Does Vitamin D Mediate the Protective Effects of Time Outdoors On Myopia? Findings From a Prospective Birth Cohort

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Purpose. More time outdoors is associated with a lesser risk of myopia, but the underlying mechanism is unclear. We tested the hypothesis that 25-hydroxyvitamin D (vitamin D) mediates the protective effects of time outdoors against myopia.

Methods. We analyzed data for children participating in the Avon Longitudinal Study of Parents and Children (ALSPAC) population-based birth cohort: noncycloplegic autorefraction at age 7 to 15 years; maternal report of time outdoors at age 8 years and serum vitamin D2 and D3 at age 10 years. A survival analysis hazard ratio (HR) for incident myopia was calculated for children spending a high- versus low-time outdoors, before and after controlling for vitamin D level ($N = 3677$).

Results. Total vitamin D and D3, but not D2, levels were higher in children who spent more time outdoors (mean [95% confidence interval (CI)] vitamin D in nmol/L: Total, 60.0 [59.4–60.6] vs. 56.9 [55.0–58.8], $P = 0.001$; D3, 55.4 [54.9–56.0] vs. 53.0 [51.3–54.9], $P = 0.014$; D2, 5.7 [5.5–5.8] vs. 5.4 [5.1–5.8], $P = 0.23$). In models including both time outdoors and sunlight-exposure–related vitamin D, there was no independent association between vitamin D and incident myopia (Total, HR = 0.83 [0.66–1.04], $P = 0.11$; D3, HR = 0.89 [0.72–1.10], $P = 0.30$), while time outdoors retained the same strong negative association with incident myopia as in unadjusted models (HR = 0.69 [0.55–0.86], $P = 0.001$).

Conclusions. Total vitamin D and D3 were biomarkers for time spent outdoors, however there was no evidence they were independently associated with future myopia.

Keywords: myopia, refractive error, epidemiology, vitamin D, light levels

A recent systematic review[1] of population-based visual impairment (VI) studies reported the prevalence of VI attributable to pathologic myopia to be 0.1% to 0.5% in Europe and 0.2% to 1.4% in Asia, and that myopia was the first-to-third most frequent cause of registered blindness. Intensive near-work/education from an early age and a lack of time spent outdoors are strong risk factors for myopia.[2–11] There is growing evidence, from both animal studies[12–14] and clinical trials (Morgan IG, et al. *IOVS* 2012;53:ARVO E-Abstract 2735 and Ref. 10), that the link between myopia and insufficient time outdoors arises from a relatively direct causal relationship, with exposure to bright light somehow influencing how the eye responds to myopiagenic visual cues.[15,16] Two main theories have been put forward to explain the relationship.[17] First,[14,18–20] that bright light's beneficial effect is due to enhanced release of dopamine in the retina,[14,18–20] and second[21,22] that its beneficial effect results from increased serum 25-hydroxyvitamin D (25(OH)D). Consistent with the second theory, serum 25(OH)D levels were found to be associated with myopia in adolescents/young adults from the United States,[22] Korea,[23] and Australia.[24]

Sunlight exposure increases production of vitamin D in the skin, therefore a relationship between myopia and 25(OH)D may be seen without vitamin D being causally linked to myopia development. We sought to test the hypothesis that vitamin D may mediate the protective effect of spending time outdoors on myopia development, by using prospectively collected data from an ongoing birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC).

Methods

ALSPAC Birth Cohort Participants

The Avon Longitudinal Study of Parents and Children recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery April 1, 1991 to December 31, 1992. Of these initial pregnancies, 13,988 children were alive at 1 year of age. An additional 713 eligible cases who had failed to join the study originally were recruited when the oldest children were approximately 7 years of age. Excluded were those mothers who had moved out of the area or were lost to follow-up, and

those participating in another study of infant development in the county of Avon, United Kingdom.[26] The ALSPAC study website contains details of all the data that is available through a fully searchable data dictionary (in the public domain http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the local research ethics committees.

Refractive Error Assessment

Participants were invited to attend regular research clinics where a number of health-related assessments and interviews took place. Vision-related tests were included in the 7-, 10-, 11-, 12-, and 15-year clinics: Refractive error was estimated by noncycloplegic autorefraction (Canon R50; Canon USA, Inc., Lake Success, NY, USA). Due to the absence of cycloplegia, we interpret these data as screening for "likely myopia," rather than as an exact measure.[26,27] Mean spherical equivalent (MSE) refractive error was calculated as the autorefraction sphere power plus one-half of the cylinder power. Subjects were classified as myopic[11] at each visit if the average of the MSEs in their two eyes was less than or equal to 1.00 diopter (D).

Serum 25(OH)D Assay

Nonfasting blood samples were collected at several of the ALSPAC clinic visits. Samples were centrifuged, and the serum stored frozen at −80°C (with no freeze/thaw cycles prior to use in the 25(OH)D assay). If a blood sample was available from the 9-year clinic, this was used for the 25(OH)D assay. If a blood sample from the 9-year clinic was unavailable, then a sample from the 7- or 11-year clinic was used. Levels of $25(OH)D_2$ and $25(OH)D_3$ in serum were measured with HPLC tandem mass spectrometry using an internal standard, by a laboratory meeting the performance target set by the vitamin D External Quality Assessment Scheme Advisory Panel for 25-hydroxyvitamin D assays (in the public domain http://www.deqas.org/). The interassay coefficients of variation for both $25(OH)D_2$ and $25(OH)D_3$ was less than 10% across the range of 1 to 250 ng/ml. Outlier removal and adjustment for year and season of collection are described in the Supplementary Information.

Time Spent Outdoors, Time Spent Reading, Parental Myopia, and Ethnicity

Variables were defined as described.[11] Children were classed as spending a high versus low amount of time outdoors per day based on responses to the following questionnaire item completed by the child's mother or guardian when the child was aged 8- to 9-years old: "On a weekend day, how much time on average does your child spend each day out of doors in summer?" Children were classified as spending a "high" amount of time outdoors if the response was "3 or more hours," and as "low" otherwise. Parental myopia was inferred[11] from a questionnaire item, "How would you rate your sight without glasses?" Analyses were restricted to children whose mother's self-reported ethnicity was "white" due to the low numbers of participants from other ethnic groups (∼2%).

Data Analysis, General Considerations

We used survival analysis[11] to study the association of risk factors with incident myopia (i.e., factors associated with how long the child "survived" in the study before being estimated as at least −1.00-D myopic). In separate analyses, linear mixed-models were used to study refractive error trajectories (i.e., the gradual change in refractive error estimates over the study period).

Depending on whether or not participants attended each of the research clinics, data were missing to varying extents for covariates, as shown in Supplementary Table S1. Analyses in which the level of serum vitamin D was modelled as a quantitative variable were considered the primary analyses. Levels of $25(OH)D_2$ and $25(OH)D_3$ levels were $\log_e$ transformed to reduce skew and heteroscedasticity. In approximately one-third of children, the measured $25(OH)D_2$ level was below the detection threshold of the assay (1.25 nmol/L). For statistical models in which $25(OH)D_2$ was included as a quantitative parameter, these subjects were excluded from the analysis, while for models in which $25(OH)D_2$ tertiles were examined, this group was assigned as the low tertile and the remaining children with measurable $25(OH)D_2$ levels were assigned by rank into the middle and high tertiles. Because $25(OH)D_3$ levels were substantially higher than $25(OH)D_2$ levels we assigned a $25(OH)D_2$ level of zero to those children with a subthreshold reading when summing the $25(OH)D_2$ and $25(OH)D_3$ levels to give the total. Outlier removal and adjustment for year and season of collection are described in the Supplementary Information and Figure S1. We used natural log-transformed levels of vitamin D, hence for back-transformed values presented, the total vitamin D level does not equal the numerical sum of the vitamin D_2 and D_3 levels.

Survival Analysis

Cox proportional hazard models were used to test whether the inclusion of serum 25(OH)D level and of time spent outdoors in the same model changed the associations that were seen with each factor when tested on its own.

We examined the previously reported association of time outdoors with myopia in initial models that also included the number of myopic parents (0, 1, or 2), time spent reading (high, low), and sex (male, female). The HR for time outdoors (Table 1) was very similar to our earlier report (which included only subjects with information available about physical activity[11]). While retaining all of the above predictors, we then included in separate models, six different estimates of vitamin D: unadjusted $25(OH)D_2$, unadjusted $25(OH)D_3$, unadjusted total 25(OH)D, and the three corresponding season and year adjusted 25(OH)D levels. Six additional models were tested using 25(OH)D tertiles instead of quantitative measures.

We set as missing any refractive error measurements that were obtained prior to the clinic visit at which the serum 25(OH)D blood sample was collected. This led to the exclusion of data for 136 (<2%) of refractive error measurements. Survival analysis models were run using SPSSv19 (IBM Corp., Armonk, NY, USA).

Refractive Error Trajectory Analysis

Longitudinal models were used to examine whether the rate of refractive error change over childhood differed between subjects classified as spending a high versus low amount of time outdoors at age 8 to 9 years, and whether controlling for 25(OH)D affected these estimates. Refractive error trajectory models were constructed analogously to those used for survival analysis, with the exception that children were required to have had at least three refractive error measurements from clinic visits beginning at, or after, the visit at which their serum 25(OH)D blood sample was collected. We set as missing any refraction measurements obtained prior to the vitamin D blood sampling visit. Details of the refractive

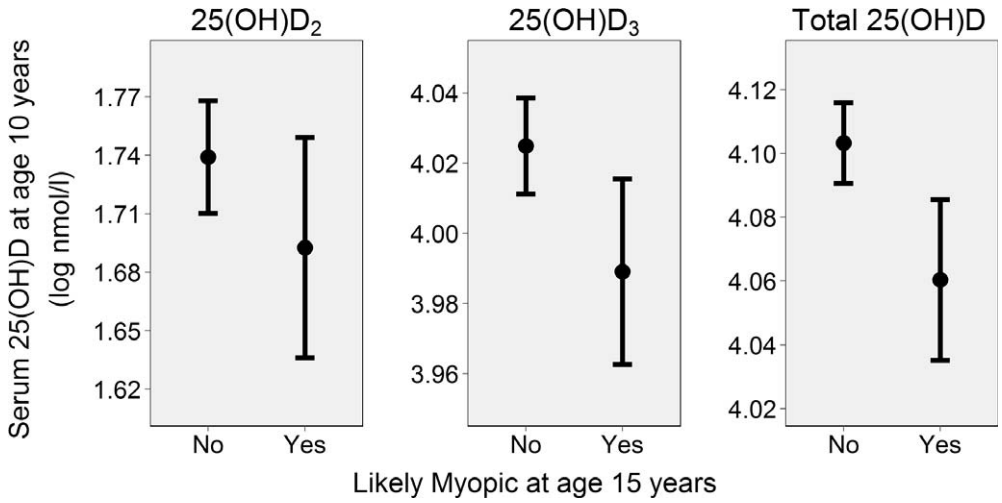

FIGURE 1. Serum 25(OH)D concentration sampled at age 10 years in children classified as likely myopic versus nonmyopic at age 15 years.

trajectory models are given in the Supplementary Information.

RESULTS

The key findings of this study can be summarized as follows: (1) time spent outdoors was positively associated with serum $25(OH)D_3$, (2) both serum $25(OH)D_2$ and $25(OH)D_3$ were inversely associated with incident myopia, and (3) the association of time spent outdoors with incident myopia was not attenuated by adjusting for serum $25(OH)D_2$ Or $25(OH)D_3$.

Association Between Serum 25(OH)D Versus Time Outdoors, and 25(OH)D Versus Myopia

There were 5126 children with information available regarding the time they spent outdoors at age 8 to 9 years, their serum 25(OH)D level, and their refractive error at one or more research clinic visits (Supplementary Table S1). The average age at which the blood samples used for serum 25(OH)D assays were collected was 9.8 (SD 1.2) years. After adjusting for year and season of collection, total serum 25(OH)D was higher in children who spent more time outdoors (60.0 [95% confidence interval (CI): 59.4–60.6] vs. 56.9 [95% CI: 55.0–58.8] nmol/L; *t*-test, $P = 0.001$) as was the case for serum $25(OH)D_3$ (55.4 [54.9–56.0] vs. 53.0 [51.3–54.9] nmol/L; $P = 0.014$) but not the level of serum $25(OH)D_2$ (5.7 [5.5–5.8] vs. 5.4 [5.1–5.8] nmol/L; $P = 0.23$). Similar trends were observed when serum 25(OH)D level was categorized into tertile groups (Supplementary Fig. S2).

Levels of season and year adjusted serum $25(OH)D_2$, $25(OH)D_3$, and total 25(OH)D sampled at age approximately 10 years were all lower in subjects classified as "likely myopic" versus "likely not myopic" at age 15 years ($25(OH)D_2$: 5.5 [95% CI = 5.2–5.8] vs. 5.7 [95% CI = 5.5–5.8] nmol/L; *t*-test, $P = 0.024$; $25(OH)D_3$: 54.0 [95% CI = 52.7–55.4] vs. 55.7 [95% CI = 55.0–56.5] nmol/L; $P = 0.030$; total 25(OH)D: 58.1 (95% CI = 56.7–59.5) vs. 60.3 [95% CI = 59.6–61.0) nmol/L; $P = 0.007$; Fig. 1). Similar trends were observed when serum 25(OH)D level was categorized into tertile groups (Supplementary Fig. S3).

The correlation between serum $25(OH)D_2$ and $25(OH)D_3$ in this cohort was weak and in a negative direction (Pearson $r = -0.14$, $P < 0.001$).

Survival Analysis

There were a maximum of $N = 3677$ subjects available for the survival analyses. Children who spent a high versus a low amount of time outdoors at age 8 to 9 years had a reduced risk of incident myopia (HR = 0.69, 95% CI = 0.55–0.86, $P = 0.001$; Table 1 and Fig. 2).

Inclusion of serum total 25(OH)D, $25(OH)D_2$, or $25(OH)D_3$ level in the model did not change this protective effect of time outdoors, while the majority of vitamin D estimates had no independent association with the outcome, when time outdoors was taken into account. An exception to this was serum $25(OH)D_2$ tertile, which showed an independent association with the risk of incident myopia (D_2 tertile after adjusting for season and year of collection: HR = 0.88, 95% CI = 0.81–0.97, $P = 0.007$; Fig. 2). However, in models that included either the unadjusted or adjusted quantitative level of $25(OH)D_2$, there was lesser evidence, possibly related to the reduced sample size for this analysis (HR ~0.89; all $P > 0.14$; Table 1). Serum $25(OH)D_3$ did not show an independent association with the risk of incident myopia in any of the models (all $P > 0.29$; Table 1).

Parental myopia and spending a high amount of time reading were both associated with an increased risk of incident myopia (both $P < 0.001$), while sex appeared unrelated to the risk of incident myopia.

Refractive Error Trajectory Analysis

In an initial model in which only the effects of age and time outdoors were considered ($N = 3924$; Supplementary Table S2) children who spent a high amount of time outdoors at age 8- to 9-years old had a refractive error 0.15 D (95% CI: 0.04–0.27; $P = 0.009$) more positive at "baseline" (age 9.8 years) than those who spent a low amount of time outdoors, with this difference between the two groups remaining relatively stable through to the age of 15 years (time outdoors × age interaction = 0.01 D/y, 95% CI: 0.00–0.03; $P = 0.12$). Controlling for serum total 25(OH)D, $25(OH)D_2$, or $25(OH)D_3$ did not affect the magnitude of these relationships (Supplementary Table S2). For instance, after controlling for year and season adjusted total 25(OH)D, children who spent a high versus low amount of time outdoors at age 8 to 9 years old still had a refractive error that was relatively more positive by 0.15 D (95% CI: 0.04–0.27; $P = 0.010$) and which remained stable as they got older (time outdoors × age interaction = 0.01 D/y, 95% CI: 0.00–0.03; $P =$

Table 1. Results of Survival Analysis Models

Serum 25(OH)D Variable Included in Model	Time Outdoors‡			25(OH)D§		
	HR	95% CI	*P*	HR	95% CI	*P*
None (initial model; $N = 3677$)	0.689	(0.551–0.861)	0.001	NA	NA	NA
Total 25(OH)D	0.693	(0.554–0.866)	0.001	0.876	(0.701–1.093)	0.241
Total 25(OH)D (adjusted†)	0.693	(0.555–0.867)	0.001	0.832	(0.664–1.043)	0.111
Total 25(OH)D tertiles	0.692	(0.553–0.864)	0.001	0.928	(0.845–1.019)	0.116
Total 25(OH)D (adjusted†) tertiles	0.691	(0.553–0.864)	0.001	0.946	(0.862–1.038)	0.243
$25(OH)D_3$	0.691	(0.552–0.863)	0.001	0.935	(0.760–1.150)	0.526
$25(OH)D_3$ (adjusted†)	0.691	(0.553–0.864)	0.001	0.893	(0.722–1.104)	0.296
$25(OH)D_3$ tertiles	0.689	(0.551–0.862)	0.001	0.967	(0.881–1.061)	0.474
$25(OH)D_3$ (adjusted†) tertiles	0.689	(0.551–0.862)	0.001	0.972	(0.886–1.066)	0.547
$25(OH)D_2$ tertiles	0.692	(0.554–0.866)	0.001	0.894	(0.815–0.980)	0.017
$25(OH)D_2$ (adjusted†) tertiles	0.692	(0.554–0.865)	0.001	0.882	(0.805–0.967)	0.007
None (initial model; $N = 2294$)*	0.690	(0.514–0.927)	0.014	NA	NA	NA
$25(OH)D_2$*	0.692	(0.515–0.929)	0.014	0.905	(0.770–1.064)	0.227
$25(OH)D_2$ (adjusted†)*	0.692	(0.516–0.929)	0.014	0.886	(0.753–1.044)	0.149

Each row of the table lists the results for one model, giving the HR for myopia associated with spending a high versus low amount of time outdoors (first column), and the HR for myopia associated with vitamin D level (second column). All models also included the predictors, number of myopic parents, time spent reading, and sex (results not shown). Note that vitamin D level was not included in the initial models.

* Sample size reduced due to the exclusion of subjects having quantitative serum $25(OH)D_2$ levels below the detection threshold. These subjects were included in the $25(OH)D_2$ tertiles model as the "low" tertile group.

† Adjusted for year and season of blood sample collection.

‡ A "low" amount of time outdoors was taken as the reference category.

§ For 25(OH)D tertiles, the lower tertile was taken as the reference category. For quantitative 25(OH)D level, the HR is shown for a one unit charge in the natural logarithm of 25(OH)D concentration in nmol/L.

0.14). Independently of time outdoors, there was some evidence for an association between $25(OH)D_3$ and refractive error trajectory (vitamin D_3 tertile × age interaction = 0.006 D/y, 95% CI: 0.000–0.012; $P = 0.049$). However, there was no indication of an independent association between $25(OH)D_3$ tertile and refractive error at baseline ($P = 0.98$).

In the model that included the full set of predictor variables, age, sex, number of myopic parents, time spent reading, and time outdoors ($N = 2852$; Table 2 and Fig. 3) children who spent a high amount of time outdoors at age 8- to 9-years old had a refractive error 0.14 D (95% CI: 0.02–0.27; $P = 0.026$) more positive at age 9.8 years than those who spent a low amount of time outdoors. However, time outdoors was not predictive of further refractive changes over the 11- to 15-year period (time outdoors × age interaction = 0.01 D/y, 95% CI: –0.01 to 0.03; $P = 0.30$). Controlling for serum total 25(OH)D, $25(OH)D_2$, or $25(OH)D_3$ did not affect the time outdoors versus myopia relationship (Table 2). For instance, after controlling for season and year adjusted total 25(OH)D, children who spent a high versus a low amount of time outdoors at age 8- to 9-years old still had a refractive error at age 9.8 years that was relatively more positive by 0.14 D (95% CI: 0.02–0.27; $P = 0.029$; Fig. 3). In the full model there was no evidence for an independent association between serum total 25(OH)D, $25(OH)D_2$, or $25(OH)D_3$ and refractive error, as regards either a main effect or an interaction with age (Table 2).

The number of myopic parents and time spent reading at age 8 to 9 years were predictive of refractive error at baseline and of future change in refractive error through to age 15 years (main effect terms both $P \leq 0.002$; age-interaction terms both $P \leq 0.004$; Fig. 3).

Discussion

In summary, these analyses do not provide support for the hypothesis that elevation of vitamin D levels is the mechanism by which spending time outdoors protects against myopia. Vitamin D is found in two forms in the blood, $25(OH)D_2$ and $25(OH)D_3$. Sunlight leads to the production of $25(OH)D_3$, but not $25(OH)D_2$, which is mainly derived from fortified foods and vitamin supplements.[28] Previous studies in the United States,[22] Korea,[23] and Australia[24] of serum 25(OH)D and myopia have analyzed only total 25(OH)D, so they were unable to address which has the stronger association with the outcome after mutual adjustment. In our analyses we saw the expected association of elevated vitamin D_3 (and total vitamin D) with spending extra time outdoors and therefore that vitamin D_3 and total vitamin D are to some extent biomarkers for time outdoors. However, there was scant evidence that vitamin D_3 or total vitamin D were themselves associated with myopia.

Relevance to Animal Studies Investigating the Roles of Vitamin D and Dopamine

Since there was no evidence to suggest a role for vitamin D in mediating the protective effects of time spent outdoors in the current study, our findings lend indirect support to the competing hypothesis that the brightness of natural light exposure outdoors alters retinal dopamine signaling, which in turn affects how children's eyes respond to myopiagenic stimuli.[15–17] There is a large body of work in animal models implicating dopamine in the regulation of eye growth, as well as, in particular, mediating the protective effects of time outdoors.[17,20,29] For instance, in chicks, daily intravitreal injection of the dopamine D2-receptor antagonist spiperone was shown to prevent the reduction in form-deprivation myopia normally produced by bright light exposure,[14] suggesting that dopamine signaling is a necessary requirement for bright lights to exert a beneficial effect. Furthermore, tree shrews fed a $25(OH)D_3$ supplement sufficient to dramatically raise their serum level developed comparable levels of form-deprivation and minus lens-induced myopia to control animals (Siegwart JT, et al. *IOVS* 2011;52:ARVO E-Abstract 6298). Finally, both form-deprivation myopia and lens-induced myopia

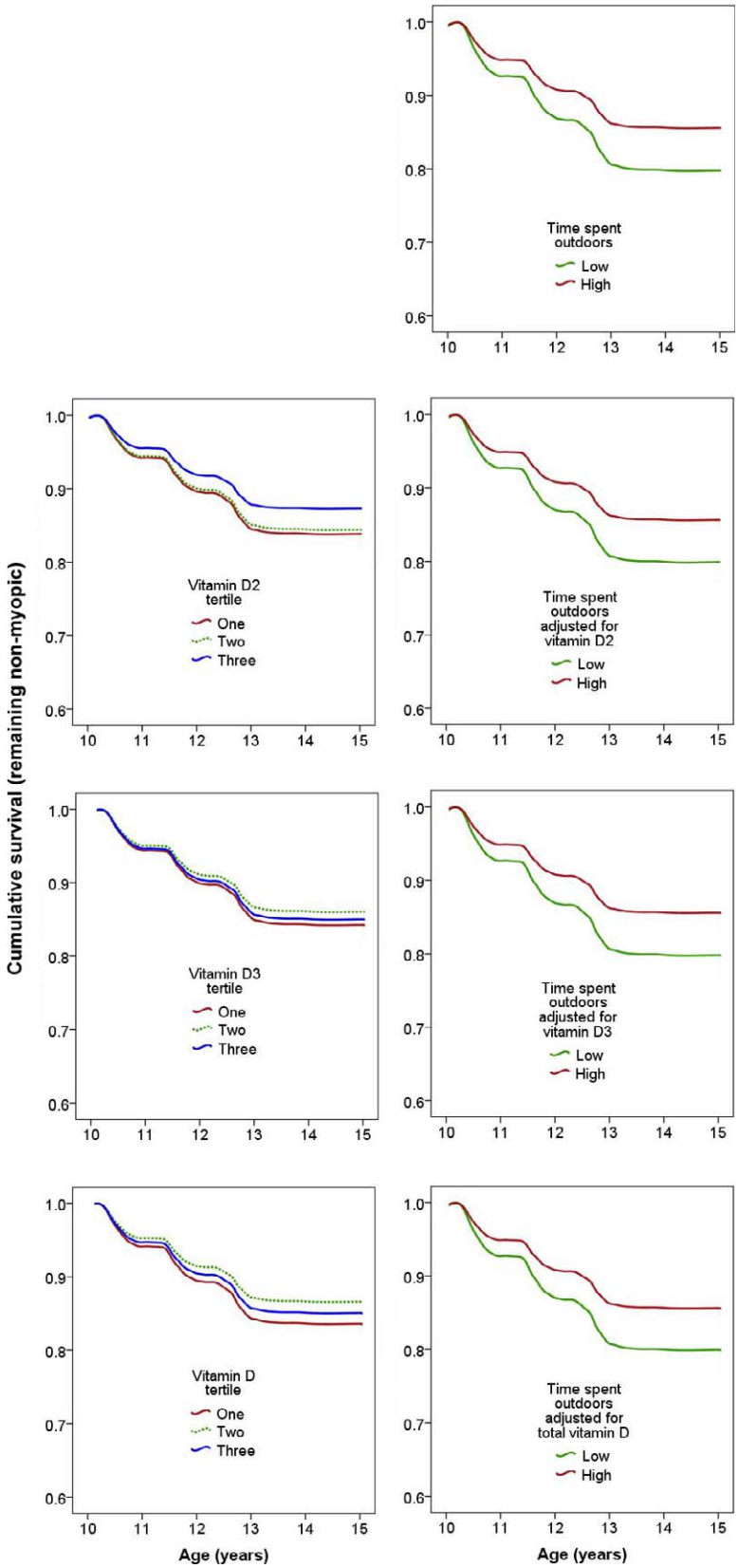

FIGURE 2. Survival curves for Cox regression models ($N = 3677$). Survival curves plotted as a function of time spent outdoors, serum 25(OH)D tertile, or time spent outdoors adjusted for serum 25(OH)D tertile. All models were adjusted for the number of myopic parents, time spent reading, and sex.

Table 2. Results of the Refraction Trajectory Analysis: Full Model

Serum 25(OH)D Variable Included in Model†	Time Outdoors			Time Outdoors × Age			25(OH)D			25(OH)D × Age		
	Beta	95% CI	*P*	Beta	95% CI	*P*	Beta	95% CI	*P*	Beta	95% CI	*P*
None (Initial model; $N = 2852$)	0.144	(0.017 to 0.271)	0.026	0.011	(−0.010 to 0.031)	0.302	NA	NA	NA	NA	NA	NA
Total 25(OH)D	0.141	(0.014 to 0.268)	0.029	0.010	(−0.010 to 0.031)	0.313	0.029	(−0.047 to 0.105)	0.455	0.003	(−0.010 to 0.015)	0.685
Total 25(OH)D (adjusted‡)	0.142	(0.015 to 0.269)	0.029	0.010	(−0.010 to 0.030)	0.322	0.030	(−0.047 to 0.108)	0.444	0.006	(−0.007 to 0.018)	0.351
Total 25(OH)D tertiles	0.141	(0.014 to 0.268)	0.030	0.010	(−0.010 to 0.030)	0.325	0.018	(−0.028 to 0.064)	0.451	0.004	(−0.004 to 0.011)	0.329
Total 25(OH)D (adjusted‡) tertiles	0.143	(0.016 to 0.270)	0.027	0.010	(−0.010 to 0.030)	0.323	0.003	(−0.042 to 0.048)	0.898	0.004	(−0.003 to 0.012)	0.241
$25(OH)D_3$	0.142	(0.015 to 0.269)	0.028	0.011	(−0.010 to 0.031)	0.306	0.020	(−0.051 to 0.091)	0.581	0.001	(−0.010 to 0.012)	0.883
$25(OH)D_3$ (adjusted‡)	0.143	(0.016 to 0.269)	0.028	0.010	(−0.010 to 0.030)	0.314	0.021	(−0.052 to 0.094)	0.567	0.004	(−0.008 to 0.015)	0.523
$25(OH)D_3$ tertiles	0.142	(0.015 to 0.269)	0.028	0.010	(−0.010 to 0.031)	0.308	0.012	(−0.033 to 0.057)	0.605	0.001	(−0.006 to 0.008)	0.750
$25(OH)D_3$ (adjusted‡) tertiles	0.144	(0.017 to 0.271)	0.027	0.010	(−0.010 to 0.030)	0.315	0.002	(−0.043 to 0.047)	0.930	0.003	(−0.004 to 0.010)	0.393
$25(OH)D_2$ tertiles	0.142	(0.015 to 0.269)	0.028	0.010	(−0.010 to 0.031)	0.309	0.027	(−0.018 to 0.072)	0.241	0.002	(−0.005 to 0.009)	0.557
$25(OH)D_2$ (adjusted‡) tertiles	0.142	(0.015 to 0.269)	0.028	0.010	(−0.010 to 0.031)	0.309	0.023	(−0.022 to 0.067)	0.323	0.003	(−0.005 to 0.010)	0.484
None (Initial model; $N = 1782$)*	0.059	(−0.106 to 0.224)	0.487	0.008	(−0.017 to 0.033)	0.532	NA	NA	NA	NA	NA	NA
$25(OH)D_2$*	0.054	(−0.111 to 0.219)	0.524	0.008	(−0.017 to 0.033)	0.539	0.044	(−0.010 to 0.098)	0.114	0.001	(−0.007 to 0.010)	0.765
$25(OH)D_2$ (adjusted‡)*	0.054	(−0.111 to 0.219)	0.519	0.008	(−0.017 to 0.033)	0.547	0.039	(−0.016 to 0.094)	0.163	0.003	(−0.005 to 0.011)	0.490

Each row of the table lists the results for one model, giving the beta coefficients (D) describing the association with refractive error. All models included time spent outdoors, time spent reading, number of myopic parents, sex, and age terms as predictor variables. Vitamin D level was not included in the initial models.

* Sample size reduced due to the exclusion of subjects having quantitative serum 25(OH)D2 levels below the detection threshold. These subjects were included in the 25(OH)D2 tertiles model as the "low" tertile group.

† The model coefficients for quantitative 25(OH)D level were multiplied by $\log_e(2)$ so that results represent the average change in refractive error per doubling of the 25(OH)D level.

‡ Adjusted for year and season of blood sample collection.

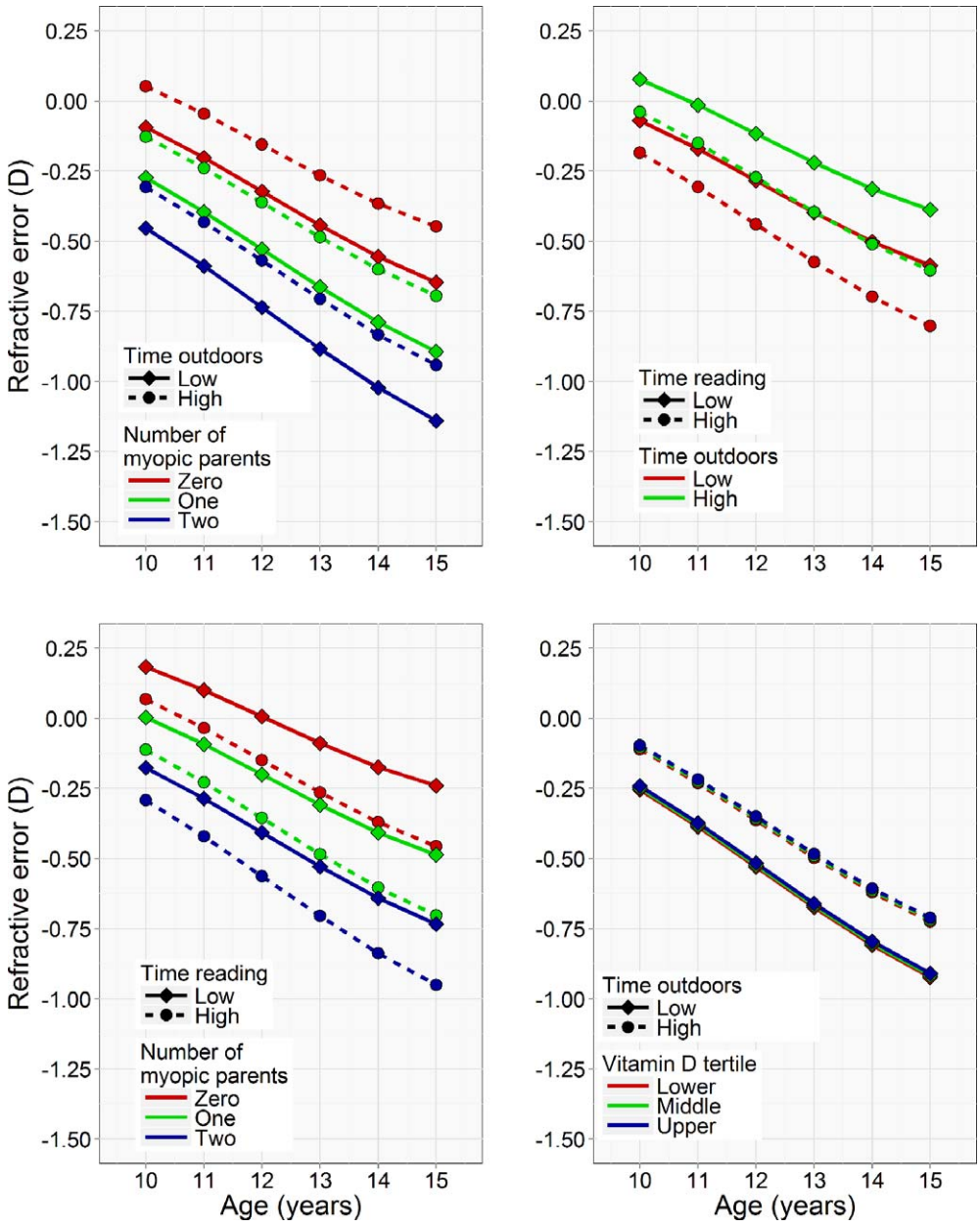

FIGURE 3. Effect of predictor variables on refractive error trajectories from age 10 to 15 years ($N = 2852$).

in tree shrews were found to be inhibited by exposure to bright lighting with minimal UV content (Siegwart JT Jr, et al. *IOVS* 2012;53:ARVO E-Abstract 3457), which again argues against bright lights exerting their effects by raising the level of serum vitamin D.

Time Outdoors and Myopia Progression

Despite the evidence that time outdoors is protective against incident myopia, it is currently uncertain whether time outdoors slows progression in individuals who are already myopic.[9,17,29] In our trajectory models for the full cohort (Table 2, Supplementary Table S2) time outdoors was associated with refractive error at "baseline" (age 9.8-years old), but did not show an interaction with age. These results suggest that children who spend less time outdoors at age 8 to 9 years will have a more negative, or less positive, refractive error at approximately age 10, while beyond this age a further influence of time spent outdoors is not detectable. It is unclear whether this lack of a time outdoors × age interaction reflects a gradually waning ability of our time outdoors variable to correctly capture the future behavior of children, or whether it reflects a lack of influence of time outdoors on refractive progression.

A Potential Role for 25(OH)D_2 or Diet?

Interestingly, serum 25(OH)D_2 tertile was positively associated with incident myopia independently of time spent outdoors (and results for quantitative serum 25(OH)D_2 level showed a trend in the same direction; Table 1). Also, in the refraction trajectory models, there was a drop in the size of the protective effect associated with spending more time outdoors when the low-25(OH)D_2 tertile group was excluded from the analysis (from 0.14 to 0.06 D; Table 2, Supplementary Table S2). These results hint that some children who go on to develop myopia

have a prior difference in diet compared with those who do not, for which the research literature provides tentative support.[30,31] However, as dietary 25(OH)D is likely to be confounded with other aspects of diet and lifestyle, further work is required to explore this relationship.

Strengths and Limitations of the Study

Strengths of this study were its large sample size, reliable measurements of both $25(OH)D_2$ and $25(OH)D_3$, and that all observations were collected prospectively, greatly reducing recall bias. A key limitation is that these data are observational, and therefore cannot indicate causality: other study designs such as randomized trials would be needed to address causation. Additional limitations are that refractive error was assessed by autorefraction without cycloplegia; time outdoors was classified based on a single survey; the method used to gauge time outdoors was a questionnaire completed by the child's mother rather than a quantitative, objective technique; differing levels of measurement error for vitamin D and time spent outdoors could have led to residual confounding and bias in estimates of "independent" effects. We discuss each of these points in more detail below.

Our approach of testing for an attenuation of the negative association between time spent outdoors and myopia when controlling for 25(OH)D relied on two assumptions. The first was that there was no residual confounding (also known[32] as "collider bias") in the path linking time outdoors and myopia (Supplementary Fig. S4), as well as no confounders of the mediator-outcome relationship (i.e., vitamin D–refractive error). The second assumption was that errors in measuring the level of time spent outdoors or serum 25(OH)D were not correlated with the levels or errors of other variables, as this could potentially have biased the results.[33,34] An inability to validate these assumptions prevented us from making claims of causality regarding 25(OH)D and the relationship between time spent outdoors and myopia.

Lack of cycloplegia will have introduced additional measurement error when refractive error was being assessed at the ALSPAC research clinics, especially when the children were young, and it will have led to the underestimation of hyperopic refractive errors in some of the hyperopic subjects. Therefore, as regards our survival analysis modelling, we expect that lack of cycloplegia would have led to the misclassification of some nonmyopic children as myopic, which would have reduced our statistical power to detect a subtle attenuation of the protective effect of time outdoors and importantly might have biased the mediation analyses in either direction depending on how measurement error in myopia assessment relates to 25(OH)D levels and/or any error in them. Exploring a range of thresholds to define myopia demonstrated that the choice of threshold did not affect the outcome: in all cases, controlling for 25(OH)D did not attenuate the association between time spent outdoors and incident myopia (Supplementary Table S3). For the refraction trajectory models, lack of cycloplegia most likely led to an underestimation of the degree of hyperopia for some individuals in a group, producing a less positive (or more negative) average refractive error for the group. Not only would this have lessened our statistical power to detect an attenuation of the protective effect of time outdoors, but for groups of subjects classified on the basis of a myopia risk factor, it would have led to bias in our estimates of the between-group differences in refractive error. Regarding the limitations of using a single, questionnaire-based assessment of time spent outdoors, this approach would have been detrimental to both our survival and trajectory models, in that it would have reduced statistical power due to misclassification of subjects. In view of all the above limitations, it seems likely that the refraction trajectory models were more severely affected than the survival analysis models (despite the expectation that by longitudinally modelling quantitative data, refraction trajectory analysis should have greater intrinsic power than survival analysis). Thus, we caution against taking the refractive error trajectory paths generated by our models as being a faithful, absolute representation of the true paths.

In summary, and as expected from the known biosynthetic pathways for vitamin D, we confirmed that vitamin D_3 was a biomarker for time spent outdoors. However, there was no statistical evidence to suggest that the participants' serum vitamin D_3 levels were associated with later myopia, once time outdoors had been taken into account. Research into other mechanisms is needed to help develop future antimyopia interventions based on the protective effects of increased time outdoors in childhood.

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