Association of serum 25-hydroxyvitamin D3 and D2 with academic performance in childhood: findings from a prospective birth cohort

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ABSTRACT

Background Higher total serum 25-hydroxyvitamin D (25(OH)D) concentrations have been associated with better cognitive function mainly in cross-sectional studies in adults. It is unknown if the associations of different forms of 25(OH)D (25(OH)D3 and 25(OH)D2) are similar.

Methods Prospective cohort study (n=3171) with serum 25(OH)D3 and 25(OH)D2 concentrations measured at mean age of 9.8 years and academic performance at age 13–14 years (total scores in English, mathematics and science) and 15–16 years (performance in General Certificates of Education examinations).

Results Serum 25(OH)D3 concentrations were not associated with any educational outcomes. Higher 25(OH)D2 concentrations were associated with worse performance in English at age 13–14 years (adjusted SD change per doubling in 25(OH)D2 (95% CI) −0.05 (−0.08 to −0.01)) and with worse academic performance at age 15–16 years (adjusted OR for obtaining ≥5 A*—C grades (95% CI) 0.91 (0.82 to 1.00)).

Conclusion The null findings with 25(OH)D3 are in line with two previous cross-sectional studies in children. It is possible that the positive association of 25(OH)D2 with academic performance seen in adults does not emerge until later in life or that the results from previous cross-sectional adult studies are due to reverse causality. The unexpected inverse association of 25(OH)D2 with academic performance requires replication in further studies. Taken together, our findings do not support suggestions that children should have controlled exposure to sunlight, or vitamin D supplements, in order to increase academic performance.

INTRODUCTION

Cognitive function in childhood and educational attainment are associated with adverse health outcomes in adulthood.1–5 These associations are approximately linear across the full distribution of cognitive function/education, rather than being driven by adverse health in those with severe learning difficulties.6 Understanding the determinants of childhood cognitive function and educational attainment are important for understanding the mechanisms linking this to later health and for developing means to improve educational attainment and later health and well-being.

Higher serum concentrations of total 25-hydroxyvitamin D (25(OH)D) have been associated with better cognitive function in some,10–15 though not all,16–17 epidemiological studies of adults. To our knowledge, only two studies have examined this association in children; both these cross-sectional studies used data from the Third US National Health and Nutrition Examination Survey (NHANES III) and found no association between total 25(OH)D and childhood cognitive function.17,18

Circulatory 25(OH)D3 consists of 25(OH)D3 (synthesised from vitamin D3 obtained mainly from ultraviolet B (UVB) induced synthesis in skin and to a lesser extent from animal food sources) and 25(OH)D2 (synthesised from vitamin D2 obtained from plant/fungal sources). There is evidence that vitamin D3 supplementation is more potent in raising and maintaining 25(OH)D concentrations than supplementation with vitamin D2.19 It is unknown if these two forms of 25(OH)D have similar associations with cognitive function.

The aim of this study was to examine the prospective association between variation in serum 25(OH)D3 and 25(OH)D2 with academic performance in children and to compare if these associations were similar with both forms of 25(OH)D.

Because vitamin D, together with parathyroid hormone (PTH), regulates calcium and phosphate homoeostasis,20,21 we investigated whether these analytes were related to academic performance and whether the association of 25(OH)D3 and or 25(OH)D2 with academic performance requires replication in further studies. Taken together, our findings do not support suggestions that children should have controlled exposure to sunlight, or vitamin D supplements, in order to increase academic performance.
Serum 25(OH)D3, 25(OH)D2, PTH, phosphate and calcium were assayed on non-fasting blood samples collected at mean age of 9.9 years for the majority of participants. If no samples were available from the 9.9 years assessment, samples from the 7.6 years assessment were used. The mean age at sample collection in the whole study population was 9.8 years (SD: 1.1). Following collection, samples were immediately spun, frozen and stored at −80°C. Assays were performed after a maximum of 12 years in storage, with no previous freeze-thaw cycles. 25(OH)D3, 25(OH)D2 and deuterated internal standard were measured with high performance liquid chromatography tandem mass spectrometer using multiple reaction mode with following mass:charge ratio transformations: 413.2 > 395.3, 401.1 > 383.3 and 407.5 > 107.2 for 25(OH)D3, 25(OH)D2 and hexa-deuterated (OH)D3, respectively. Interassay coefficients of variation for the assay were <6% from 2 to 50 pmol/l. The assay sensitivity (replicates of the zero standard) was 1 pmol/l.

**Outcomes**

KS3 and KS4 results were obtained by linkage to National Pupil Database, a central repository for pupil-level annual school census, using unique pupil numbers as identifiers. KS3 tests are performed at 13–14 years (school year 9) when performance in English, maths and science is assessed. Total point scores in each subject were used in the analyses. KS4 is performed at 15–16 years when pupils take their General Certificates of Education in a range of academic and applied subjects. General Certificates of Education are graded from A* to G, with A* indicating the highest performance. We examined associations with three outcomes from KS4 assessments: (1) those achieving five or more A*—C grades, including maths and English (this variable is provided in the National Pupil Data set and is the level of achievement generally required to go on to advance level study and ultimately university; many semi-skilled jobs that do not require a university degree also consider this an important achievement); (2) those achieving nine or more A*—A (an indicator of the highest performers) and (3) those achieving no A*—C grades (an indicator of poor performance).

**Confounders**

Data on ethnicity, maternal and paternal education and occupational social class were obtained from parent-completed questionnaires. Data on subsidised school meals were obtained from the National Pupil Database. Data on time spent outdoors and protection from UVB exposure (use of sunblock, covering clothing or hat and avoidance of midday sun) were obtained from parent-completed questionnaires at mean age of 8.5 years. Body mass index (BMI) was calculated from height and weight measured at the same time as blood samples were taken. IQ was assessed by the Wechsler Intelligence Scale for Children (WISC-III UK) at the 8–9-year assessment.

**Statistical analyses**

Statistical analyses were conducted with Stata V.11.0 (Stata Corp LP). To adjust for seasonal variability, 25(OH)D3 was modelled according to date of blood sampling using linear regression with trigonometric sine and cosine functions. 25(OH)D3 was log-transformed to reduce heteroscedasticity. The residual from the sine–cosine date of sampling regression model was then used as the primary 25(OH)D3 exposure variable in subsequent regression analyses. To include all participants on whom a 25(OH)D3 was assayed, those with a value below the detectable limit of 1.25 nmol/l were imputed using a random intercept logistic regression model.

Age and gender SD (z) scores for serum 25(OH)D3 and 25(OH)D2, PTH, calcium and phosphate were generated using the internal cohort data with age in 1 month categories. The association of potential confounders with KS3 outcomes and with exposures was assessed with linear regression, and the association of confounders with KS4 outcomes was assessed with logistic regression.

The associations of 25(OH)D3 and 25(OH)D2 with KS3 and KS4 results were assessed with non-parametric bootstrap procedure in conjunction with multilevel mixed-effects linear regression (for KS3 outcomes) or random-effects logit models (for KS4 outcomes) based on 10,000 replications. The multilevel mixed-effects linear regression and random-effects logit models were used to take into account the school-level variance in the KS.
results, and the bootstrapping procedure enabled us to statistically compare associations of 25(OH)D$_3$ to those of 25(OH)D$_2$. The difference between the association of 25(OH)D$_2$ and 25(OH)D$_3$ was calculated from the bootstrap replicate distribution. Estimates and SEs were empirically calculated from the mean and SD of the bootstrap distribution, respectively. All p values were calculated using bootstrap means and SEs and compared to a z-distribution. To numerically compare the associations of two forms of 25(OH)D, $\beta$-coefficients from the regression models were multiplied by $\log_2(2)$. These are then interpreted as mean differences or ORs per doubling of exposure. For the main analyses presented here, we compared the associations of season-adjusted 25(OH)D$_3$ to those of 25(OH)D$_2$. The associations of unadjusted (for season) 25(OH)D$_3$ and total 25(OH)D (25(OH)D$_2$ + 25(OH)D$_3$) with no seasonal adjustment, plus 25(OH)D$_3$ with all outcomes are shown in the supplementary material.

**RESULTS**

The mean (SD) seasonally adjusted serum 25(OH)D$_3$, phosphate and albumin-adjusted calcium concentration were 62.1 (0.8) nmol/l, 1.53 (0.16) mmol/l and 2.38 (0.12) mmol/l, respectively. The median (IQR) for serum 25(OH)D$_2$ and PTH were 3.2 (1.3–6.7) nmol/l and 4.4 (3.3–5.8) pmol/l, respectively. Mean (SD) score in KS3 English, maths and science examinations were 54 (15), 89 (20) and 102 (22), respectively.

Supplementary table 1 shows the distribution of outcomes and confounders in participants with data on outcomes who were excluded due to missing data in exposures or confounders and in complete cases. Complete cases were more likely to perform better in both KS examinations, have higher IQ and lower BMI, to be of white ethnicity, less meticulous about protection from UVB and from higher socioeconomic backgrounds than those with missing data on exposures or confounders.

Supplementary table 2 shows the associations of potential confounders with 25(OH)D$_3$, 25(OH)D$_2$, PTH, calcium and phosphate. Those with non-white ethnicity had higher PTH and lower 25(OH)D$_3$ and 25(OH)D$_2$ concentrations. BMI was negatively associated with serum 25(OH)D$_3$ and positively with PTH concentrations. Higher socioeconomic position was associated with higher concentrations of 25(OH)D$_3$ and phosphate and lower concentrations of 25(OH)D$_2$ and calcium. Children who were protected more from UVB exposure had lower 25(OH)D$_2$, phosphate and PTH concentrations and higher calcium concentrations. Time spent outdoors during summer was positively associated with 25(OH)D$_3$ concentrations. Pupils who were eligible for free school meals had lower 25(OH)D$_3$ and higher 25(OH)D$_2$ and PTH concentrations.

Supplementary table 3 shows the associations of potential confounders with KS3 scores. Non-white ethnicity, higher BMI, lower socioeconomic position, being more meticulous about avoiding UVB exposure and spending less time outdoors were all associated with lower examination scores in at least one of English, maths or science. Supplementary table 4 summarises the associations of potential confounders and academic performance in KS4. In general, these associations were similar to those found with KS3 scores.

Table 1 shows the prospective associations of serum 25(OH)D$_3$, 25(OH)D$_2$, calcium, phosphate and PTH concentrations with KS3 scores. 25(OH)D$_3$ concentrations were not associated with any KS outcomes. Higher 25(OH)D$_2$ concentrations were associated with worse performance in English in the unadjusted model (model 1) and after adjusting for confounders including ethnicity, socioeconomic position, protection from UVB (model 2) and for other analytes, including 25(OH)D$_3$ (model 3). 25(OH)D$_2$ was not associated with performance in maths or science. The associations of 25(OH)D$_3$ and 25(OH)D$_2$ with English were different from each other ($p$=0.01). None of PTH, calcium or phosphate was associated with KS3 outcomes in confounder-adjusted models.

Table 2 shows the prospective association between exposures and KS4 outcomes. These were essentially consistent with findings seen for KS3 outcomes. 25(OH)D$_3$ was not associated with any of the KS4 outcomes, and higher serum 25(OH)D$_2$ concentrations were associated with worse academic performance (less likely to obtain ≥5 A*-A grades or ≥9 A*-C grades) in the unadjusted model (model 1). These inverse associations attenuated slightly with adjustment for potential

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>SD change in outcome per doubling of exposure (95% CI)</th>
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<tr>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
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<td>Model 2</td>
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<td>Model 3</td>
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<tr>
<td>25(OH)D$_3$</td>
<td>English total score</td>
<td>0.01 (–0.01 to 0.03)</td>
</tr>
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<td></td>
<td>Maths total score</td>
<td>0.00 (–0.02 to 0.03)</td>
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<tr>
<td></td>
<td>Science total score</td>
<td>0.01 (–0.01 to 0.03)</td>
</tr>
<tr>
<td>25(OH)D$_2$</td>
<td>English total score</td>
<td>–0.07 (–0.10 to –0.04)</td>
</tr>
<tr>
<td></td>
<td>Maths total score</td>
<td>0.02 (–0.01 to 0.06)</td>
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<tr>
<td></td>
<td>Science total score</td>
<td>–0.03 (–0.06 to 0.01)</td>
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<tr>
<td>Albumin-adjusted calcium</td>
<td>English total score</td>
<td>–0.01 (–0.03 to 0.01)</td>
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<td></td>
<td>Maths total score</td>
<td>0.00 (–0.02 to 0.02)</td>
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<td></td>
<td>Science total score</td>
<td>0.00 (–0.03 to 0.02)</td>
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<tr>
<td>Phosphate</td>
<td>English total score</td>
<td>0.01 (–0.01 to 0.03)</td>
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<tr>
<td>Parathyroid hormone</td>
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<td>–0.01 (–0.03 to 0.01)</td>
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<td></td>
<td>Maths total score</td>
<td>0.00 (–0.02 to 0.03)</td>
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Model 1 is unadjusted (the exposures are standardised for age and gender and 25(OH)D$_3$ is adjusted for season and ethnicity).
Model 2 same as model 1 plus adjustment for ethnicity, head of household social class, mothers and partners education, time spent outdoors during summer (age 8.5 years), use of sunblock, hat, covering clothing, avoidance of midday sun, body mass index and eligibility for free school meals.
Model 3 same as model 2 plus adjustment for serum concentrations of other hormones/metabolites, which are related 25(OH)D$_3$ homoeostasis (eg, association of 25(OH)D$_3$ is adjusted for 25(OH)D$_2$, phosphate, albumin-adjusted calcium and parathyroid hormone).
confounders (model 2), with additional adjustment for other analytes, including 25(OH)D₃ not changing results further (model 3). There was statistical evidence that the associations of 25(OH)D₃ and 25(OH)D₂ with obtaining ≥5 A*–C grades differed from each other (p = 0.02). PTH, calcium and phosphate concentrations were not associated with any KS4 outcomes, with the exception of phosphate having a modest positive association with obtaining ≥5 A*–C grades. The associations of unadjusted (for season) 25(OH)D₃ and total 25(OH)D concentrations with educational outcomes were similar to those of season-adjusted 25(OH)D₃ shown here (supplementary tables 5 and 6).

Supplementary tables 7 and 8 show the results of sensitivity analyses restricted to participants with data on IQ at age 8.5 years and exposure measurements at age 9.9 or 11.8 years. Adjustment for IQ at age 8.5 years, in addition to other confounders, did not alter the inverse associations of 25(OH)D₂ with outcomes.

**DISCUSSION**

Serum 25(OH)D₃ concentrations, at age 7.6–11.8 years, were not associated with academic performance assessed at age 13–14 or 15–16 years in our prospective study. Somewhat surprisingly serum concentrations of 25(OH)D₂ were negatively associated with academic achievements. This association was consistent with the associations of indicators of lower socioeconomic position (e.g., eligibility for free school meals, lower parental education and socioeconomic position) with lower educational attainment and higher 25(OH)D₂ concentrations. It is therefore possible that it represents residual confounding. There was little evidence that PTH, calcium or phosphate were associated with educational attainment. These findings build on two previous cross-sectional studies in children using NHANES data that reported no associations of total 25(OH)D or calcium with cognitive function. Since 25(OH)D₂ is the major contributor to total 25(OH)D, our findings of no association of 25(OH)D₃ or calcium with academic performance are consistent with these studies. This is illustrated in our cohort by the lack of association of total 25(OH)D (supplementary table 6) with any of the educational outcomes.

Most cross-sectional studies in adults have shown a positive association between total 25(OH)D concentrations and cognitive function, though a recent second prospective study found no association between total 25(OH)D and cognitive decline. The difference between the overall body of evidence in adults (suggesting better cognitive function in those with higher vitamin D) compared with children (no association) could be because the key effects of 25(OH)D only emerge in adults either because it is related more to cognitive decline than development or because it is a lifetime cumulative amount of vitamin D that is important with respect to cognitive function. Alternatively, it could be that studies of adults (which are mostly cross-sectional) are more likely to be affected by reverse causality than those in childhood (with adults with poorer cognitive function spending less time outdoors and hence having lower 25(OH)D concentrations).

Experimental studies suggest that vitamin D and its metabolites are important for neuronal function. The findings from these studies suggest that vitamin D may be linked to cognition by different pathways, including mediation of inflammatory response, regulation of genes that mediate neuronal density, synaptic plasticity, neurotransmitter synthesis and survival and differentiation of neurons, as well as direct functions as a neurotrophic and neuroprotective factor. The majority of the animal studies have used vitamin D receptor knockout models or restricted the UV irradiation/dietary intake of vitamin D₃, which would provide more information on the effects of vitamin D₃ and its metabolites. Thus, it is difficult to suggest a biological mechanism, which could explain the negative association between 25(OH)D₂ and academic performance based on these possible pathways. One possibility is that this is a chance finding given the number of associations that have been examined in this paper and the lack of any previous studies (that we are aware of) that have examined the associations of 25(OH)D₂ specifically with cognitive or educational outcomes. It is also
possible that despite extensive adjustment for potential confounders, the association is explained by residual confounding and different confounder structures between the two forms of 25(OH)D. The main source of 25(OH)D$_2$ are diet and supplements, whereas that of 25(OH)D$_3$ is UVB exposure. Dietary behaviours may be more likely to be affected by multiple confounders that could generate the 25(OH)D$_2$ association than is UVB exposure. However, for this to be the case, we would need to think of a (or several) confounders that are related to higher levels of consumption of food that is a source of 25(OH)D$_2$ (mostly supplements) and lower educational attainment.

Strengths and weaknesses
In addition to examining prospective associations, the strengths of the present study include large sample size, ability to adjust for multiple confounders and the measurement methodology for 25(OH)D concentrations. With one exception, previous studies of associations in adults and children have used immunoassays, which may underestimate the amount of 25(OH)D. Studies of associations in adults and children have used IQ tests, with other studies using IQ tests. Academic performance with similar vitamin D concentrations but may not generalise across populations. In cross-sectional data, ranging between 0.29 and 0.66. Lastly, in ALSPAC are similar to these published associations (based on multiple confounders that could generate the 25(OH)D association than is UVB exposure. However, for this to be the case, we would need to think of a (or several) confounders that are related to higher levels of consumption of food that is a source of 25(OH)D$_2$ (mostly supplements) and lower educational attainment.

What is already known on this subject

- Higher total serum 25-hydroxyvitamin D (25(OH)D) concentrations have been associated with better cognitive function mainly in cross-sectional studies of adults. There are few prospective studies, and none of these are in children.
- 25(OH)D is present in two forms: 25(OH)D$_3$ and 25(OH)D$_2$, but it is unknown if the associations of these two forms with cognitive performance are similar.

What this study adds

- Our prospective study suggests that 25(OH)D$_2$ is an important determinant of academic performance in children and that 25(OH)D$_3$ is inversely associated with performance, though this may be a chance finding or due to residual confounding.
- Protection of children from ultraviolet B exposure, which has been associated with low concentrations of vitamin D, but which protects against skin damage and skin cancer, is unlikely to have any detrimental effect on academic achievement.

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Contributors
Planned the research project: A-MT and DAL. Obtained funding: DAL. Provided essential materials: WDF (laboratory analyses). Data analysis: A-MT and AS. Supervised the analyses and project: DAL. Wrote the first draft of the manuscript: A-MT and DAL. Accepted the final manuscript and made important intellectual contribution to draft versions: A-MT, AS, WDF and DAL. Acts as guarantor: A-MT.

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Competing interests
None.
Research report

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