies identified different risk alleles within the *VDR*, and the *VDR* (and related) gene polymorphisms for which there is evidence of functional effects are not those that show an association. These inconsistencies do cast some doubt on a causal role of polymorphisms in the *VDR*, and replication studies are needed.

In many populations, the main source of vitamin D is endogenous synthesis following sun exposure of the skin.²¹ Vitamin D deficiency is reportedly widespread,²² and population 25(OH)D levels have been decreasing over time,²³ possibly due to behavioral changes to decrease sun exposure. Taken together, the environmental and genetic associations and the correlative temporal pattern provide compelling evidence that myopia risk is linked to vitamin D-related factors.

Previous refractive error studies did not take account of individual ocular and nonocular sun exposure when exploring the relationship of myopia and vitamin D levels. The purpose of our current study was to examine the association between serum 25(OH)D₃ concentrations and the prevalence of myopia, adjusting for potential confounders including a marker of ocular sun exposure, in a large cohort of young adults of mainly Northern European ancestry but with a subset of East Asian ancestry.

METHODS

Study Participants

The study comprised participants who were enrolled in the 20-year follow-up of the Western Australian Pregnancy Cohort (Raine) Study conducted between March 2010 and April 2012.²⁴ The Raine Study methodology has been described previously.²⁵ In brief, a total of 2900 pregnant women attending the public antenatal clinic at King Edward Memorial Hospital, or nearby private practices, were recruited into the Raine Study between May 1989 and November 1991. A total of 2868 of their offspring have since undergone serial assessment. The current study was conducted in accordance with the tenets of the Declaration of Helsinki. The protocol was approved by the Human Research Ethics Committee at the University of Western Australia. Informed consent was

obtained from all participants at the initiation of the eye examination session at 20 years.

Questionnaire

Each participant completed a questionnaire providing socio-demographic data and information on current education status (studying part- or full-time) and parental myopia, that is, whether one or both parents were myopic or short-sighted. Individuals were asked to report their time spent outdoors and had four possible responses to the question “In the summer, when not working at your job or at school, what part of the day do you spend outside?”: none, <¼ of the day, approximately half of the day, and >¾ of the day. “None” and “<¼ of the day” groups were combined due to low numbers in the “none” category.

Assessment of Myopia and Ocular Sun Exposure

As part of a comprehensive eye examination,²⁶ postcycloplegic autorefractometry was measured using the Nidek ARK-510A (NIDEK Co. Ltd., Gamagori, Japan) autorefractor. The mean of three consecutive measurements was recorded for each eye. Myopia was defined as mean spherical equivalent (MSE, sum of spherical error and half of cylindrical error) of both eyes ≤ −0.5 diopters (D). This definition was adopted due to its wide use and validated reliability in young individuals.²⁷ The MSE of two eyes was calculated for each participant to determine the prevalence of myopia. We used a camera system developed by Ooi and colleagues²⁸ to derive a score for a biomarker of ocular sun exposure by measuring conjunctival UV autofluorescence (CUVAF). The area of fluorescence (mm²) for each photograph was determined using Adobe Photoshop CS4 Extend (Adobe Systems, Inc., San Jose, CA, USA). Total ocular sun exposure of individuals was determined as the summed area of the CUVAF in four photographs (left and right eyes, nasal and temporal conjunctiva) of each individual. The reliability of CUVAF as a biomarker of subacute sunlight exposure has been previously validated.²⁹

Assessment of Serum 25(OH)D₃ Concentrations

Participants provided a fasting blood sample for analysis of serum 25(OH)D concentration at the age 20 years follow-up. Venous blood samples were taken from an antecubital vein after an overnight fast, and samples were stored at −80°C until analyzed using liquid chromatography–tandem mass spectrometry (RDDT, Bundoora, VIC, Australia), according to published methodology.³⁰ The interassay coefficients of variation ranged from 5.8% to 9.2% at 28.2 and 180.8 nmol/L 25(OH)D₃, respectively.

Statistical Analysis

A comparison of participants completing the 20-year follow-up with those who were part of the original cohort but did not attend an eye examination was performed using the data from the year 1 follow-up to examine the sociodemographic characteristics between the two groups. These characteristics included sex, ethnicity, family structure (sole-parent versus couple families), income levels, and Socioeconomic Index for Areas (SEIFA) Index of Relative Advantage and Disadvantage (IRSAD) of parents/carers. For the latter, a higher score reflects higher relative socioeconomic advantage (www.abs.gov.au [in the public domain]).

The fasting blood samples were collected year-round between March 2010 and April 2012. Therefore, the seasonal component was removed (deseasonalized) according to

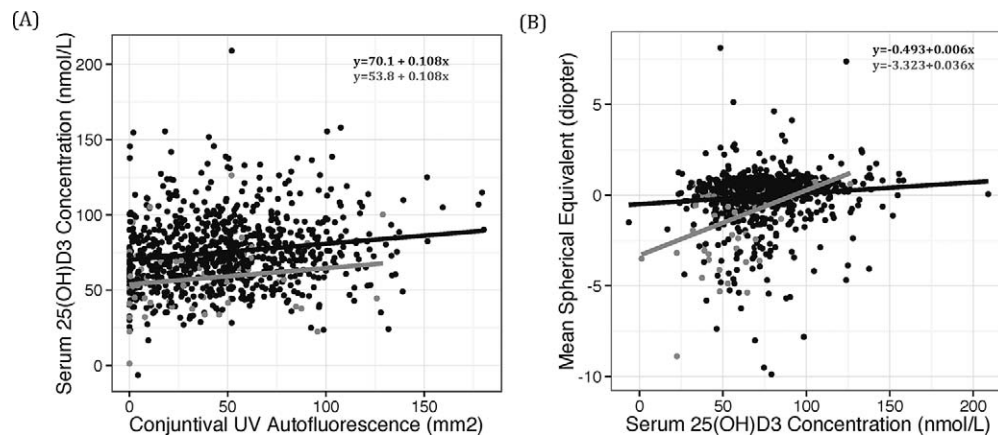


FIGURE 1. Simple linear regression equations of 25(OH)D₃ concentration (nmol/L) with (A) ocular sun exposure (conjunctival UV autofluorescence) and (B) refractive error in young adults (*black*, Northern Europeans; *gray*, East Asians).

published methodology³¹ by fitting a sinusoidal model to serum 25(OH)D₃ concentrations incorporating the month the blood sample was taken. Serum 25(OH)D₂ concentrations were detectable in just 10 participants and at levels below 7 nmol/L; therefore only deseasonalized serum 25(OH)D₃ concentrations were included in the analysis.

Mean spherical equivalent and total 25(OH)D₃ concentrations were not normally distributed (evidence from Kolmogorov-Smirnov test, $P < 0.001$, and nonlinear quantile-quantile [Q-Q] plots); thus summary data are presented as medians and interquartile ranges (IQRs). The difference in serum 25(OH)D₃ concentrations between myopic and nonmyopic participants was assessed using the Mann-Whitney U test.

We defined vitamin D status as being sufficient when concentrations of serum 25(OH)D₃ were ≥ 75 nmol/L, insufficient when they were 50 to 74.9 nmol/L, and deficient when they were < 50 nmol/L.³² Differences between categorical variables were assessed with χ^2 tests. We used a χ^2 test for trend to assess a possible dose-response relationship with myopia prevalence across categories of vitamin D status. A simple linear regression model was generated to describe the relationship between levels of 25(OH)D₃ and MSE using the least squares method. We used simple logistic regression to estimate the odds ratios (OR) and confidence intervals (95% CI) of myopia prevalence in relation to each covariate, testing for trend by replacing categorical predictors with a single predictor, taking category rank scores. A P value < 0.05 was considered statistically significant. A multivariable logistic regression model was constructed to assess the association between myopia prevalence and 25(OH)D₃ concentration (or vitamin D status) while adjusting for age, sex, and other covariates identified as being significant in univariable analysis. Separate multivariable models containing either total CUVAF or time spent outdoors were constructed due to the expected collinearity between these.

Two subgroups were created based on self-reported ethnicity, Australians with Northern European ancestry and East Asians, and the above analyses repeated.

Statistical analyses were performed using the statistical software R version 2.15.1 (R Foundation for Statistical Computing, <http://www.r-project.org/> [in the public domain]).

RESULTS

Compared with individuals from the original cohort who did not participate in the 20-year follow-up, participants who attended eye examinations were more likely to have been born

into couple families (84% vs. 61%) and families with a combined income of more than US \$23,500 (59% vs. 38%) at a time when the average income of a full-time worker in Australia was approximately US \$27,500. Similarly, the mean IRSAD score was higher for parents/carers of the participants from the 20-year follow-up (1039 ± 89) compared to parents/carers of their peers who were not examined (1001 ± 86 , $P < 0.001$). There was no significant difference in sex and ethnicity between the two groups.

Of 1344 participants who attended an eye examination, 198 (14.7%) participants did not have a 25(OH)D level measurement, and 200 (14.9%) participants had incomplete clinical examination or questionnaire data. Serum 25(OH)D₃ concentration and potential confounders including age, sex, ethnicity, parental myopia, education, and ocular sun exposure were available for 946 participants (70.4%); just over half ($n = 480$; 50.7%) of these were female. Only 837 participants had data for time outdoors and potential confounders. The mean (\pm standard deviation) age was 20.0 ± 0.4 years (range, 18.3–22.1 years), and the majority ($n = 798$, 84.4%) of the participants had Northern European ancestry. Of the 946 participants, 837 (88.5%) reported their time spent outdoors during summer. Of these, 406 (48.5%) spent less than a quarter of an average summer day outside; 332 (39.7%) spent approximately half of their day outside; and 99 (11.8%) spent the majority of their day outside. Serum 25(OH)D₃ concentration was lower in males (70.9 nmol/L; IQR = 56.1–84.8) compared to females (71.7 nmol/L [IQR = 58.6–85.2]; $P = 0.015$), and East Asian individuals had lower serum 25(OH)D₃ concentrations compared to their peers with Northern European ancestry (55.3 nmol/L [IQR = 42.4–70.1] vs. 73.0 nmol/L [IQR = 59.6–87.9], $P < 0.001$). Serum 25(OH)D₃ concentration increased with increasing CUVAF (Fig. 1A) and time spent outdoors (β estimate = 9.0 nmol/L increase per one category of time outdoors; standard error = 1.2, P trend < 0.001).

Over one-fifth of participants ($n = 221$ [23.4%]) had myopia (MSE ≤ -0.5 D). Median MSE was -1.56 D (IQR = -3.19 to -0.88) and $+0.44$ D (IQR = $+0.13$ to $+0.75$) in the myopia and nonmyopia groups, respectively ($P < 0.001$). The demographic data for myopic and nonmyopic individuals are displayed in Table 1. Serum 25(OH)D₃ concentrations were significantly lower in the myopic compared to the nonmyopic participants: median of 67.6 nmol (IQR = 52.3–79.6) and 72.5 nmol (IQR = 59.4–87.2), respectively ($P = 0.003$). Figure 1B shows the positive association between serum 25(OH)D₃ concentrations and MSE. The prevalence of

TABLE 1. The Demographic Characteristics of the Study Cohort ($n = 946$)

| | Myopic Participants, $n = 221$ (%) | Nonmyopic Participants, $n = 725$ (%) | <i>P</i> Value |
|------------------------------------|--|---|------------------|
| Sex, male | 102 (53.8) | 364 (49.7) | 0.328 |
| Ethnicity | | | |
| Northern European ancestry | 170 (76.9) | 628 (86.6) | |
| East Asian ancestry | 29 (13.1) | 31 (4.3) | |
| Other | 22 (10.0) | 66 (9.1) | <0.001 |
| Education (currently studying) | 166 (75.1) | 430 (59.3) | <0.001 |
| Parental myopia | | | |
| Neither parent | 125 (56.6) | 559 (77.1) | |
| One parent | 65 (29.4) | 126 (17.4) | |
| Both parents | 31 (14.0) | 40 (5.5) | <0.001 |
| Time spent outdoors during summer* | | | |
| Less than ¼ of day | 115 (56.9) | 291 (45.8) | |
| ½ day | 69 (34.2) | 263 (41.4) | |
| More than ¾ of day | 18 (8.9) | 81 (12.8) | 0.020 |
| Conjunctival UV autofluorescence† | | | |
| First quartile | 72 (32.6) | 165 (22.8) | |
| Second quartile | 73 (33.0) | 163 (22.5) | |
| Third quartile | 40 (18.1) | 196 (27.0) | |
| Fourth quartile | 36 (16.3) | 201 (27.7) | <0.001 |
| Vitamin D status‡ | | | |
| Deficient | 51 (31.7) | 90 (12.4) | |
| Insufficient | 100 (45.2) | 309 (42.6) | |
| Optimal | 70 (31.8) | 326 (45.0) | <0.001 |

P values < 0.05 are shown in bold.

* Numbers of participants may not equal 946, due to missing data in the time spent outdoors.

† Conjunctival UV autofluorescence was divided into quartiles: first quartile: 0 to 20.80; second quartile: 20.81 to 45.10; third quartile: 45.11 to 70.40; fourth quartile: 70.41 to 180.0, used here as a marker of ocular sun exposure.

‡ Vitamin D deficiency is defined as a deasonalized serum 25(OH)D₃ concentration below 50 nmol/L and vitamin D insufficiency as deasonalized serum 25(OH)D₃ concentration of 50 to 74.9 nmol/L.

myopia decreased in association with higher 25(OH)D₃ concentration (OR = 0.88, 95% CI 0.82–0.94, per 10 nmol/L increase) and across categories of increasing vitamin D status (χ^2 for linear trend = 19.63, $P < 0.001$). Presence of myopia was also positively associated with currently studying and parental myopia and was inversely associated with higher CUVAF area, Northern European ethnicity, and greater time spent outdoors in univariable analyses (Table 2).

Table 3 shows the results of the multivariable logistic regression models. In the model adjusted for age and sex only, the odds of being myopic decreased with increasing 25(OH)D₃ concentration (OR: 0.88, 95% CI: 0.82–0.94 per 10 nmol/L increase, $P < 0.001$), and increased across categories of decreasing vitamin D status (OR: 2.67, 95% CI: 1.72–4.11 for vitamin D deficiency versus sufficiency). The significant association with 25(OH)D₃ as a continuous variable was retained in the multivariable model adjusted for age, sex, ethnicity, parental myopia, education, and CUVAF (OR: 0.91, 95% CI: 0.85–0.98 per 10 nmol/L increase, $P = 0.013$). Across categories of vitamin D status, the odds of being myopic were significantly increased only in the comparison of vitamin D

deficiency to vitamin D sufficiency in the fully adjusted model; but there was evidence of a significant trend (OR: 1.42, 95% CI: 1.12–1.79 per category increase, P for trend < 0.001).

In the subgroup analysis including only participants with Northern European background, the myopic group had a significantly lower median 25(OH)D₃ concentration compared to the nonmyopic group (Fig. 2). The odds of having myopia decreased significantly with increasing 25(OH)D₃ concentration in the age- and sex-adjusted model. This effect was no longer statistically significant after adjustment for the other factors in the fully adjusted model, although the strength of the association was very similar. Nevertheless, vitamin D deficiency was associated with an increased risk of myopia compared to vitamin D sufficiency in the fully adjusted model, with evidence of a trend across the categories (OR: 1.35, 95% CI: 1.04–1.75, P for trend = 0.024).

Among the 60 participants with East Asian background, 48.3% ($n = 29$) were myopic. Serum 25(OH)D₃ concentration was significantly lower in the myopic compared to nonmyopic East Asian participants (53.0 nmol/L [IQR: 11.2–94.8] vs. 61.0 nmol/L [IQR: 13.8–108.3], $P = 0.035$). Similarly, higher 25(OH)D₃ concentration was associated with a decreased odds of having myopia in the age- and sex-adjusted model as well as the fully adjusted model (Table 3), but the trend across categories of vitamin D status was not statistically significant ($P = 0.078$), probably because of the small sample size. The point estimates suggest that the odds of being myopic were increased 6-fold in association with vitamin D deficiency compared to sufficiency. We were unable to undertake further adjustment in this subgroup because of the small sample size.

In analyses of both the full sample and the Northern European subgroup, CUVAF, parental myopia, and education remained significant in the adjusted multivariable regression model for myopia that also included serum 25(OH)D₃ concentration (all $P \leq 0.001$).

Separate models constructed to adjust for time spent outdoors provided results similar to those adjusting for CUVAF. For example, in the full sample, the odds of being myopic decreased with increasing 25(OH)D₃ concentrations (adjusted OR: 0.89, 95% CI: 0.83–0.97 per 10 nmol/L increase, $P = 0.008$). Similarly, the adjusted odds of having myopia were 0.93 (95% CI: 0.85–1.01) and 0.56 (95% CI: 0.33–0.83) with every 10 nmol/L increase in 25(OH)D₃ concentrations in Northern European ($P = 0.091$) and East Asian subsets ($P = 0.011$), respectively. The odds of being myopic were significantly increased in the comparison of vitamin D deficiency to vitamin D sufficiency in the fully adjusted model (OR: 2.14, 95% CI: 1.27–3.58, $P = 0.008$). In individuals with Northern European ancestry ($n = 707$), the odds of being myopic were not significantly different between those who were vitamin D deficient and sufficient (OR: 1.78; 95% CI: 0.96–3.27, $P = 0.065$).

East Asian ethnicity was associated with both lower 25(OH)D₃ levels and greater prevalence of myopia. We therefore examined these associations in more detail by comparing a model containing only ethnicity as a risk factor with a second model that included ethnicity and serum 25(OH)D₃ concentrations. Addition of serum 25(OH)D₃ concentration improved the model fit, and East Asian ethnicity remained significant with an Akaike information criterion (AIC) difference of 7.8 ($P < 0.001$) and the effect of ethnicity slightly reduced (β estimate of -0.664 to -0.518).

Finally, a binary variable for serum 25(OH)D concentration was generated using a cut-off point of <75 vs. ≥ 75 nmol/L. We used this to explore the determinants of vitamin D insufficiency by setting this variable as the dependent parameter and using a model that included factors that were associated with vitamin D concentrations as independent variables. Presence

TABLE 2. Univariable Logistic Regression Analysis of Associations With Myopia

| Covariate | All Participants, <i>n</i> = 946 | | Northern European Participants, <i>n</i> = 798 | | East Asian Participants, <i>n</i> = 60 | |
|---|----------------------------------|----------------|--|----------------|--|----------------|
| | OR (95% CI) | <i>P</i> Value | OR (95% CI) | <i>P</i> Value | OR (95% CI) | <i>P</i> Value |
| Age, per year | 1.25 (0.90-1.74) | 0.180 | 1.26 (0.85-1.85) | 0.235 | 2.75 (0.82-11.10) | 0.119 |
| Sex | | | | | | |
| Female | Reference | | Reference | | Reference | |
| Male | 1.17 (0.87-1.59) | 0.292 | 1.07 (0.76-1.51) | 0.691 | 1.07 (0.76-1.51) | 0.691 |
| Ethnicity | | | | | | |
| Non-Northern European | Reference | | - | - | - | - |
| Northern European | 0.51 (0.35-0.76) | <0.001 | - | - | - | - |
| 25(OH)D ₃ level Per 10 nmol/L increase | 0.88 (0.82-0.94) | <0.001 | 0.92 (0.85-0.99) | 0.033 | 0.68 (0.48-0.90) | 0.013 |
| Vitamin D status* | | | | | | |
| Sufficient | Reference | | Reference | | Reference | |
| Insufficient | 1.51 (1.07-2.13) | <0.001 | 1.57 (1.09-2.28) | 0.017 | 3.25 (0.64-24.58) | 0.185 |
| Deficient | 2.63 (1.71-4.05) | 0.019 | 2.03 (1.19-3.42) | 0.008 | 4.90 (0.95-37.84) | 0.078 |
| CUVAF Per mm ² | 0.99 (0.98-0.99) | <0.001 | 0.98 (0.98-0.99) | <0.001 | 0.99 (0.97-1.00) | 0.150 |
| CUVAF† | | | | | | |
| First quartile | Reference | | Reference | | Reference | |
| Second quartile | 1.03 (0.69-1.52) | 0.896 | 1.02 (0.65-1.59) | 0.925 | 1.49 (0.39-6.04) | 0.565 |
| Third quartile | 0.47 (0.30-0.72) | <0.001 | 0.55 (0.34-0.89) | 0.016 | 0.46 (0.10-1.85) | 0.289 |
| Fourth quartile | 0.41 (0.26-0.64) | <0.001 | 0.45 (0.27-0.75) | 0.002 | 0.56 (0.10-2.74) | 0.479 |
| Time outdoors in summer‡ | | | | | | |
| Less than ¼ of day | Reference | | Reference | | Reference | |
| ½ day | 0.66 (0.47-0.93) | 0.019 | 0.66 (0.45-0.98) | 0.040 | 0.28 (0.07-0.92) | 0.044 |
| More than ¾ of day | 0.56 (0.31-0.96) | 0.042 | 0.64 (0.34-1.13) | 0.135 | 0.33 (0.01-3.80) | 0.388 |
| Parental myopia | | | | | | |
| No parent affected | Reference | | Reference | | Reference | |
| One parent affected | 2.31 (1.61-3.29) | <0.001 | 2.44 (1.64-3.61) | <0.001 | 0.82 (0.21-2.95) | 0.764 |
| Both parents affected | 3.47 (2.08-5.75) | <0.001 | 3.01 (1.68-5.30) | <0.001 | 5.25 (1.13-38.08) | 0.053 |
| Education | | | | | | |
| Not studying | Reference | | Reference | | Reference | |
| Currently studying | 2.07 (1.48-2.93) | <0.001 | 1.84 (1.27-2.68) | 0.001 | 1.67 (0.37-8.80) | 0.513 |

P values < 0.05 are shown in bold.

* Vitamin D deficiency is defined as a deaseasonalized serum 25(OH)D₃ concentration below 50 nmol/L and vitamin D insufficiency as deaseasonalized serum 25(OH)D₃ concentration of 50 to 74.9 nmol/L.

† CUVAF was divided into quartiles: first quartile: 0 to 20.80; second quartile: 20.81 to 45.10; third quartile: 45.11 to 70.40; fourth quartile: 70.41 to 180.0.

‡ Only 837 participants included in this univariate analysis due to missing data in the time spent outdoors.

of myopia was associated with an increased odds of being vitamin D insufficient in this model (adjusted OR: 1.63; 95% CI: 1.18-2.27; *P* = 0.003).

DISCUSSION

In this study of young adults at 20 years of age, lower serum 25(OH)D₃ concentrations were associated with higher prevalence of myopia as previously identified in Caucasian teens²⁸ and Korean adolescents.¹⁰ This association could be evidence of an underlying biochemical mechanism between serum 25(OH)D concentrations and myopia and explain previous findings that greater time spent outdoors is associated with reduced risk of myopia development. Alternatively, the 25(OH)D₃ concentration could be simply a biomarker of sun exposure, with some other non-vitamin D element being a protective factor. Although it might be suggested that our results reflect reverse causality, whereby myopic young adults prefer to spend more time indoors and thus have lower self-reported time outdoors and lower 25(OH)D₃ levels, this

explanation is not supported by the findings from the study of Jones-Jordan et al.³³ In that prospective study, sports/outdoor activities were decreased in myopic subjects 3 years before onset, thus pointing to a causal relationship between outdoor exposure and myopia development. Hence, the results of the present study should not be interpreted in the sense of reflecting reverse causality. Our results are consistent with previous findings of environmental or demographic risk factors for myopia including Asian ethnicity, history of parental myopia, higher education, lower levels of CUVAF, and less time spent outdoors³⁴ (McKnight CM, manuscript submitted, 2014).

Country of origin, genetic traits, and cultural behavior are important factors determining serum 25(OH)D₃ concentrations.³⁵ A higher risk of vitamin D deficiency in individuals with Asian, Middle Eastern, and African origins is well described.³⁶⁻⁴⁰ Lower serum 25(OH)D levels in dark-skinned individuals are likely due to both behavioral factors⁴¹ and decreased efficiency of vitamin D production in darker-skinned individuals.⁴² Lower 25(OH)D₃ concentrations among East Asian individuals have been reported previously.⁴³ Therefore,

TABLE 3. Adjusted Multivariable Logistic Regression Analysis of the Association Between 25(OH)D₃ Levels and Presence of Myopia

| | Age- and Sex-Adjusted Model | | Multivariable Model* | |
|--------------------------------------|-----------------------------|--------------|----------------------|--------------|
| | OR (95% CI) | P Value | Adjusted OR (95% CI) | P Value |
| 25(OH)D ₃ , per 10 nmol/L | | | | |
| All participants | 0.88 (0.82-0.94) | <0.001 | 0.91 (0.85-0.98) | 0.013 |
| Northern European participants | 0.92 (0.85-0.99) | 0.037 | 0.94 (0.87-1.02) | 0.138 |
| East Asian participants | 0.66 (0.46-0.88) | 0.009 | 0.58 (0.38-0.82) | 0.005 |
| Vitamin D status† | | | | |
| All participants | | | | |
| Deficient | 2.67 (1.72-4.11) | <0.001 | 2.07 (1.29-3.32) | 0.002 |
| Insufficient | 1.51 (1.07-2.13) | 0.019 | 1.28 (0.89-1.84) | 0.177 |
| Sufficient | Reference | | Reference | |
| Northern European participants | | | | |
| Deficient | 2.01 (1.17-3.39) | 0.010 | 1.77 (1.01-3.07) | 0.043 |
| Insufficient | 1.57 (1.09-2.28) | 0.017 | 1.38 (0.94-2.03) | 0.104 |
| Sufficient | Reference | | Reference | |
| East Asian participants‡ | | | | |
| Deficient | 6.24 (1.07-55.27) | 0.060 | - | - |
| Insufficient | 3.59 (0.65-29.71) | 0.173 | - | - |
| Sufficient | Reference | | Reference | |

P values < 0.05 are shown in bold.

* Multivariate model adjusted for age, sex, ethnicity (where applicable), parental myopia, CUVAF (mm²), education.

† Vitamin D deficiency is defined as a deaseasonalized serum 25(OH)D₃ concentration below 50 nmol/L and vitamin D insufficiency as deaseasonalized serum 25(OH)D₃ concentration of 50 to 74.9 nmol/L.

‡ The sample size was too small to undertake further adjustment in this subgroup.

the markedly lower 25(OH)D₃ concentrations in participants with East Asian compared to Northern European ancestry in our cohort was not unexpected. However, it was interesting to note that the East Asian group also had a higher prevalence of myopia. Further probing of this finding led us to identify that adjusting for 25(OH)D concentration accounted for part of the association between ethnicity and prevalence of myopia. Hence we suggest that lower vitamin D status is one factor that mediates the difference in myopia prevalence between various ethnicities.

Many mechanisms have been postulated to explain the apparent protective effect of time spent outdoors for myopia

development. Given that the association between near work and myopia is weak and inconsistent, substitution of outdoor activities for near work does not appear to be the important factor, nor does participating in sport per se.^{13,44} In animal models, emmetropization is an active process by which optical defocus adjusts the rate of axial elongation during growth and development of the eye. Therefore, it was proposed that improved retinal image quality during distance viewing with a smaller pupil size and accommodative errors may inhibit ocular growth, thus decreasing the risk of development of myopia. However, evidence from animal models did not support this hypothesis.^{45,46} Another hypothesis was that greater light

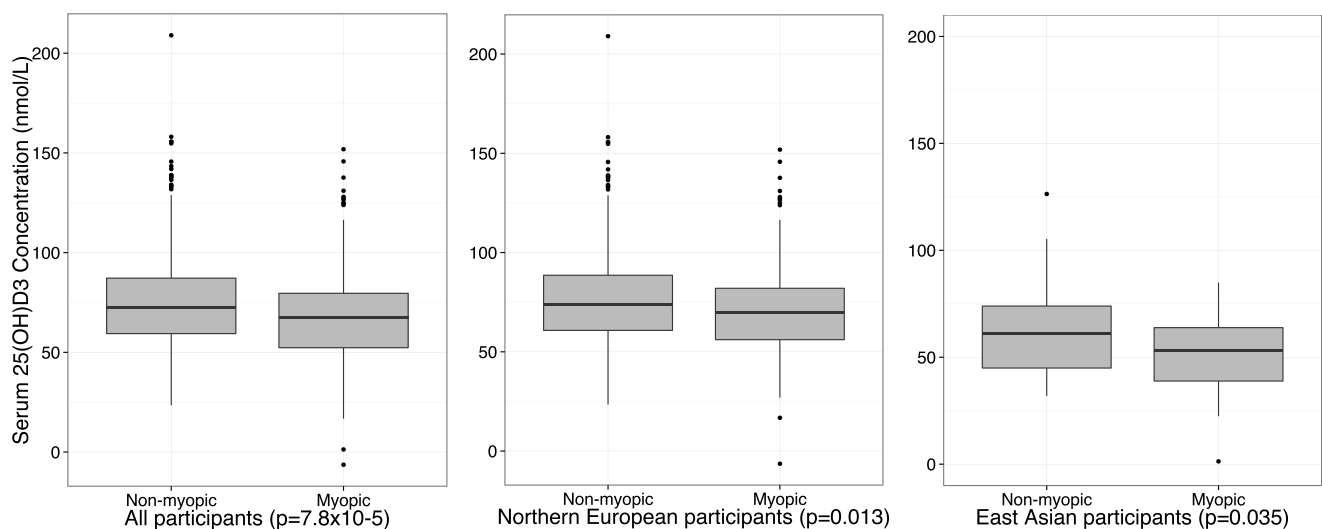


FIGURE 2. Comparison of 25(OH)D₃ Levels (nmol/L) between myopic and nonmyopic participants in individuals with Northern European and East Asian ancestries.

intensities outside may alter the release of dopamine, known to inhibit ocular growth, in the retina,¹³ and this has been tested in chick models.⁴⁷ In support of this hypothesis, high ambient lighting was found to retard development of myopia in chick and Rhesus monkey studies.⁴⁸⁻⁵⁰

Matrix metalloproteinases (MMP) have been implicated in the scleral remodeling of experimental myopia⁵¹ and in the development of simple myopia.⁵² Recently an inverse correlation between blood MMP9 and 25(OH)D levels has been found in submariners.⁵³ A possible explanation for this might be that suboptimal serum 25(OH)D levels (from either sun avoidance⁵⁴ or Western diets that are typically low in vitamin D⁵⁵) may modulate blood and perhaps tissue levels of MMP with a downstream effect on scleral morphology and refraction.

This study is unique in having a large sample size and an objective measure of ocular sun exposure that correlates well with time spent outdoors. The only method to determine vitamin D deficiency or sufficiency in an individual is to measure circulating 25(OH)D concentration. In this study, serum 25(OH)D concentrations were measured as the common pathway in vitamin D metabolism for both dietary and sun-induced vitamin D. Moreover, this study contains a wealth of supporting data reinforcing the identified association between myopia and vitamin D status. One caveat relating to this study that must be acknowledged is that the study used cross-sectional data collection from a birth cohort and refractive error was measured at only one point in time; therefore causality cannot be inferred. Furthermore, the highest incidence of myopia occurs in children aged 5 to 15 years. As no data on 25(OH)D concentrations were available from younger time points at the time of this analysis, we made the assumption that 25(OH)D levels in young adults are consistent with those in younger years during which myopia may have developed. Information on time spent outdoors and other potential risk factors including ethnicity, education, and parental myopia was extracted from self-reported questionnaires and is thus subject to recall bias. This, though, is likely to be nondifferential across the groups defined by having myopia or not, so would have resulted in a bias toward null findings; that is, our findings may be an underestimate of the true associations. It is also possible that the findings in participants were different from those in nonparticipants, given that more than 50% of the original cohort were lost to follow-up and a relatively high proportion (30%) of participants in the 20-year follow-up had incomplete data. Further investigations are necessary to validate the findings from this cohort and to assess effects of population differences.

In conclusion, findings from this study suggest that there could be a biological association between the risk of myopia and reduced 25(OH)D₃ concentrations within different populations. However it is important to bear in mind that the 25(OH)D₃ could be acting as a proxy for ocular sun exposure, with the latter the important factor. Therefore, future studies prospectively investigating the effects of 25(OH)D₃ concentrations and ocular sun exposure in the development of refractive error are warranted.

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