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

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39 **Abstract**

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

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

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

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

59 **Conclusions:** Myopia in young adulthood was most strongly associated with recent
60 25(OH)D concentrations, a marker of time spent outdoors.

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

62 INTRODUCTION

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

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

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

89 Serum 25-hydroxyvitamin D [25(OH)D] concentration is the usual marker of vitamin D status
90 and, in Australians not taking vitamin D supplements, is predominantly derived from
91 endogenous synthesis following exposure of the skin to ultraviolet radiation (Nowson &
92 Mergerison 2002). Serum 25(OH)D concentration is an objective biomarker of recent
93 (weeks/months) time spent outdoors in children and adults (Jones et al. 1999; van der Mei et
94 al. 2006; Bener et al. 2009; Hanwell et al. 2010), being most strongly associated with
95 cumulative sun exposure over the preceding 6 weeks (Nair-Shalliker et al. 2013), but also
96 associated with reported sun exposure over the preceding 3 years ($r=0.31$, $p<0.01$) (van der
97 Mei et al. 2006). Lower 25(OH)D concentration is associated with higher risk of myopia
98 (Mutti & Marks 2011; Choi et al. 2014; Yazar et al. 2014; Tideman et al. 2016; Tang et al.
99 2019), but it seems unlikely that this relationship is causal (Guggenheim et al. 2014; Cuellar-
100 Partida et al. 2017); rather that 25(OH)D concentration acts as a biomarker of time spent
101 outdoors.

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

107 **MATERIALS & METHODS**

108 **Participants**

109 The Raine Study is a multi-generation, longitudinal cohort study. We analysed data from
110 Generation 2 (Gen2) of the Raine Study (hereafter referred to as the “participants”) a cohort
111 of individuals who have been followed longitudinally since birth. Between 1989 and 1992,
112 pregnant mothers of Gen2 participants were recruited into the Raine Study when the
113 participants were between 16 and 20 weeks of gestation ($n=2968$). There were 2868
114 (98.9%) live births (50.7% male). Since birth, participants of the Gen2 cohort have been
115 invited to participate in regular follow-ups including at age 6, 14, 17 and 20 years (Yazar et
116 al. 2013; Straker et al. 2017). Height and weight were measured at all follow-ups. The
117 number of participants in each successive follow-up has gradually declined over time
118 (Straker et al. 2017). There is no difference in infant birth characteristics between those who
119 did and did not participate in the Gen2 20-year follow-up, with the exception that those who
120 participated were more likely to be of Caucasian race (79.5% vs 85.5%, $p<0.001$) (Straker et

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

125 **Questionnaire data**

126 At the 20-year follow-up, participants completed questionnaires on current studying status
127 (yes/no), past ocular history, and parental myopia status (none, one or two). Parents of the
128 participants self-reported their race; participants were classified as Caucasian if both parents
129 reported being of Caucasian race. Participants also reported the average proportion of their
130 non-work day spent outdoors in summer (none, less than $\frac{1}{4}$ of the day, $\frac{1}{2}$ of the day, greater
131 than $\frac{3}{4}$ of the day, cannot judge), and average proportion of leisure time spent outdoors in
132 winter (mostly indoors, $\frac{1}{2}$ and $\frac{1}{2}$, mostly outdoors, don't know). These questionnaire data
133 were previously validated in a study showing that greater self-reported time spent outdoors
134 in summer and winter is associated with larger conjunctival ultraviolet autofluorescence area,
135 an objective measure of time spent outdoors (McKnight et al. 2015). At a later follow-up (23-
136 year follow-up), participants reported the age when they first started wearing glasses or
137 contact lenses.

138 **Assessment of 25(OH)D concentration**

139 Fasting blood samples were collected from participants at the 6-, 14-, 17- and 20-year
140 follow-ups. Sera were stored at -80°C until analysis. Total serum 25(OH)D concentrations of
141 samples from the 6- and 14-year follow-ups were measured by enzyme immunoassay (EIA;
142 Immunodiagnostic Systems Ltd., USA). At the 17- and 20-year follow-ups, 25(OH)D₂ and
143 25(OH)D₃ concentrations were measured using isotope-dilution liquid chromatography-
144 tandem mass spectrometry (LC-MS/MS) according to a published methodology (Maunsell et
145 al. 2005; Zhu et al. 2017). For consistency with EIA results, 25(OH)D₂ and 25(OH)D₃
146 concentrations were summed to calculate total 25(OH)D concentration. Interbatch
147 coefficients of variation for the low, medium and high standards ranged from 4.6% to 8.7%
148 for the EIA and 5.0% to 8.8% for the LC-MS/MS and are detailed in the supporting
149 information (Yazar et al. 2014; Zhu et al. 2017).

150 Serum 25(OH)D concentration was re-measured using LC-MS/MS in 50 of the 6-year
151 samples and 12 of the 14-year samples. There was a high correlation between LC-MS/MS
152 and EIA in the 12 re-measured samples from the 14-year follow-up ($r^2=0.933$) (Hollams et al.
153 2011). Compared to LC-MS/MS, EIA was found to overestimate 25(OH)D concentration in
154 the 50 re-measured samples from the 6-year follow-up (Hollams et al. 2011; Anderson et al.

155 2014). We therefore used a previously developed weighted Deming regression equation to
156 adjust for this overestimation as follows: $Adjusted\ 25(OH)D = 22.3 + 0.58 \times EIA$ (Anderson
157 et al. 2014; Zhu et al. 2017). Vitamin D status was defined as low (25(OH)D concentration <
158 50nmol/L), medium (≥ 50 nmol/L and <75nmol/L) and high (≥ 75 nmol/L) (Zhu et al. 2017).

159 **Eye examination**

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

166 **Statistical analysis**

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

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

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

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

190 *Age-specific 25(OH)D concentration and myopia*

191 We used regression modelling to analyse the association between myopia status at 20 years
192 (logistic regression) or spherical equivalent at 20 years (linear regression) and 25(OH)D
193 concentration as a continuous variable at ages 6, 14, 17 and 20 years separately, before
194 and after adjusting for confounders. Based on a previously identified threshold (Yazar et al.
195 2014), we also tested vitamin D status as a categorical variable as low (25(OH)D <50nmol/L)
196 vs medium and high (≥50nmol/L) at ages 14, 17 and 20 years (age 6 years not included
197 because only 5 participants had a low vitamin D status).

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

203 *Trajectory analyses*

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

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

220 *Age of onset*

221 To investigate 25(OH)D concentration and age of onset of myopia, we used data on age
222 when first started wearing glasses or contact lenses to code participants as: “not myopic” if
223 they were not myopic at the age the 25(OH)D sample was collected and remained not
224 myopic at the next follow-up, “became myopic” if they were not myopic at the age the
225 25(OH)D sample was collected but became myopic prior to the next follow-up and “myopic”
226 if they were myopic at the time of 25(OH)D sample collection. The same LMM was then
227 fitted as above but 25(OH)D concentrations were stratified by age of onset of myopia status
228 rather than myopia status or myopia severity.

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

231 **RESULTS**

232 At the 20-year follow-up, 1344 participants attended an eye examination (46.9% of original
233 cohort). Of these, 27 (2.0%) met ocular exclusion criteria or had missing autorefractometry data,
234 and 57 (4.2%) did not have at least one 25(OH)D measurement, leaving 1260 (93.8%) for
235 this analysis. Table 1 shows the participant characteristics at each follow-up. Participants
236 were predominantly Caucasian and there was a slight preponderance of males. In this study,
237 276 (21.9%) participants were myopic at the 20-year follow-up and of these, 203 (16.1%), 57
238 (4.5%) and 16 (1.3%) participants had low, moderate and high myopia, respectively.

239 Participants were more likely to be myopic at 20 years if they were non-Caucasian (32.1% vs
240 20.2%, $p < 0.001$), had more parents who were myopic (0 vs 1 vs 2; 18.2% vs 34.1% vs
241 40.8%, respectively, $p < 0.001$), or if they were currently studying (27.4% vs 15.9%, $p < 0.001$).

242 Greater self-reported time spent outdoors was associated with higher raw 25(OH)D
243 concentrations in both summer and winter. In those who attended the 20-year follow-up
244 between the months of December and March, reporting a greater proportion of the day spent
245 outdoors in summer was associated with higher raw 25(OH)D concentration at the 20-year
246 follow-up (per one category increase [none, $< \frac{1}{4}$ of the day, $\frac{1}{2}$ of the day, $> \frac{3}{4}$ of the day],
247 $\beta = 9.2$ nmol/L, 95% CI: 4.3, 14.1, $p < 0.001$) and explained an additional 5.5% of the variation
248 in 25(OH)D concentration after adjusting for covariates. In those who attended the 20-year
249 follow-up between June and September, reporting a higher proportion of leisure time spent
250 outdoors in winter was associated with higher raw 25(OH)D concentration at the 20-year
251 follow-up (per one category increase [mostly indoors, $\frac{1}{2}$ and $\frac{1}{2}$, mostly outdoors], $\beta = 7.1$
252 nmol/L, 95% CI: 3.8, 10.4, $p < 0.001$) and explained an additional 4.1% of the variation in
253 25(OH)D concentration after adjusting for covariates.

254 *Insert Table 1 here*

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

256 Table 2 shows the univariate and multivariable associations between 25(OH)D concentration
257 at ages 6, 14, 17 and 20 years and myopia or spherical equivalent at the 20-year follow-up.
258 The association between 25(OH)D concentration and myopia or spherical equivalent was
259 not significantly different between males and females at any follow-up. Lower 25(OH)D
260 concentration at 20 years and low vitamin D status at 17 and 20 years were significantly
261 associated with increased risk of myopia and lower 25(OH)D concentration/status at 20
262 years was associated with a more negative (i.e. more myopic) spherical equivalent in the
263 multivariable adjusted analysis. The sensitivity analysis of those with complete 25(OH)D
264 data at all follow-ups and with complete data at the 6- and 20-year or the 14- and 20-year
265 follow-ups are shown in Tables S1a, S1b and S2. Results were similar for analyses of
266 myopia. Analyses of spherical equivalent (Table S2) did differ slightly in that a higher
267 25(OH)D concentration at the 14-year follow-up was associated with a more positive
268 spherical equivalent after adjusting for confounders (Beta=0.07, 95% CI: 0.01, 0.13).

269 *Insert Table 2 here*

270 *Trajectory analysis*

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

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

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

288 25(OH)D concentration largely overlap at all ages, with the exception of age 15 years and
289 onwards in the non-Caucasian group, suggesting that the age-specific 25(OH)D
290 concentrations are predominantly not significantly different.

291 *Insert Figure 1 here*

292 In model 2, there was a significant interaction between age and myopia severity only, such
293 that, relative to those without myopia, those with more severe myopia had declining
294 25(OH)D concentrations with increasing age. The myopia groups had similar 25(OH)D
295 concentrations at age 6 years, but differences were pronounced by age 20 years.

296 *Insert Figure 2 here*

297 *Age of onset*

298 Age of onset data was available for 225/276 (81.4%) individuals with myopia. There was no
299 significant difference in reported age of onset between Caucasians and non-Caucasians
300 (mean: 14.1 vs 15.4 years, $p=0.09$). Using LMM, those in the “became myopic” and “myopic”
301 groups had on average a significantly lower 25(OH)D concentration by approximately 3.8
302 nmol/L (coefficients: became myopic=-0.24, 95% CI: -0.45, -0.03; myopic=-0.24, 95% CI: -
303 0.41, -0.07), compared to the not myopic group across all follow-ups.

304 **DISCUSSION**

305 In summary, we found that low 25(OH)D concentration at 20 years of age and a low vitamin
306 D status at 17 and 20 years of age were associated with increased risk of being myopic by
307 age 20 years. Low 25(OH)D concentration was also associated with a more myopic
308 spherical equivalent at the 20-year follow-up. These findings agree with a previous cross-
309 sectional analysis of this same cohort in which a 25(OH)D₃ concentration <50nmol/L was
310 associated with higher odds of myopia compared with a 25(OH)D₃ concentration ≥50nmol/L
311 (Yazar et al. 2014). In the trajectory analysis, those who were myopic at 20 years, or who
312 had more severe myopia, had 25(OH)D concentration trajectories that declined relative to
313 those without myopia as they became older. Consistent with this, the difference in total
314 25(OH)D concentration between those with and without myopia was greatest at 20 years
315 and less at younger ages. Finally, those who developed myopia between the 6- and 20-year
316 follow-ups had significantly lower 25(OH)D concentration prior to the onset of myopia.

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

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

325 Previous longitudinal studies and randomised controlled trials have demonstrated that
326 spending more time outdoors in childhood protects against the onset of myopia in the
327 subsequent 3- to 6-year period (Jones-Jordan et al. 2011; Guggenheim et al. 2012; French
328 et al. 2013; Wu et al. 2013; He et al. 2015). The Avon Longitudinal Study of Parents and
329 Children (ALSPAC), showed that greater primary caregiver-reported time spent outdoors at
330 ages 3, 4, 4.5, 5.5, 6.5 and 8.5 years were all associated with reduced likelihood of
331 becoming myopic between ages 10 and 15 years (Shah et al. 2017), although this study was
332 limited to non-cycloplegic autorefraction data, which overestimate myopia (Fotedar et al.
333 2007; Sanfilippo et al. 2014). An Australian study found that the 5- to 6-year risk of incident
334 myopia was lower in children who spent high compared to low amounts of time outdoors as
335 measured by parent questionnaires at both ages 6 and 12 years, but the effect was slightly
336 greater for the younger cohort (French et al. 2013).

337 In our study, we did not detect an association between myopia status or spherical equivalent
338 at 20 years and 25(OH)D concentration at 6 or 14 years, despite previous studies showing
339 an association between time outdoors and risk of myopia at these ages. We may not have
340 detected such an association for a number of reasons. First, we may have lacked power due
341 to the lower number of participants with 25(OH)D measurements at 6 years (n=618) or with
342 a low vitamin D status (n=5 and n=39 at 6 and 14 years, respectively). The smaller
343 difference in mean 25(OH)D concentration at younger ages, as indicated in the trajectory
344 analysis, would also reduce our power to detect an effect at these ages. Second, EIA was
345 used to assess 25(OH)D concentration at the 6- and 14-year follow-ups; lower accuracy
346 and/or precision of the EIA could have reduced the ability to detect an association (Lai et al.
347 2012). Third, time spent outdoors and 25(OH)D concentration at 6 and 14 years may be
348 associated with myopia incidence over the short- or medium-term but less strongly
349 associated with myopia at 20 years. Fourth, it is possible that 25(OH)D concentration is a
350 poorer marker of time spent outdoors at these ages, although associations between time
351 outdoors and 25(OH)D concentration have been reported in pre-pubertal children (Jones et
352 al. 1999).

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

355 shape of the trajectories between those with and without myopia. This this does not
356 necessarily mean that there were significant differences in the age-specific estimates of
357 25(OH)D concentration. Indeed, the substantial overlap of the 95% confidence intervals in
358 Figure 1 suggests the age-specific 25(OH)D distributions are not significantly different,
359 particularly at younger ages.

360 In Caucasian individuals (85% of cohort), the 25(OH)D concentration trajectories were
361 similar between myopic and non-myopic individuals in early childhood but then diverged with
362 the differences becoming more apparent at older ages. Thus, we were able to find
363 differences in 25(OH)D concentration in myopic and non-myopic individuals only for older
364 ages. On the other hand, non-Caucasian children who were myopic at 20 years had lower
365 25(OH)D concentration from early childhood compared to non-myopic peers. It is possible
366 that that differences in the amount of time spent outdoors between myopic and non-myopic
367 individuals start to arise in childhood, but we were unable to detect a significant difference in
368 our study. Our trajectory results agree with those from the ALSPAC, which found that
369 primary caregivers of children who became myopic between ages 10 and 15 years reported
370 declining amounts of time spent outdoors between ages 2 and 8 years, relative to those who
371 remained non-myopic (Shah et al. 2017).

372 It is unclear why the difference in 25(OH)D trajectory between myopes and non-myopes was
373 apparent at an earlier age in non-Caucasians compared to Caucasians. Non-Caucasians,
374 both with and without myopia, had lower 25(OH)D concentrations overall. This may be
375 related to having darker skin pigmentation, which reduces endogenous synthesis of vitamin
376 D (Mithal et al. 2009), or due to non-Caucasian individuals spending less time outside and
377 having less sun exposure (Rose et al. 2008; Guo et al. 2014). Non-Caucasians also had a
378 higher prevalence of myopia in this study and children of East or South-East Asian ethnicity
379 have been noted to have a higher incidence (French et al. 2013) and progression (Pärssinen
380 et al. 2020) of myopia compared to Caucasian populations. A previous study found higher
381 25(OH)D concentration was associated with a larger reduction in risk of myopia among
382 participants of East Asian ethnicity compared to Caucasians (Yazar et al. 2014). Thus, the
383 larger difference in 25(OH)D trajectory between individuals with and without myopia among
384 non-Caucasian participants could be reflective of the generally higher prevalence of myopia
385 among these participants or could suggest that greater amounts of time spent outdoors are
386 required to reduce risk of myopia among non-Caucasian individuals.

387 It was somewhat unexpected that serum 25(OH)D trajectories of those with no, low,
388 moderate and high myopia were similar in early childhood (Figure 2), with model
389 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.

426 2016), as well as near work, a known risk factor for myopia (Huang et al. 2015). Myopia
427 status was not measured at younger ages and we were therefore unable to thoroughly
428 investigate the short-term effects of time outdoors at ages 6 and 14 years on myopia and
429 relied on recall data when investigating age of onset of myopia.

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

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

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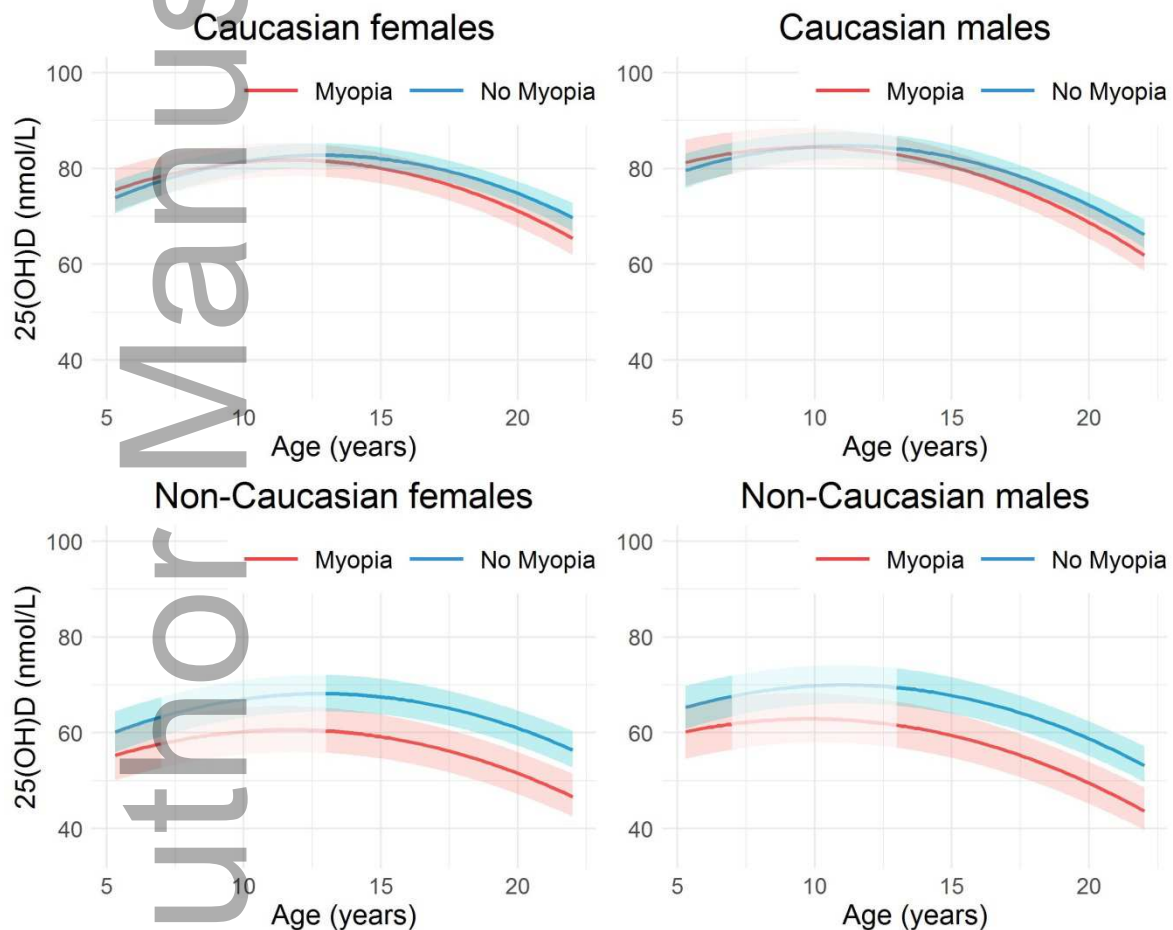
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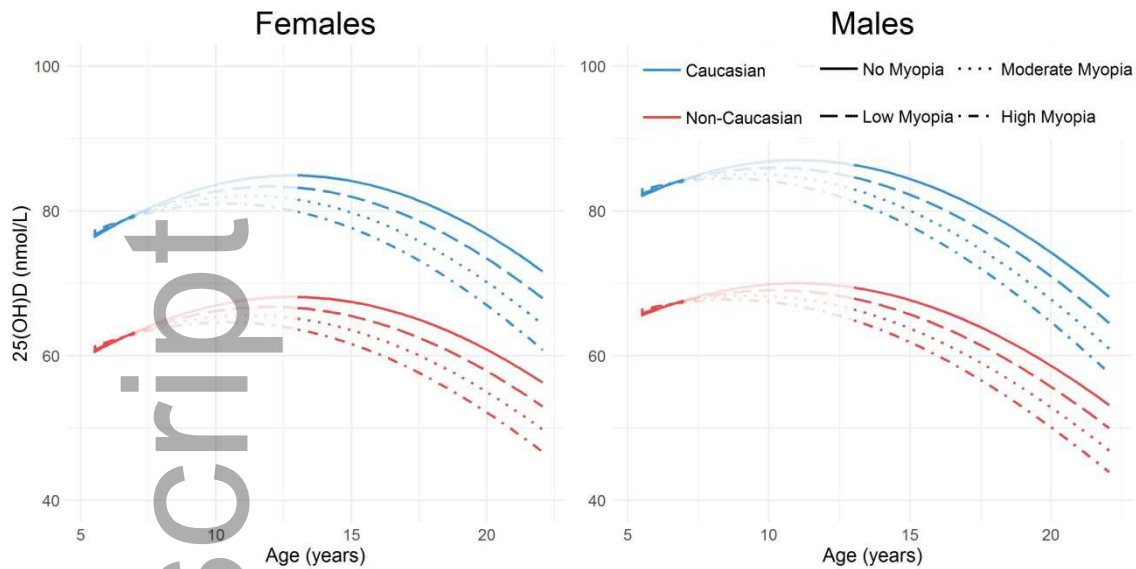
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 611



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



618

619 **Figure 2** Best-fit model estimates of 25(OH)D trajectory in those who had no myopia, low
 620 myopia, moderate myopia and high myopia at 20 years of age, stratified by sex and race.

621 The faded areas of the plots correspond to ages where no 25(OH)D concentration data were
 622 available in this study and plots are extrapolated from model fit.

623 **Table 1:** Participant characteristics at each of the 6-, 14-, 17- and 20-year Raine Study
 624 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%)

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

627 ‡1127 (89.4%) participants had a 25(OH)D measurement at this follow-up

628 §Deseasonalised by adjusting for month of collection

629 **Table 2** Associations between myopia or spherical equivalent at 20 years and age-specific
630 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)*

631 *p<0.05

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

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

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

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

639 SUPPORTING INFORMATION

640 25-hydroxyvitamin D Assay Coefficients of Variation

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

643 Table S1b Sensitivity Analysis comparing results of overall analysis of myopia (Table
644 2) to a subset of participants who have complete 25(OH)D data at both the 6- and
645 20-year follow-ups or complete data at both the 14- and 20-year follow-ups

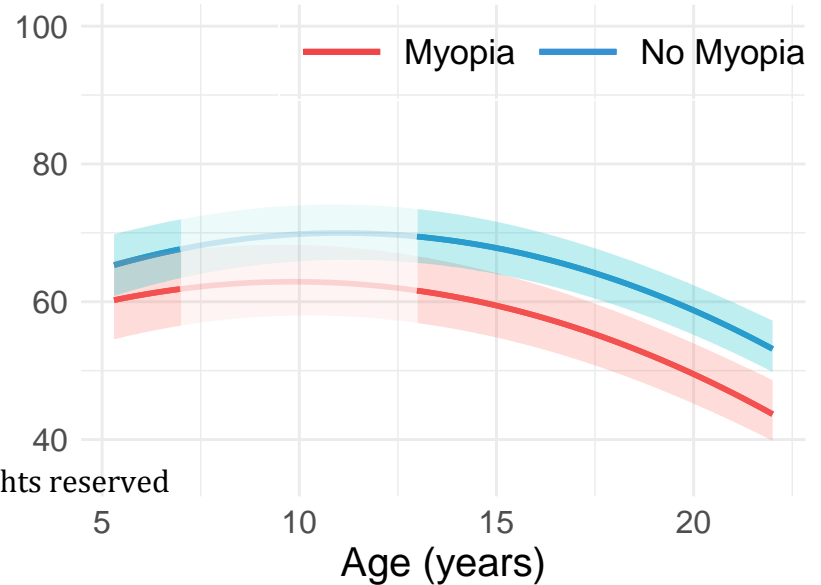
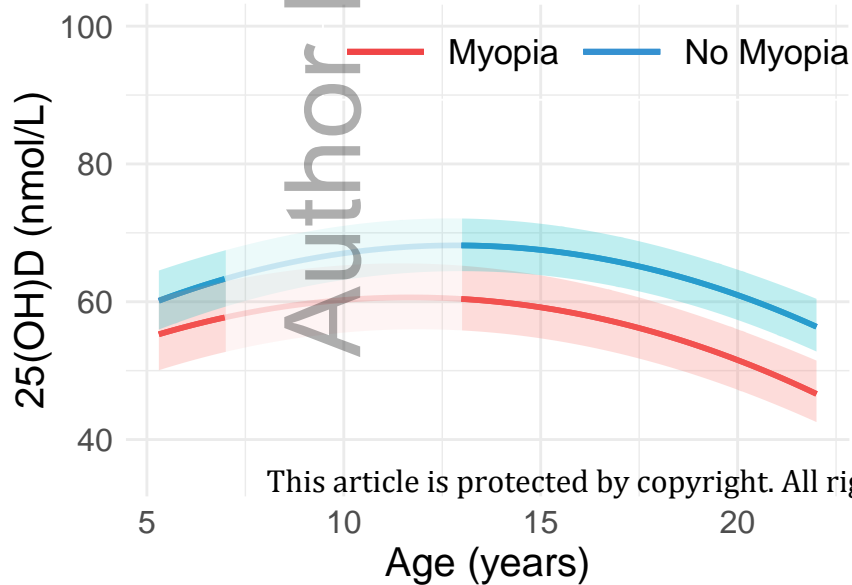
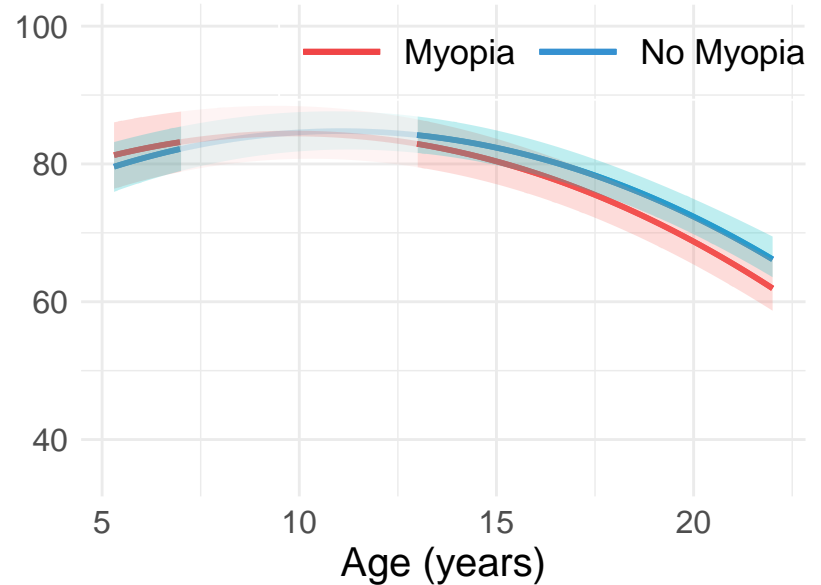
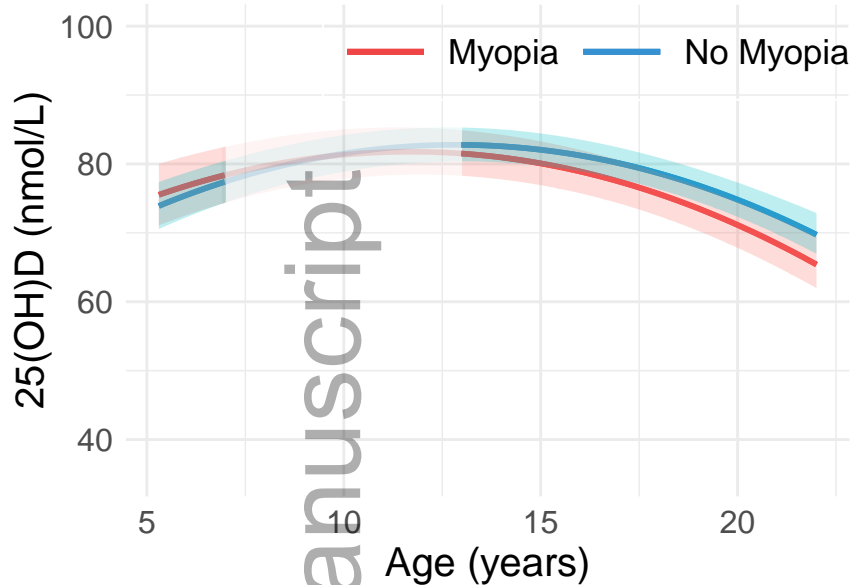
646 Table S2 Sensitivity Analysis comparing results of overall analysis of spherical
647 equivalent (Table 2) to a subset of participants who have complete 25(OH)D data at
648 all follow-ups

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

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

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

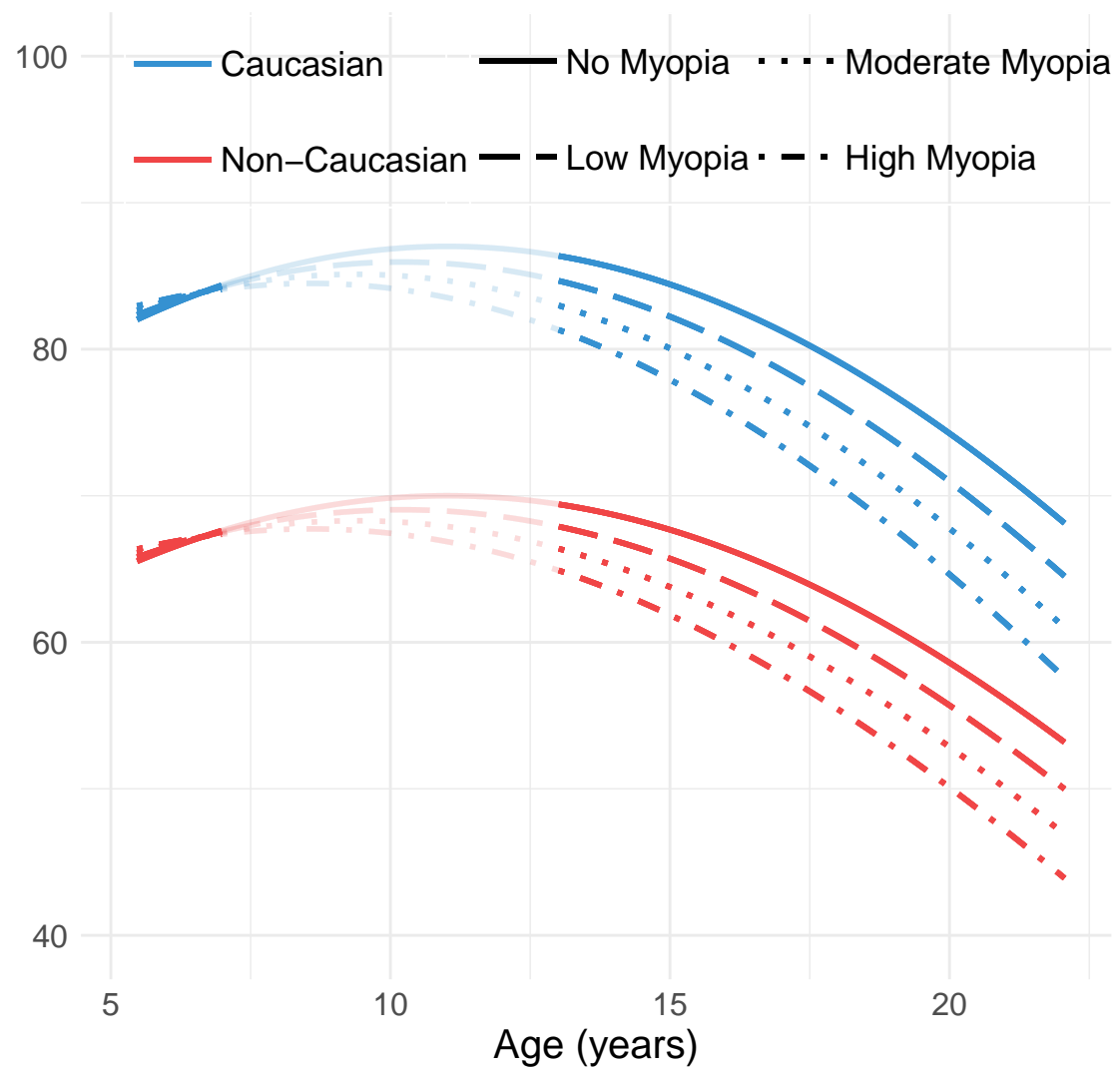
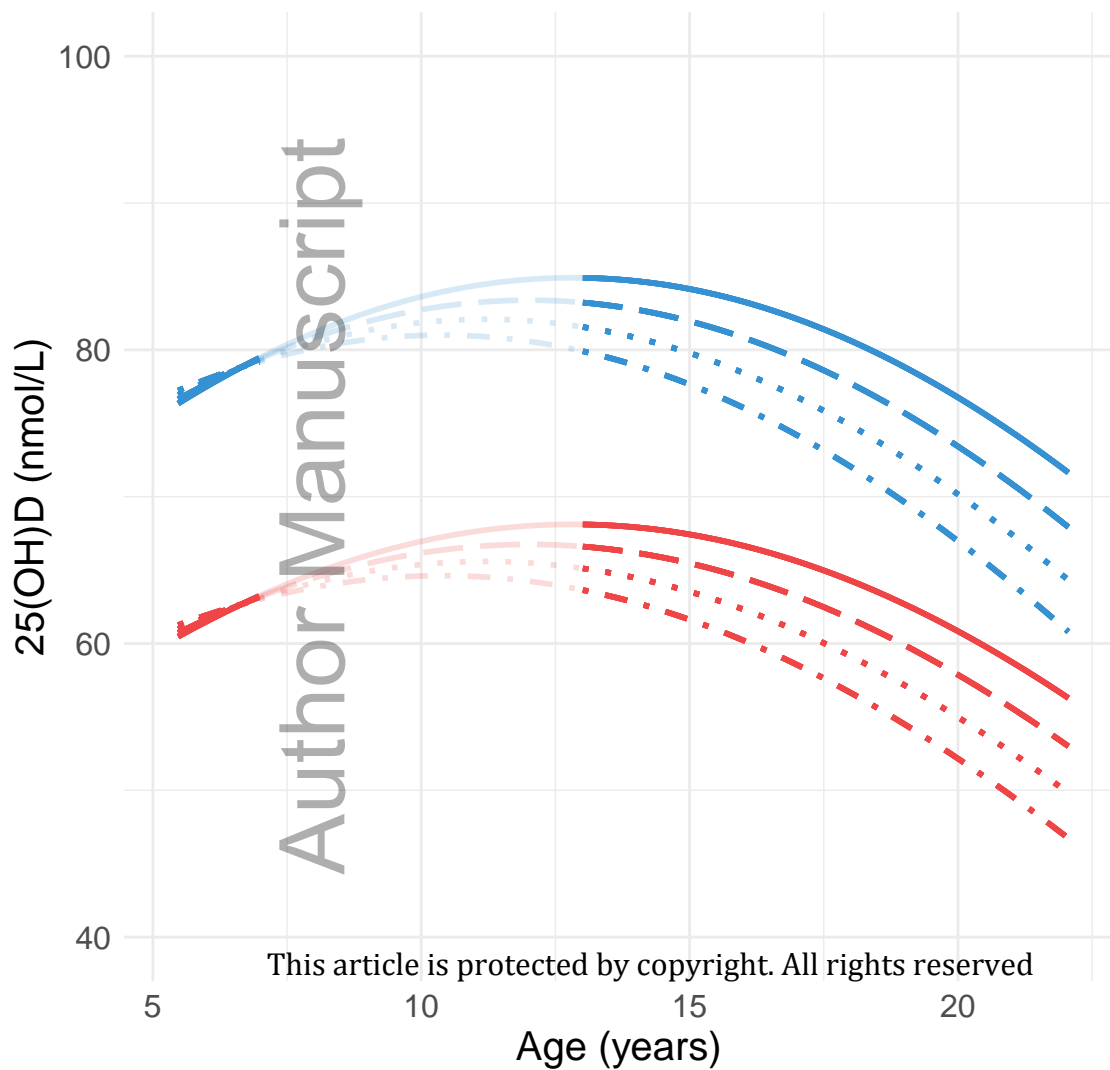
655 Figure S2: Quantile-quantile plots of all available 25(OH)D data (i.e. at 6, 14, 17 and
656 20 years combined) after common transformations



Females

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Males





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Title:

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