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Article type : Original Article

Time spent outdoors through childhood and adolescence – assessed by 25-hydroxyvitamin D concentration – and risk of myopia at 20 years

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Abstract

Purpose: To investigate the relationship between time spent outdoors, at particular ages in childhood and adolescence, and myopia status in young adulthood using serum 25-hydroxyvitamin D [25(OH)D] concentration as a biomarker of time spent outdoors.

Methods: Participants of the Raine Study Generation 2 cohort had 25(OH)D concentrations measured at the 6- 14-, 17- and 20-year follow-ups. Participants underwent cycloplegic autorefraction at age 20 years and myopia was defined as a mean spherical equivalent -0.50 dioptres or more myopic. Logistic regression was used to analyse the association between risk of myopia at age 20 years and age-specific 25(OH)D concentrations. Linear mixed-effects models were used to analyse trajectory of 25(OH)D concentrations from 6 to 20 years.

Results: After adjusting for sex, race, parental myopia, body mass index and studying status, myopia at 20 years was associated with lower 25(OH)D concentration at 20 years (per 10 nmol/L decrease, odds ratio (aOR)=1.10, 95%CI: 1.02, 1.18) and a low vitamin D

status [25(OH)D<50 nmol/L] at 17 years (aOR=1.71, 95%CI: 1.06, 2.76) and 20 years (aOR=1.71, 95%CI: 1.14, 2.56), compared to those without low vitamin D status. There were no associations between 25(OH)D at younger ages and myopia. Individuals who were myopic at 20 years had a 25(OH)D concentration trajectory that declined, relative to non-myopic peers, with increasing age. Differences in 25(OH)D trajectory between individuals with and without myopia were greater among non-Caucasians compared to Caucasians.

Conclusions: Myopia in young adulthood was most strongly associated with recent 25(OH)D concentrations, a marker of time spent outdoors.

Key words: Vitamin D, myopia, the Raine Study, time outdoors

INTRODUCTION

Myopia affects over one-fifth of young adult Australians (McKnight et al. 2014). Myopia is linked to higher risk of visual impairment from conditions such as retinal detachment and myopic maculopathy (Vongphanit et al. 2002; Mitry et al. 2010; Marcus et al. 2011) and risk escalates with increasing severity of myopia (Tideman et al. 2016). The prevalence of myopia is rising globally (Holden et al. 2016) and this will incur considerable economic costs into the future (Zheng et al. 2013; Naidoo et al. 2019).

Over the last decade, spending little time outdoors has emerged as a risk factor for myopia and a potential target for intervention (Jones et al. 2007; Rose et al. 2008; Guggenheim et al. 2012; French et al. 2013; He et al. 2015; Wu et al. 2018). Indeed, to combat the rising prevalence of myopia, countries such as Singapore and Taiwan have implemented public health interventions aimed at increasing children's time spent outdoors. These interventions predominantly target children since an earlier age of onset of myopia is associated with more myopic refractive error in later life and consequently greater risk of myopia-associated visual impairment (Pärssinen et al. 2014; Chua et al. 2016). It is not clear whether time spent outdoors is more important at particular ages, and whether reductions in myopia risk from spending more time outdoors in childhood are sustained into adulthood, when myopia typically ceases to develop. Previous studies investigating the effect of time spent outdoors at particular ages on risk of future myopia did not extend their follow-ups beyond adolescence (i.e. 15-17 years) and may have been limited by use of questionnaires to assess time spent outdoors, which can be relatively coarse (Guggenheim et al. 2012; French et al. 2013; Shah et al. 2017). Time spent outdoors as measured by questionnaire is subject to recall error, although it is moderately correlated with objective measures (questionnaire vs dosimeter, r=0.46, p=0.003) (Cargill et al. 2013; Køster et al. 2017). To address this, it is possible to investigate the association between myopia in adulthood, when myopia has

stabilized, and an objective biomarker of time spent outdoors measured during childhood and adolescence.

Serum 25-hydroxyvitamin D [25(OH)D] concentration is the usual marker of vitamin D status and, in Australians not taking vitamin D supplements, is predominantly derived from endogenous synthesis following exposure of the skin to ultraviolet radiation (Nowson & Mergerison 2002). Serum 25(OH)D concentration is an objective biomarker of recent (weeks/months) time spent outdoors in children and adults (Jones et al. 1999; van der Mei et al. 2006; Bener et al. 2009; Hanwell et al. 2010), being most strongly associated with cumulative sun exposure over the preceding 6 weeks (Nair-Shalliker et al. 2013), but also associated with reported sun exposure over the preceding 3 years ($r=0.31$, $p<0.01$) (van der Mei et al. 2006). Lower 25(OH)D concentration is associated with higher risk of myopia (Mutti & Marks 2011; Choi et al. 2014; Yazar et al. 2014; Tideman et al. 2016; Tang et al. 2019), but it seems unlikely that this relationship is causal (Guggenheim et al. 2014; Cuellar-Partida et al. 2017); rather that 25(OH)D concentration acts as a biomarker of time spent outdoors.

Using measurements of 25(OH)D concentration as a biomarker of time outdoors, we aimed to investigate how 25(OH)D concentrationat ages 6, 14, 17 and 20 years, is related to myopia risk at 20 years. Additionally, we performed a trajectory analysis to assess how changes in 25(OH)D concentration, and consequently time spent outdoors, between ages 6 and 20 years differed between those with and without myopia at 20 years.

MATERIALS & METHODS

Participants

The Raine Study is a multi-generation, longitudinal cohort study. We analysed data from Generation 2 (Gen2) of the Raine Study (hereafter referred to as the “participants”) a cohort of individuals who have been followed longitudinally since birth. Between 1989 and 1992, pregnant mothers of Gen2 participants were recruited into the Raine Study when the participants were between 16 and 20 weeks of gestation (n=2968). There were 2868 (98.9%) live births (50.7% male). Since birth, participants of the Gen2 cohort have been invited to participate in regular follow-ups including at age 6, 14, 17 and 20 years (Yazar et al. 2013; Straker et al. 2017). Height and weight were measured at all follow-ups. The number of participants in each successive follow-up has gradually declined over time (Straker et al. 2017). There is no difference in infant birth characteristics between those who did and did not participate in the Gen2 20-year follow-up, with the exception that those who participated were more likely to be of Caucasian race (79.5% vs 85.5%, $p<0.001$) (Straker et

al. 2017). Participants provided written informed consent prior to participating in any follow-up of the Raine Study. Follow-ups in this analysis were approved by the University of Western Australia Human Research Ethics Committee and adhered to the Tenets of the Declaration of Helsinki.

Questionnaire data

At the 20-year follow-up, participants completed questionnaires on current studying status (yes/no), past ocular history, and parental myopia status (none, one or two). Parents of the participants self-reported their race; participants were classified as Caucasian if both parents reported being of Caucasian race. Participants also reported the average proportion of their non-work day spent outdoors in summer (none, less than ¼ of the day, ½ of the day, greater than ¾ of the day, cannot judge), and average proportion of leisure time spent outdoors in winter (mostly indoors, ½ and ½, mostly outdoors, don't know). These questionnaire data were previously validated in a study showing that greater self-reported time spent outdoors in summer and winter is associated with larger conjunctival ultraviolet autofluorescence area, an objective measure of time spent outdoors (McKnight et al. 2015). At a later follow-up (23-year follow-up), participants reported the age when they first started wearing glasses or contact lenses.

Assessment of 25(OH)D concentration

Fasting blood samples were collected from participants at the 6-, 14-, 17- and 20-year follow-ups. Sera were stored at -80˚C until analysis. Total serum 25(OH)D concentrations of samples from the 6- and 14-year follow-ups were measured by enzyme immunoassay (EIA; Immunodiagnostic Systems Ltd., USA). At the 17- and 20-year follow-ups, $25(OH)D_2$ and $25(OH)D_3$ concentrations were measured using isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to a published methodology (Maunsell et al. 2005; Zhu et al. 2017). For consistency with EIA results, $25(OH)D_2$ and $25(OH)D_3$ concentrations were summed to calculate total 25(OH)D concentration. Interbatch coefficients of variation for the low, medium and high standards ranged from 4.6% to 8.7% for the EIA and 5.0% to 8.8% for the LC-MS/MS and are detailed in the supporting information (Yazar et al. 2014; Zhu et al. 2017).

Serum 25(OH)D concentration was re-measured using LC-MS/MS in 50 of the 6-year samples and 12 of the 14-year samples. There was a high correlation between LC-MS/MS and EIA in the 12 re-measured samples from the 14-year follow-up (r^2=0.933) (Hollams et al. 2011). Compared to LC-MS/MS, EIA was found to overestimate 25(OH)D concentration in the 50 re-measured samples from the 6-year follow-up (Hollams et al. 2011; Anderson et al.

2014). We therefore used a previously developed weighted Deming regression equation to adjust for this overestimation as follows: $Adjusted\ 25(OH)D = 22.3 + 0.58\ \times EIA$ (Anderson et al. 2014; Zhu et al. 2017). Vitamin D status was defined as low (25(OH)D concentration < 50nmol/L), medium (≥50nmol/L and <75nmol/L) and high (≥75nmol/L) (Zhu et al. 2017).

Eye examination

At the 20-year follow-up (2010-2012), participants underwent a comprehensive eye examination. Refractive error was measured by autorefraction (Nidek ARK-510A, Nidek Co. Ltd, Japan) after instillation of 1 drop of tropicamide 1% and phenylephrine 10% in each eye (Yazar et al. 2013). Myopia was defined as a mean spherical equivalent of both eyes ≤-0.50 dioptres (D) (Yazar et al. 2014), and was further classified into low (≤-0.50D and >-3.00D), moderate (≤-3.00D and >-6.00D) and high (≤-6.00D) myopia.

Statistical analysis

Participants were excluded from the analysis if they: did not have any 25(OH)D measurements; did not have post-cycloplegic autorefraction data; or, had a history of an ocular or genetic condition or previous ocular surgery known to affect refractive error.

We assessed the usefulness of 25(OH)D concentration as a marker of time spent outdoors in this study by examining the relationship between raw 25(OH)D concentration and self-reported time spent outdoors at 20 years. For participants who attended the 20-year follow-up between December and March (Australian summer is December to February), we analysed the association between 25(OH)D concentration and self-reported time spent outdoors in summer (summer analysis). For participants who attended the 20-year follow-up between June and September (Australian winter is June to August), we analysed the association between 25(OH)D concentration and self-reported time spent outdoors in winter (winter analysis). Linear regression models were constructed for both the summer and winter analyses adjusting for age, sex, race and body mass index (BMI).

As blood samples were collected throughout the year, we deseasonalised 25(OH)D concentration measurements prior to all analyses by fitting a sinusoidal model as previously described (van der Mei et al. 2006). We identified the following potential confounders between myopia and 25(OH)D concentration from prior studies: sex (Hollams et al. 2011), number of myopic parents (McKnight et al. 2014; Yazar et al. 2014; Shah et al. 2017), BMI (Mai et al. 2012; Black et al. 2014), studying status at 20-year follow-up (yes/no) (McKnight et al. 2014; Yazar et al. 2014), and race (Caucasian/non-Caucasian) (Yazar et al. 2014). Potential confounders were included as covariates in all multivariable models (see below)

investigating the association between myopia and 25(OH)D concentrations or vitamin D status.

Age-specific 25(OH)D concentration and myopia

We used regression modelling to analyse the association between myopia status at 20 years (logistic regression) or spherical equivalent at 20 years (linear regression) and 25(OH)D concentration as a continuous variable at ages 6, 14, 17 and 20 years separately, before and after adjusting for confounders. Based on a previously identified threshold (Yazar et al. 2014), we also tested vitamin D status as a categorical variable as low (25(OH)D <50nmol/L) vs medium and high (≥50nmol/L) at ages 14, 17 and 20 years (age 6 years not included because only 5 participants had a low vitamin D status).

To assess whether incomplete 25(OH)D data at ages 6, 14 or 17 years was introducing any bias to this analysis, we conducted a sensitivity analysis using logistic regression to analyse the association between myopia or spherical equivalent and 25(OH)D concentration or vitamin D status for those with complete 25(OH)D data for all follow-ups (n=390, 31.0%) or with complete data at both the 6- and 20-year or 14- and 20-year follow-ups.

Trajectory analyses

We investigated whether those who were myopic at 20 years had different 25(OH)D concentration trajectories compared to those who were not myopic by constructing linear mixed-effects models (LMM) using the 'lme4' package, similar to previous studies (Jones-Jordan et al. 2011; Shah et al. 2017). LMMs are robust to missing data and can account for the correlation between consecutive 25(OH)D measurements within an individual. The outcome variable in LMM was 25(OH)D concentration from the 6- to the 20-year follow-ups. Because the distribution of 25(OH)D concentrations was positively skewed, we square root-transformed the deseasonalised 25(OH)D concentration as this most closely approximated a normal distribution (Figures S1 and Figure S2). Random intercepts for each subject were included in LMMs to account for within-subject correlation.

We then fitted two models, first stratifying 25(OH)D trajectories by myopia status at 20 years (yes/no) and second stratifying 25(OH)D trajectory by severity of myopia at 20 years (none, low, moderate, high). Both models were adjusted for all potential confounders (sex, race, parental myopia, BMI, studying status). Interactions between myopia and age, sex and Caucasian/non-Caucasian race were tested using Wald Chi Square tests. A quadratic term was used to test for non-linear 25(OH)D concentration trajectories.

Age of onset

To investigate 25(OH)D concentration and age of onset of myopia, we used data on age when first started wearing glasses or contact lenses to code participants as: “not myopic” if they were not myopic at the age the 25(OH)D sample was collected and remained not myopic at the next follow-up, “became myopic” if they were not myopic at the age the 25(OH)D sample was collected but became myopic prior to the next follow-up and “myopic” if they were myopic at the time of 25(OH)D sample collection. The same LMM was then fitted as above but 25(OH)D concentrations were stratified by age of onset of myopia status rather than myopia status or myopia severity.

The significance level was set at 5%. All analyses were conducted using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

At the 20-year follow-up, 1344 participants attended an eye examination (46.9% of original cohort). Of these, 27 (2.0%) met ocular exclusion criteria or had missing autorefraction data, and 57 (4.2%) did not have at least one 25(OH)D measurement, leaving 1260 (93.8%) for this analysis. Table 1 shows the participant characteristics at each follow-up. Participants were predominantly Caucasian and there was a slight preponderance of males. In this study, 276 (21.9%) participants were myopic at the 20-year follow-up and of these, 203 (16.1%), 57 (4.5%) and 16 (1.3%) participants had low, moderate and high myopia, respectively. Participants were more likely to be myopic at 20 years if they were non-Caucasian (32.1% vs 20.2%, $p<0.001$), had more parents who were myopic (0 vs 1 vs 2; 18.2% vs 34.1% vs 40.8%, respectively, $p<0.001$), or if they were currently studying (27.4% vs 15.9%, $p<0.001$).

Greater self-reported time spent outdoors was associated with higher raw 25(OH)D concentrations in both summer and winter. In those who attended the 20-year follow-up between the months of December and March, reporting a greater proportion of the day spent outdoors in summer was associated with higher raw 25(OH)D concentration at the 20-year follow-up (per one category increase [none, <¼ of the day, ½ of the day, > ¾ of the day]., $\beta=9.2$ nmol/L, 95% CI: 4.3, 14.1, $p<0.001$) and explained an additional 5.5% of the variation in 25(OH)D concentration after adjusting for covariates. In those who attended the 20-year follow-up between June and September, reporting a higher proportion of leisure time spent outdoors in winter was associated with higher raw 25(OH)D concentration at the 20-year follow-up (per one category increase [mostly indoors, ½ and ½, mostly outdoors], $\beta=7.1$ nmol/L, 95% CI: 3.8, 10.4, $p<0.001$) and explained an additional 4.1% of the variation in 25(OH)D concentration after adjusting for covariates.

Insert Table 1 here

Age-specific 25(OH)D concentration, vitamin D status and myopia risk at 20 years

Table 2 shows the univariate and multivariable associations between 25(OH)D concentration at ages 6, 14, 17 and 20 years and myopia or spherical equivalent at the 20-year follow-up. The association between 25(OH)D concentration and myopia or spherical equivalent was not significantly different between males and females at any follow-up. Lower 25(OH)D concentration at 20 years and low vitamin D status at 17 and 20 years were significantly associated with increased risk of myopia and lower 25(OH)D concentration/status at 20 years was associated with a more negative (i.e. more myopic) spherical equivalent in the multivariable adjusted analysis. The sensitivity analysis of those with complete 25(OH)D data at all follow-ups and with complete data at the 6- and 20-year or the 14- and 20-year follow-ups are shown in Tables S1a, S1b and S2. Results were similar for analyses of myopia. Analyses of spherical equivalent (Table S2) did differ slightly in that a higher 25(OH)D concentration at the 14-year follow-up was associated with a more positive spherical equivalent after adjusting for confounders (Beta=0.07, 95% CI: 0.01, 0.13).

Insert Table 2 here

Trajectory analysis

The estimated 25(OH)D concentration trajectories for those with or without myopia at 20 years (model 1) or with none, low, moderate or high myopia at 20 years (model 2; myopia severity treated as an ordinal variable) are shown in Figure 1 and Figure 2 (model estimates provided in Table S3 and Table S4). In both models, there was a significant interaction between age and sex such that, compared to females, males had a 25(OH)D trajectory that was initially higher at 6 years but declined to become lower at 20 years.

In model 1, there was a significant interaction between myopia status at 20 years and both race and age, indicating that the shape of the 25(OH)D trajectory was significantly different between those with and without myopia and Caucasians vs non-Caucasians. The difference in 25(OH)D concentration trajectory between participants with and without myopia was smaller in Caucasians than in non-Caucasians. Relative to those who remained non-myopic, those who were myopic at age 20 years had a decline in 25(OH)D concentration as they aged.

The interaction terms in the LMMs indicate that the slopes of the trajectories of 25(OH)D concentration are significantly different between myopic and non-myopic individuals. This does not necessarily indicate that the mean age-specific 25(OH)D concentrations are significantly different and Figure 1 shows that the 95% confidence intervals for the mean

25(OH)D concentration largely overlap at all ages, with the exception of age 15 years and onwards in the non-Caucasian group, suggesting that the age-specific 25(OH)D concentrations are predominantly not significantly different.

Insert Figure 1 here

In model 2, there was a significant interaction between age and myopia severity only, such that, relative to those without myopia, those with more severe myopia had declining 25(OH)D concentrations with increasing age. The myopia groups had similar 25(OH)D concentrations at age 6 years, but differences were pronounced by age 20 years.

Insert Figure 2 here

Age of onset

Age of onset data was available for 225/276 (81.4%) individuals with myopia. There was no significant difference in reported age of onset between Caucasians and non-Caucasians (mean: 14.1 vs 15.4 years, p=0.09). Using LMM, those in the "became myopic" and "myopic" groups had on average a significantly lower 25(OH)D concentration by approximately 3.8 nmol/L (coefficients: became myopic=-0.24, 95% CI: -0.45, -0.03; myopic=-0.24, 95% CI: -0.41, -0.07), compared to the not myopic group across all follow-ups.

DISCUSSION

In summary, we found that low 25(OH)D concentration at 20 years of age and a low vitamin D status at 17 and 20 years of age were associated with increased risk of being myopic by age 20 years. Low 25(OH)D concentration was also associated with a more myopic spherical equivalent at the 20-year follow-up. These findings agree with a previous cross-sectional analysis of this same cohort in which a $25(OH)D_3$ concentration <50nmol/L was associated with higher odds of myopia compared with a $25(OH)D_3$ concentration ≥50nmol/L (Yazar et al. 2014). In the trajectory analysis, those who were myopic at 20 years, or who had more severe myopia, had 25(OH)D concentration trajectories that declined relative to those without myopia as they became older. Consistent with this, the difference in total 25(OH)D concentration between those with and without myopia was greatest at 20 years and less at younger ages. Finally, those who developed myopia between the 6- and 20-year follow-ups had significantly lower 25(OH)D concentration prior to the onset of myopia.

Serum 25(OH)D concentration appeared to be a reasonable marker of time spent outdoors in young adulthood in our study. Reported time outdoors accounted for around 5% of the variation in 25(OH)D concentration, similar to a smaller Australian study which found that 8%

of the variance in 25(OH)D concentration was accounted for by reported solar ultraviolet radiation exposure over the preceding 16 weeks. We could not internally validate the usefulness of 25(OH)D concentration as a marker of time outdoors at ages 6, 14 and 17 years, but other studies have shown that time spent outdoors and 25(OH)D concentration are associated at these ages (Jones et al. 1999; Bener et al. 2009).

Previous longitudinal studies and randomised controlled trials have demonstrated that spending more time outdoors in childhood protects against the onset of myopia in the subsequent 3- to 6-year period (Jones-Jordan et al. 2011; Guggenheim et al. 2012; French et al. 2013; Wu et al. 2013; He et al. 2015). The Avon Longitudinal Study of Parents and Children (ALSPAC), showed that greater primary caregiver-reported time spent outdoors at ages 3, 4, 4.5, 5.5, 6.5 and 8.5 years were all associated with reduced likelihood of becoming myopic between ages 10 and 15 years (Shah et al. 2017), although this study was limited to non-cycloplegic autorefraction data, which overestimate myopia (Fotedar et al. 2007; Sanfilippo et al. 2014). An Australian study found that the 5- to 6-year risk of incident myopia was lower in children who spent high compared to low amounts of time outdoors as measured by parent questionnaires at both ages 6 and 12 years, but the effect was slightly greater for the younger cohort (French et al. 2013).

In our study, we did not detect an association between myopia status or spherical equivalent at 20 years and 25(OH)D concentration at 6 or 14 years, despite previous studies showing an association between time outdoors and risk of myopia at these ages. We may not have detected such an association for a number of reasons. First, we may have lacked power due to the lower number of participants with 25(OH)D measurements at 6 years (n=618) or with a low vitamin D status (n=5 and n=39 at 6 and 14 years, respectively). The smaller difference in mean 25(OH)D concentration at younger ages, as indicated in the trajectory analysis, would also reduce our power to detect an effect at these ages. Second, EIA was used to assess 25(OH)D concentration at the 6- and 14-year follow-ups; lower accuracy and/or precision of the EIA could have reduced the ability to detect an association (Lai et al. 2012). Third, time spent outdoors and 25(OH)D concentration at 6 and 14 years may be associated with myopia incidence over the short- or medium-term but less strongly associated with myopia at 20 years. Fourth, it is possible that 25(OH)D concentration is a poorer marker of time spent outdoors at these ages, although associations between time outdoors and 25(OH)D concentration have been reported in pre-pubertal children (Jones et al. 1999).

The trajectory analysis showed that the trajectories of 25(OH)D concentration were different between those with and without myopia at 20 years; that is, a significant difference in the

shape of the trajectories between those with and without myopia. This this does not necessarily mean that there were significant differences in the age-specific estimates of 25(OH)D concentration. Indeed, the substantial overlap of the 95% confidence intervals in Figure 1 suggests the age-specific 25(OH)D distributions are not significantly different, particularly at younger ages.

In Caucasian individuals (85% of cohort), the 25(OH)D concentration trajectories were similar between myopic and non-myopic individuals in early childhood but then diverged with the differences becoming more apparent at older ages. Thus, we were able to find differences in 25(OH)D concentration in myopic and non-myopic individuals only for older ages. On the other hand, non-Caucasian children who were myopic at 20 years had lower 25(OH)D concentration from early childhood compared to non-myopic peers. It is possible that that differences in the amount of time spent outdoors between myopic and non-myopic individuals start to arise in childhood, but we were unable to detect a significant difference in our study. Our trajectory results agree with those from the ALSPAC, which found that primary caregivers of children who became myopic between ages 10 and 15 years reported declining amounts of time spent outdoors between ages 2 and 8 years, relative to those who remained non-myopic (Shah et al. 2017).

It is unclear why the difference in 25(OH)D trajectory between myopes and non-myopes was apparent at an earlier age in non-Caucasians compared to Caucasians. Non-Caucasians, both with and without myopia, had lower 25(OH)D concentrations overall. This may be related to having darker skin pigmentation, which reduces endogenous synthesis of vitamin D (Mithal et al. 2009), or due to non-Caucasian individuals spending less time outside and having less sun exposure (Rose et al. 2008; Guo et al. 2014). Non-Caucasians also had a higher prevalence of myopia in this study and children of East or South-East Asian ethnicity have been noted to have a higher incidence (French et al. 2013) and progression (Pärssinen et al. 2020) of myopia compared to Caucasian populations. A previous study found higher 25(OH)D concentration was associated with a larger reduction in risk of myopia among participants of East Asian ethnicity compared to Caucasians (Yazar et al. 2014). Thus, the larger difference in 25(OH)D trajectory between individuals with and without myopia among non-Caucasian participants could be reflective of the generally higher prevalence of myopia among these participants or could suggest that greater amounts of time spent outdoors are required to reduce risk of myopia among non-Caucasian individuals.

It was somewhat unexpected that serum 25(OH)D trajectories of those with no, low, moderate and high myopia were similar in early childhood (Figure 2), with model extrapolations suggesting that these trajectories only diverge around 8-10 years of age,

390 although we had no 25(OH)D concentration data at these ages. Those with more severe
391 myopia typically have onset at an earlier age (Pärssinen et al. 2014; Chua et al. 2016). We
392 therefore expected those with moderate or high myopia, who most likely developed myopia
393 early in life, to have an associated low 25(OH)D concentration, compared to non-myopic
394 peers, in early childhood. When we investigated whether 25(OH)D concentrations were
395 lower prior to, or after, the onset of myopia, compared to those who remained non-myopic,
396 we found that those who became myopic had a lower 25(OH)D concentration prior to onset
397 of myopia and this was comparable to those who were already myopic. This tentatively
398 indicates there is a decrease in time spent outdoors prior to myopia onset, as shown in other
399 studies (Jones-Jordan et al. 2011), and this decrease is sustained after the onset of myopia.

400 There are two clinically relevant findings from this study. First, spending more time outdoors
401 in early adulthood was associated with reduced risk of myopia. Thus, to prevent myopia in
402 adulthood, individuals will need to ensure regular time spent outdoors through late
403 adolescence and early adulthood and it seems likely that behavioural interventions will be
404 effective in this period. It is possible that we detected a significant association between
405 myopia and low vitamin D status, but not 25(OH)D concentration, at age 17 years because
406 of a threshold effect, in which those who spend very little time outside are at much higher
407 risk of myopia (Yazar et al. 2014). Second, our data suggested that those who were myopic
408 had trajectories of 25(OH)D concentration that were similar to their peers in early childhood,
409 but diverged from their peers with increasing age, showing lower concentration. Therefore,
410 behavioural interventions to prevent myopia by increasing time spent outdoors may be best
411 targeted to childhood to prevent this divergence.

412 A limitation of our study was the change in 25(OH)D assay method between follow-ups. This
413 could potentially induce false associations or mask true associations, particularly in trajectory
414 models. However, rank order should be approximately preserved between EIA and LC-
415 MS/MS measurements (Farrell et al. 2012) and a previous analysis of the same Raine Study
416 data found relatively consistent intraclass correlations between any two 25(OH)D
417 measurements at ages 6, 14, 17 or 20 years (0.40–0.67) (Zhu et al. 2017). Retention of
418 participants is a challenge in long-term cohort studies and nearly half the Raine Study cohort
419 did not participate in the 20-year follow-up. Our study may therefore have suffered from
420 attrition bias. Participant characteristics were similar between those who did and did not
421 participate in this study, but, as those who participated were more likely to be Caucasian and
422 race was associated with myopia in our study, it is possible that those who did not participate
423 were more or less likely to be myopic and we cannot rule out any impact of attrition bias. Our
424 study also lacked data on vitamin D supplementation, which increases serum 25(OH)D
425 concentration and could reduce its value as a marker of time spent outdoors (Black et al.

2016), as well as near work, a known risk factor for myopia (Huang et al. 2015). Myopia status was not measured at younger ages and we were therefore unable to thoroughly investigate the short-term effects of time outdoors at ages 6 and 14 years on myopia and relied on recall data when investigating age of onset of myopia.

The strengths of our study are the use of cycloplegic autorefraction to determine myopia status, the assessment of myopia at an age when further myopia is unlikely to develop, the objective assessment of time outdoors using 25(OH)D concentration, the relatively long period over which 25(OH)D samples were collected and the availability of data on race and parental myopia.

Our results show that less time spent outdoors – as assessed by an objective biomarker – at ages 17 and 20 years is associated with increased risk of myopia. Those who were myopic at 20 years had a 25(OH)D concentration trajectory that declined relative to those who remained non-myopic with increasing age; suggesting these individuals spent less time outdoors as they became older. To reduce risk of myopia in young adulthood, high amounts of time spent outdoors may need to be sustained through late adolescence and into young adulthood.

ACKNOWLEDGEMENTS:

We thank the Raine Study participants and their families and the Raine Study team for cohort coordination and data collection. We thank Denise Anderson for developing the weighted Deming regression equation for adjusting the 6-year 25(OH)D measures.

This work has been presented at the International Myopia Conference on the 12th September 2019 and at the Orthoptics Australia Annual Scientific Meeting on the 10th November 2019.

Funding Sources: The Raine Study is supported by the University of Western Australia, Curtin University, the Raine Medical Research Foundation, Telethon Kids Institute, Edith Cowan University, Women and Infants Research Foundation, Murdoch University and The University of Notre Dame Australia. The 5-, 14-, 17- and 20-year follow-ups of the Raine Study were all supported by the National Health and Medical Research Council (specifically, grants 963209, 211912, 003209, 353514, 403981, 1022134 and 1021105). Analysis of the 17-year 25(OH)D samples was funded by the Ada Bartholomew Medical Research Trust. The 20-year follow-up of the Raine Study also received funding from Ophthalmic Research Institute of Australia (ORIA), Lions Eye Institute, the Australian Foundation for the Prevention of Blindness, and Alcon Research Institute. GL is supported by an Australian Government

Research Training Stipend, RML is supported by a NHMRC Senior Research Fellowship, SY by a NHMRC CJ Martin Biomedical Fellowship, LJB by an MS Research Australia Postdoctoral Fellowship and a Curtin University Research Fellowship, and DAM by a NHMRC Practitioner Fellowship. The funders had no role in the study.

Conflict of Interest: There are no conflicts of interest to declare

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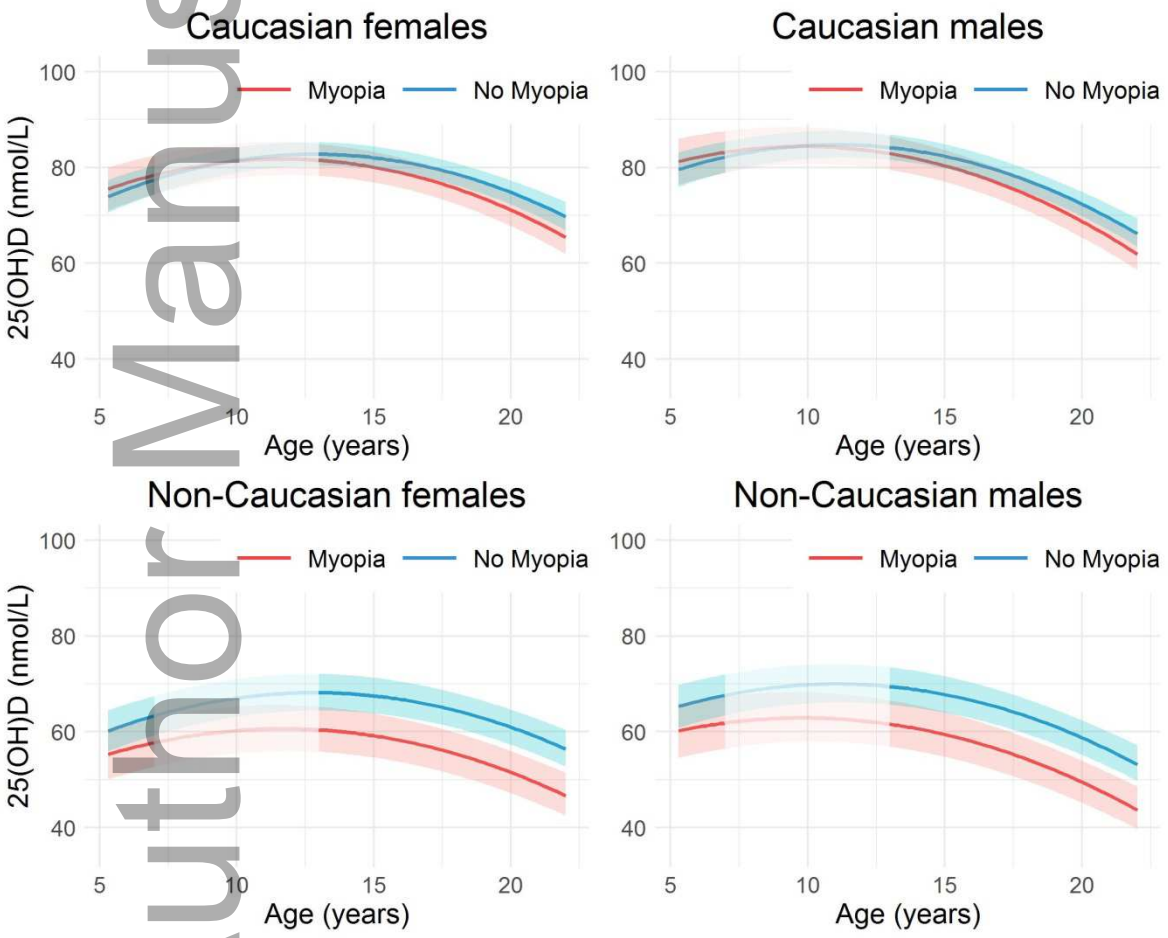


612
613 **Figure 1** Best-fit model estimates of change in 25(OH)D trajectory in those with and without
614 myopia at 20 years, stratified by sex and race. Shaded areas are 95% confidence intervals
615 and were calculated using the emmeans package. The faded areas of the plots correspond
616 to ages where no 25(OH)D concentration data were available in this study and plots are
617 extrapolated from model fit.

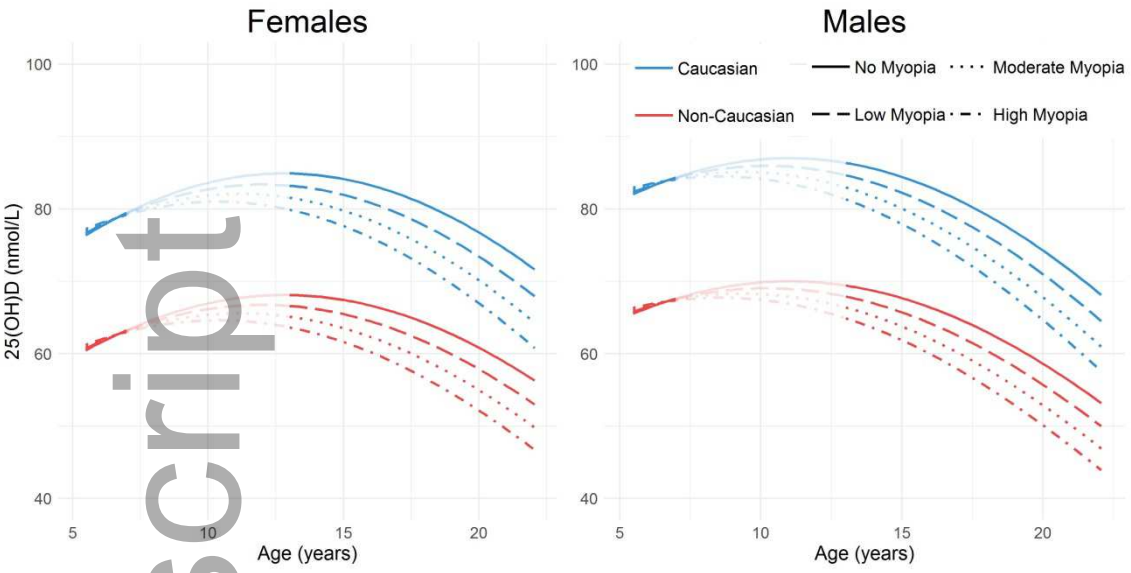


Figure 2 Best-fit model estimates of 25(OH)D trajectory in those who had no myopia, low myopia, moderate myopia and high myopia at 20 years of age, stratified by sex and race. The faded areas of the plots correspond to ages where no 25(OH)D concentration data were available in this study and plots are extrapolated from model fit.

Table 1: Participant characteristics at each of the 6-, 14-, 17- and 20-year Raine Study follow-ups

Follow-up	6-year (n=618)†	14-year(n=988)†	17-year (n=873)†	20-year (n=1260)‡
Age (years), mean (range)	5.91 (5.4, 6.8)	14.1 (13.0, 15.1)	17.0 (15.7, 18.9)	20.0 (19.1, 22.1)
Sex, n (%)				
Female	280 (45.31%)	477 (48.3%)	425 (48.7%)	604 (47.9%)
Male	338 (54.69%)	511 (51.7%)	448 (51.3%)	656 (52.1%)
Parent myopia (at 20 years)				
0 parents	364 (72.9%)	593 (71.9%)	523 (71.0%)	762 (72.6%)
1 parent	98 (19.6%)	169 (20.5%)	154 (20.9%)	211 (20.1%)
2 parent	37 (7.4%)	63 (7.6%)	60 (8.1%)	76 (7.2%)
Race, n (%)				
Caucasian	528 (85.4%)	855 (86.5%)	744 (85.2%)	1076 (85.4%)
Non-Caucasian	90 (14.6%)	133 (13.5%)	129 (14.8%)	184 (14.6%)
Body Mass Index (kg/m^2), median (IQR)	15.5 (14.7, 16.5)	20.4 (18.6, 22.9)	22.1 (20.0, 24.3)	23.4 (21.1, 26.2)
25(OH)D concentration (nmol/L)§, median (IQR) [range]	79.4 (70.9, 90.4) [40.6–210.0]	82.8 (68.5, 98.5) [22.9–260.0]	72.3 (57.7, 86.9) [5.1–179.1]	69.8 (56.6, 85.0) [5.7–209.3]
Vitamin D Status				
Low	5 (0.8%)	39 (3.9%)	122 (14.0%)	186 (16.5%)

Medium	231 (37.4%)	319 (32.3%)	360 (41.2%)	490 (43.5%)
High	382 (61.8%)	630 (63.8%)	391 (44.8%)	451 (40.0%)

†Includes only those with 25(OH)D measurements at this follow-up and who participated in the 20-year follow-up

‡1127 (89.4%) participants had a 25(OH)D measurement at this follow-up

§Deseasonalised by adjusting for month of collection

Table 2 Associations between myopia or spherical equivalent at 20 years and age-specific 25(OH)D measurements

	Odds Ratio for Myopia (95% Confidence Interval)		Beta for Spherical Equivalent (95% Confidence Interval)	
	Univariate	Multivariable†	Univariate	Multivariable†
Deseasonalised 25(OH)D Concentration (per 10nmol/L)				
Year 6 (n=499‡)	0.88 (0.78, 0.98)*	0.94 (0.83, 1.07)	0.05 (-0.01, 0.12)	0.01 (-0.07, 0.08)
Year 14 (n=823‡)	0.95 (0.89, 1.01)	0.99 (0.93, 1.06)	0.05 (0.02, 0.09)*	0.03 (-0.01, 0.07)
Year 17 (n=733‡)	0.91 (0.85, 0.97)*	0.94 (0.87, 1.02)	0.07 (0.03, 0.11)*	0.03 (-0.01, 0.08)
Year 20 (n=933‡)	0.90 (0.85, 0.96)*	0.91 (0.85, 0.98)*	0.08 (0.04, 0.12)*	0.07 (0.02, 0.11)*
Low vitamin D status (Reference: Medium/high vitamin D status)§				
Year 6 (n=5)	NA	NA	NA	NA
Year 14 (n=35¶)	0.97 (0.44, 2.14)	0.62 (0.25, 1.56)	-0.30 (-0.80, 0.19)	0.01 (-0.52,0.53)
Year 17 (n=110¶)	1.94 (1.28, 2.94)*	1.71 (1.06, 2.76)*	-0.49 (-0.77, -0.20)*	-0.28 (-0.58, 0.02)
Year 20 (n=156¶)	1.76 (1.24, 2.19)*	1.71 (1.14, 2.56)*	-0.40 (-0.65, -0.16)*	-0.29 (-0.57, -0.01)*

*p<0.05

†Adjusted for sex, number of myopic parents, body mass index, studying at 20-year follow-up (yes/no) and race (Caucasian/non-Caucasian)

‡Number of participants with complete data for all variables in multivariable analysis

§Low vitamin D Status defined as 25(OH)D concentration <50 nmol/L; Medium/high vitamin D status defined as 25(OH)D concentration ≥50 nmol/L

¶Number of participants in the low vitamin D category with complete data for all variables in multivariable analysis

SUPPORTING INFORMATION

25-hydroxyvitamin D Assay Coefficients of Variation

Table S1a Sensitivity Analysis comparing results of overall analysis of myopia (Table 2) to a subset of participants who have complete 25(OH)D data at all follow-ups

Table S1b Sensitivity Analysis comparing results of overall analysis of myopia (Table 2) to a subset of participants who have complete 25(OH)D data at both the 6- and 20-year follow-ups or complete data at both the 14- and 20-year follow-ups

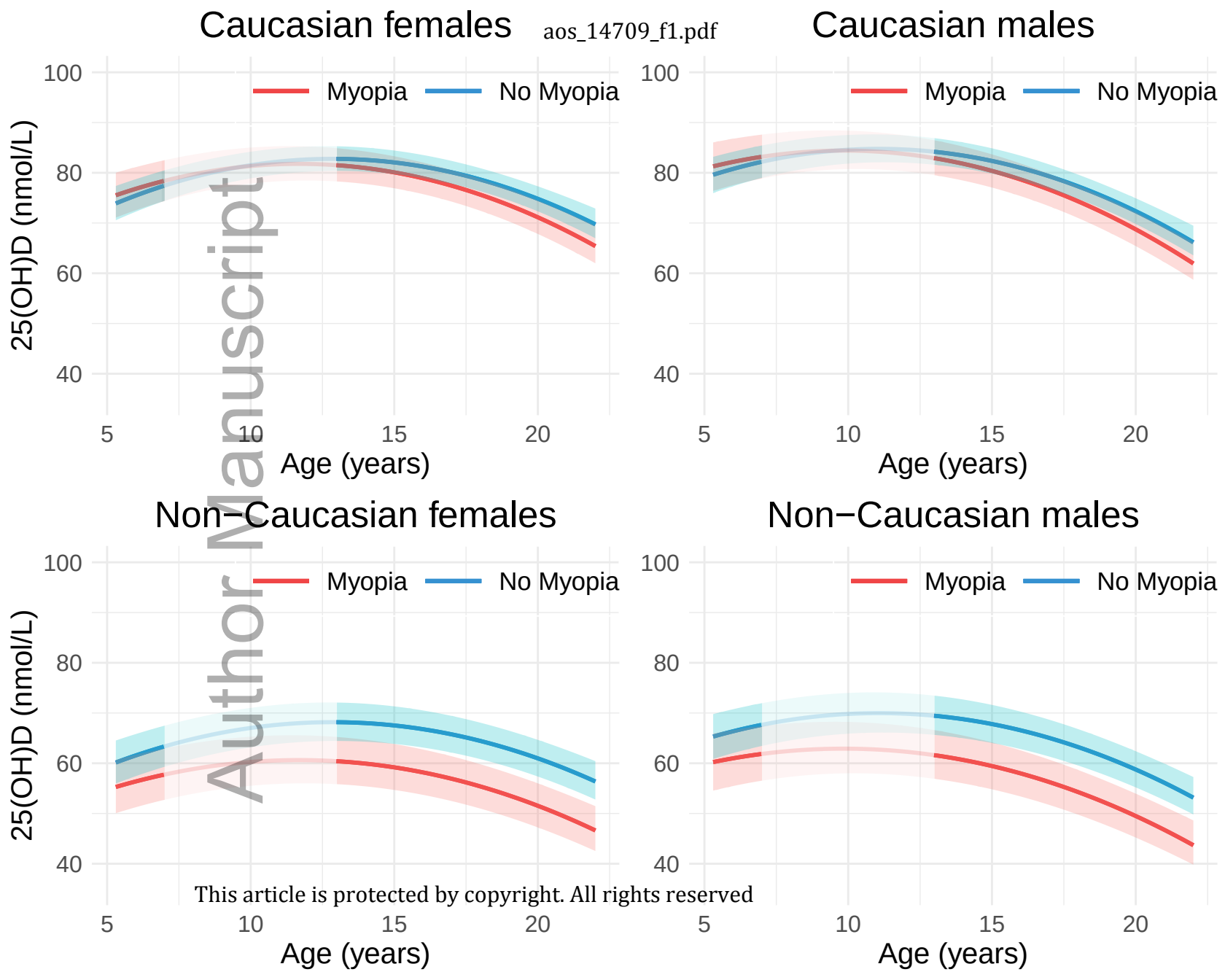
Table S2 Sensitivity Analysis comparing results of overall analysis of spherical equivalent (Table 2) to a subset of participants who have complete 25(OH)D data at all follow-ups

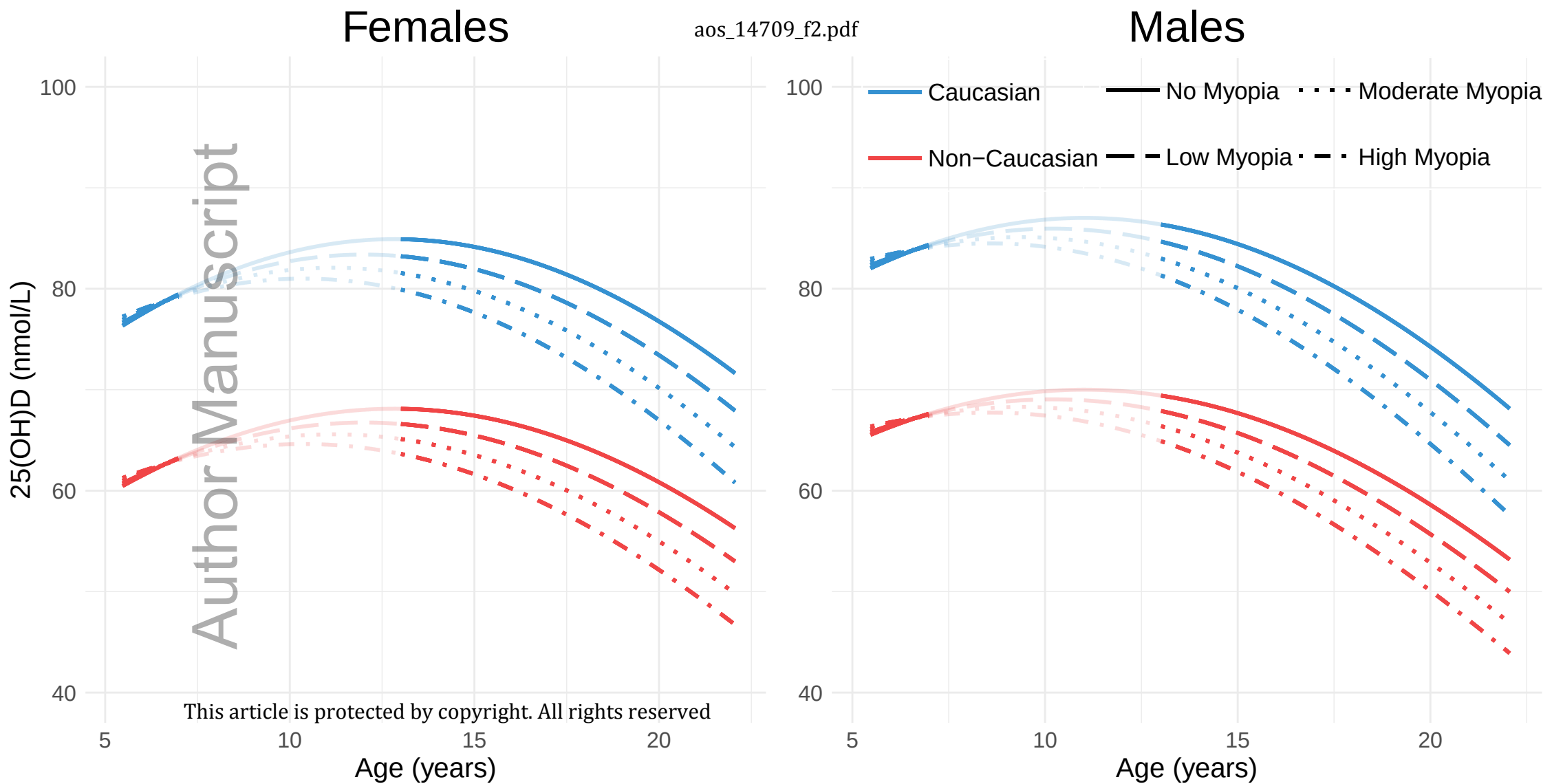
Table S3 Best-fit linear mixed-effects model estimates using square root of 25(OH)D at all follow-ups as outcome and myopia status at 20 years as predictor

Table S4 Best-fit linear mixed-effects model estimates using square root of 25(OH)D at all follow-ups as outcome and myopia severity at 20 years as predictor

Figure S1: Histograms of all available 25(OH)D data (i.e. at 6, 14, 17 and 20 years combined) after common transformations.

Figure S2: Quantile-quantile plots of all available 25(OH)D data (i.e. at 6, 14, 17 and 20 years combined) after common transformations





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Title:
Time spent outdoors through childhood and adolescence - assessed by 25-hydroxyvitamin D concentration - and risk of myopia at 20 years

Date:
2021-01-10

Citation:
Lingham, G., Mackey, D. A., Zhu, K., Lucas, R. M., Black, L. J., Oddy, W. H., Holt, P., Walsh, J. P., Sanfilippo, P. G., Chan She Ping-Delfos, W. & Yazar, S. (2021). Time spent outdoors through childhood and adolescence - assessed by 25-hydroxyvitamin D concentration - and risk of myopia at 20 years. ACTA OPHTHALMOLOGICA, 99 (6), pp.679-687. https://doi.org/10.1111/aos.14709.

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