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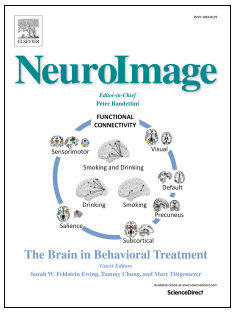

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Credit Author Statement

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A prospective population-based study of gestational vitamin D status and brain morphology in preadolescents

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Abstract

Low vitamin D level during pregnancy has been associated with adverse neurodevelopmental outcomes such as autism spectrum disorders (ASD) in children. However, the underlying neurobiological mechanism remains largely unknown. This study investigated the association between gestational 25-hydroxyvitamin D [25(OH)D] concentration and brain morphology in 2597 children at the age of 10 years in the population-based Generation R Study. We studied both 25(OH)D in maternal venous blood in mid-gestation and in umbilical cord blood at delivery, in relation to brain volumetric measures and surface-based cortical metrics including cortical thickness, surface area, and gyrification using linear regression. We found exposure to higher maternal 25(OH)D concentrations in mid-gestation was associated with a larger cerebellar volume in children (b=0.02, 95%CI 0.001 to 0.04), however this association did not remain after correction for multiple comparisons. In addition, children exposed to persistently deficient (i.e., <25 nmol/L) 25(OH)D concentration from mid-gestation to delivery showed less cerebral gray matter and white matter volumes, as well as smaller surface area and less gyrification at 10 years than those with persistently sufficient (i.e., ≥ 50 nmol/L) 25(OH)D concentration. These results suggest temporal relationships between gestational vitamin D concentration and brain morphological development in children.

Keywords: epidemiology, neuroimaging, pregnancy, vitamin D

1. Introduction

Vitamin D is an essential micronutrient that is mainly synthesized in the skin by exposure to sunlight (Bendik et al., 2014). In fetal life, vitamin D is mainly transported from mother to fetus through the placenta in the form of 25-hydroxyvitamin D [25(OH)D] (McAree et al., 2013). Maternal serum 25(OH)D concentration and that of the fetus measured in cord blood are highly correlated (Glorieux et al., 1981; Kimball et al., 2008), suggesting maternal vitamin D level is a reliable indicator of fetal vitamin D status.

Vitamin D deficiency is prevalent worldwide, with people living in Europe, the Middle East, and Asia at particular risk (Lips, 2007). It is also known that women, especially those in pregnancy, are more likely to be vitamin D deficient (Gellert et al., 2017; Vinkhuyzen et al., 2016). Maternal vitamin D deficiency has repeatedly been associated with adverse birth outcomes such as fetal growth restriction and preterm birth (Bodnar et al., 2015; Leffelaar et al., 2010). In recent years, emerging evidence also associates low maternal vitamin D level during pregnancy with long-term cognitive and neuropsychiatric outcomes of the offspring. For instance, Keim et al. (Keim et al., 2014) found maternal vitamin D concentration in mid and late gestation was positively associated with child IQ at age 7. Two neonatal studies reported an association of vitamin D deficiency and the risk of schizophrenia (Eyles et al., 2018; McGrath et al., 2010). In addition, animal studies using rodents and epidemiological studies in humans showed that gestational vitamin deficiency was associated with an increased risk of autism spectrum disorders (ASD) or more autism-related phenotypes in offspring (Ali et al., 2019; Chen et al., 2016; Magnusson et al., 2016; Vuillermot et al., 2017), which has also been supported by the evidence from our present cohort (Vinkhuyzen et al., 2018; Vinkhuyzen et al., 2017). However, these relationships remain inconclusive due to some inconsistent findings. For example, maternal vitamin D levels during

pregnancy were not related to ASD symptoms in children from 5 to 18 years old in a Spanish birth cohort (Lopez-Vicente et al., 2019), and a case-control study in Southern California, Unite States reported no association between neonatal vitamin D levels and ASD in childhood (Windham et al., 2019).

Using neuroimaging techniques, clear associations have been established between cognitive, emotional, and behavioral phenotypes and brain morphology. For instance, reduced brain volumes and less gyrification are frequently reported in children with poor cognitive outcomes or ASD (Arhan et al., 2017; Blanken et al., 2015; Duret et al., 2018; Libero et al., 2014; Pangelinan et al., 2011). To date, however, the evidence linking early life exposure to low vitamin D and brain development remains scarce. There are a few animal studies showing that rats exposed to vitamin D deficiency in gestation had a smaller brain volume and larger cerebral ventricles (Eyles et al., 2003; Feron et al., 2005). Similarly, in studies in older adults using a cross-sectional design, a lower vitamin D concentration was associated with smaller brain volume and larger cerebral ventricles (Annweiler et al., 2013; Hooshmand et al., 2014). However, we know of no study exploring maternal gestational vitamin D status and brain morphology in children. Such studies may further our understanding of the biological mechanism underlying the established association between gestational vitamin D and child neurodevelopmental outcomes, and justify interventions such as vitamin D supplementation during pregnancy. Therefore, we investigated the association of maternal vitamin D concentration during pregnancy with offspring brain morphology at age 10 years. Based on the existing literature, we hypothesized that low gestational vitamin D level was associated with global alterations in brain morphology in children.

2. Material and methods

2.1 Study design

This study is embedded in the Generation R Study (Kooijman et al., 2016), a population-based prospective cohort in Rotterdam, the Netherlands. Pregnant women living in the study area with an expected delivery date between April 2002 to January 2006 were recruited. In total 8879 mothers were enrolled in the study prenatally, who gave birth to 8976 live-born children. The study has been approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

2.2 Participants

Of the 8976 mother-child dyads, we excluded 966 without any information on gestational vitamin D concentration. This left 8010 children, of which 6156 visited the research center at age 9-11 years and were invited for a magnetic resonance imaging (MRI) assessment of the brain (White et al., 2018); among the 3363 children that underwent brain MRI assessment, 2715 children had usable brain morphological data after quality inspection. We also randomly excluded 118 siblings to rule out potential clustered data (i.e., children born to the same mother and thus exposed to shared genetic or environmental factors shaping their brain development), leaving 2597 children as the study population. Of these children, 2427 had vitamin D concentration information in mid-gestation, 1706 had vitamin D concentration information at delivery, and 1536 had information on both assessments (see Supplementary Figure S1 for the flow diagram).

2.3 Vitamin D concentration

Maternal venous blood samples were collected during mid-pregnancy at a median gestational age of 20.4 (range 18.1-24.9) weeks. Cord blood from the umbilical vein was collected at delivery, at

a median gestational age of 40.3 (range 27.6-43.4) weeks. Samples were analyzed using isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Eyles Laboratory of the Queensland Brain Institute, University of Queensland, Australia. Vitamin D status was assessed by measuring 25(OH)D, defined as the sum of 25-hydroxyvitamin D_2 [$25(OH)D_2$] and 25-hydroxyvitamin D_3 [$25(OH)D_3$] in serum (Eyles et al., 2009). Assay accuracy was assessed using certified reference materials purchased from the Australian National Institute of Standards and Technology (NIST SRM 972a Levels 1-4). Further details of the assay methodology have been described elsewhere (Vinkhuyzen et al., 2016).

2.4 Structural neuroimaging

Prior to neuroimaging, all children were familiarized with MRI scanning during a mock scanning session. All images were acquired using the same sequence on the same scanner (3 Tesla GE MR 750w Discovery). Following a three-plane localizer scan, a high-resolution T1-weighted inversion recovery fast spoiled gradient recalled sequence was acquired. Detailed information on the sequence and imaging procedure can be found elsewhere (White et al., 2018).

Volumetric segmentation and cortical reconstruction were performed with FreeSurfer v.6.0.0 (http://surfer.nmr.mgh.harvard.edu/). The standard reconstruction stream was applied, and surface-based models of white matter and gray matter were generated. Thickness maps for each subject were smoothed with a 10 mm full-width half-maximum Gaussian kernel. Local gyrification index (LGI) maps were smoothed using a 5 mm full-width half-maximum Gaussian kernel. The quality of surface reconstruction was visually inspected, after which data with insufficient quality were eliminated.

2.5 Covariates

Possible confounders were chosen based on prior literature (Morales et al., 2015; Vinkhuyzen et al., 2018; Whitehouse et al., 2012) and directed acyclic graphs (Shrier and Platt, 2008). Information on maternal age at intake, ethnicity, marital status, education, household income, smoking and alcohol use in pregnancy, and vitamin supplement use in pregnancy was collected at enrollment of the study with questionnaire. Maternal ethnicity was determined from the country of birth of the parents according to the largest ethnic groups in our study population and used to define broad categories based on similarities in skin color and/or cultural background (Eilers et al., 2013; Voorburg/Heerlen, 2004a; Voortman et al., 2015). The categories were Dutch, Non-Dutch Western (European, North American, and Oceanian); Turkish and Moroccan; African (Cape Verdean, other African, Surinamese-Creole, and Dutch Antillean); and Other (Asian, Surinamese-Hindu, Surinamese-unspecified, and South and Central American). Educational level was categorized into primary or low, secondary, and higher (Voorburg/Heerlen, 2004b). Household income in pregnancy was categorized into less than €1200, €1200 to €2000, and more than €2000 per month. Maternal smoking and alcohol use in pregnancy were assessed in each trimester of pregnancy. Maternal smoking was categorized into 'never smoked in pregnancy', 'smoked until pregnancy was known', and 'continued to smoke in pregnancy' (Roza et al., 2007). Maternal alcohol use was categorized into 'never drank in pregnancy', 'drank until pregnancy was known', 'continued to drink in pregnancy occasionally', and 'continued to drink in pregnancy frequently (defined as one or more glass/week for at least two trimesters)'. Child date of birth and sex were obtained from medical record at birth. Season of blood sampling was recorded at the moment of blood sampling of 25(OH)D.

2.6 Statistical analysis

Information on maternal or child characteristics were presented as mean (standard error) or median (95% range) for continuous variables and number (percentage) for categorical variables. First, 25(OH)D concentration was studied as a continuous variable. Second, 25(OH)D concentration was categorized to 'deficient', 'insufficient' and 'sufficient' groups using the cut-off of 25 nmol/L and 50 nmol/L (Garcia et al., 2017; Osteoporosis, 2003; Vinkhuyzen et al., 2018); the 'sufficient' group was set as the reference. For a region of interest (ROI) approach, we used multiple linear regression to examine the association of 25(OH)D status in mid-gestation and at delivery with child brain volumetric measures, including total brain volume, cerebral gray matter volume, cerebral white matter volume, and cerebellar volume. Additionally, we associated 25(OH)D status across the two assessments with these measures to investigate whether exposure to consistently low 25(OH)D levels from mid-gestation to delivery was related to brain volumes in children. In a supplementary analysis, we also examined whether children exposed to low 25(OH)D at one assessment and sufficient 25(OH)D at the other assessment had different brain volumes than those with sufficient 25(OH)D levels as assessed in maternal blood in mid-gestation and in umbilical cord blood. We also investigated 25(OH)D concentration in relation to volumes of subcortical structures (i.e., the thalamus, amygdala, hippocampus, putamen, pallidum, caudate, and accumbens) and the lateral ventricles in secondary analyses. For an exploratory surface-based brain analysis we used linear regression run in a custom-in-house package ('QdecR', http://github.com/slamballais/QDECR) at each cortical vertex to examine the association of gestational 25(OH)D concentration with cortical thickness, surface area, and gyrification. In the surface-based models for single vitamin D assessment, 25(OH)D concentration was introduced as a continuous variable only. Regression analyses were run in two models. The first analyses (Model 1) were adjusted for age at the neuroimaging assessment and

sex of the child. In a second step, we further adjusted for other potential confounders (Model 2). In particular, we adjusted for season of blood sampling in mid-gestation and season of blood sampling at delivery simultaneously in the fully adjusted model (Model 2) when investigating 25(OH)D levels across the two assessments in relation to brain morphology.

We performed two sensitivity analyses to test the robustness of the primary analyses. First, information on 25(OH)D concentration and covariates between the participants and non-participants was compared to apply inverse probability weighting (IPW). This approach addresses selection bias and helps obtain results more representative for the initial population (Forns et al., 2018; Nohr and Liew, 2018). Inverse probability weights were calculated with logistic regression. Second, using information from genomic components (Medina-Gomez et al., 2015), we re-ran analyses including only the 1092 children of European ancestry to eliminate effect modification by ethnicity due to genetic or dietary variations.

Missing covariate data (proportions ranging from 1.5% to 15.1%) were accounted for by multiple imputation with the 'Mice' package (missing at random indicated by Little's test) (van Buuren and Groothuis-Oudshoorn, 2011). A total of 10 imputed datasets were generated with 10 iterations. Only pooled results are reported. Statistical significance was set as $\alpha < 0.05$ (2-sided). Furthermore, a false discovery rate (FDR) correction was applied to the two primary and two secondary analyses separately to minimize false positive findings due to multiple testing (Benjamini and Hochberg, 1995). For the surface-based brain analyses, correction for multiple testing was performed using built-in Gaussian Monte Carlo Simulations (Hagler et al., 2006). Cluster-wise p-values were Bonferroni-corrected for two hemispheres ($p<0.025$), and a cluster forming threshold (CFT) of $p=0.001$ was selected for significance testing because it has shown high correspondence with actual permutation testing at the smoothing kernels used (Greve and

Fischl, 2018; Muetzel et al., 2019). All analyses were run using the R statistical software (version 3.5.1).

3. Results

3.1 Descriptive statistics

Table 1 shows the demographic information of the study population. Children (49.5% boys) were scanned at an average age of 10.1 years. Over half of them (57.3%) were of Dutch national origin. The median 25(OH)D concentration in mid-pregnancy was 53.8 nmol/L, and the median 25(OH)D concentration at delivery was 31.0 nmol/L. 25(OH)D concentration at delivery was significantly correlated with 25(OH)D concentration in mid-pregnancy (r=0.56).

3.2 Gestational 25(OH)D concentration and child brain volumes

25(OH)D concentration in mid-gestation was positively associated with child total brain volume at 10 years [b=0.49, representing 0.49 cm^3 difference (larger) in total brain volume per 1 nmol/L increase of 25(OH)D concentration, 95% CI 0.37 to 0.61, p<0.001] in Model 1. However, after adjusting for the additional covariates, this association did not remain (b=0.06, 95% CI -0.08 to 0.21, p=0.39). Higher 25(OH)D concentration in mid-pregnancy was also associated with larger volumes of cerebral gray matter, cerebral white matter, and the cerebellum in children when correcting for age at the neuroimaging assessment and sex, but only the association with cerebellar volume remained after adjustment, as shown in Table 2. However, this association did not survive after correcting for multiple testing. Likewise, 25(OH)D concentration at delivery was associated with child total brain volumes (b=0.73, 95% CI 0.52 to 0.94, p<0.001) in Model 1, but not in Model 2 (b=0.06, 95% CI -0.20 to 0.32, p=0.65). A similar pattern was observed for

the associations of 25(OH)D concentration at delivery with volumes of cerebral gray and white matter, and cerebellar volume.

Next we tested the associations of categories of 25(OH)D levels with brain volumetric measures. There was no evidence suggesting brain volume differences in children exposed to 'deficient' or 'insufficient' 25(OH)D concentration in gestation compared to those exposed to 'sufficient' 25(OH)D concentration after taking into account covariates and multiple testing.

Of the 1536 children with 25(OH)D concentration data at both assessments, using the same cut-offs as above, 230 were defined as exposed to 'consistently deficient' 25(OH)D concentration. Likewise, 168 were determined as 'consistently insufficient' and 291 were determined as 'consistently sufficient'. Table 3 shows the adjusted and unadjusted results, here we focus on the adjusted results only. Children exposed in utero to 'consistently insufficient' 25(OH)D concentration had a smaller total brain volume (b=-18.20, 95% CI -35.81 to -0.59, p=0.04) and a smaller cerebral gray matter volume than the reference group [i.e., the 291 children exposed to 'consistently sufficient' 25(OH)D concentration from mid-gestation to delivery]. Also children exposed to 'consistently deficient' 25(OH)D concentration showed a smaller total brain volume (b=-36.47, 95% CI -62.83 to -10.12, p=0.007), and smaller cerebral gray and white matter volumes at 10 years of age than the reference group. After FDR correction most association remained, only the difference in total brain volume between the 'consistently insufficient' group and the reference group disappeared. No association was found between 25(OH)D status from mid-gestation to delivery and cerebellar volume. In addition, no brain volumetric differences were observed between children exposed to low 25(OH)D at one assessment only (n=606) and those with 'consistently sufficient' 25(OH)D concentration (data not shown).

Figure 1 shows the results of the subcortical structures. After adjusting for covariates, although a marginal positive association between 25(OH)D concentration in mid-gestation and the volume of the pallidum was observed, gestational 25(OH)D concentration was not associated with the volume of any subcortical structures in children after correcting for multiple testing. We found no association between gestational 25(OH)D concentration and lateral ventricle volume. 25(OH)D status from mid-gestation to delivery was not associated with the volume of the subcortical structures or the lateral ventricle (data not shown).

3.3 Gestational 25(OH)D concentration and child surface-based brain morphometry

In the analyses only adjusted for child age at the neuroimaging assessment and sex, 25(OH)D concentration in mid-gestation and at delivery were associated with widespread differences in cortical thickness, surface area, and gyrification in both hemispheres. However, after full adjustment for confounding variables, no association remained. Compared to children exposed to 'consistently sufficient' 25 (OH)D concentration from mid-gestation to delivery, those exposed to 'consistently insufficient' 25 (OH)D concentration showed smaller surface area in the temporal region in the right hemisphere, and those exposed to 'consistently deficient' 25(OH)D concentration showed smaller surface area in the frontal and occipital region in the right hemisphere, as well as less gyrification in the temporal region in the left hemisphere after adjustment for all covariates (Figure 2).

3.4 Sensitivity analyses

As shown in Table S1 and Table S2, results from inverse probability weighted regression were generally consistent with the main analyses. Table S3 and Table S4 demonstrate that there were no associations between gestational 25(OH)D concentration and brain volumes at 10 years in

children of European ancestry. However, the sample sizes of these analyses were considerably smaller.

4. Discussion

In this population-based study, we found that exposure to persistently low vitamin D levels was associated with a smaller brain (specially less cerebral gray and white matter volumes) and differed surface-based cortical metrics such as surface area and gyrification in children, using repeated assessment of 25(OH)D concentration from mid-gestation to delivery. Also there was a positive association between mid-gestational 25(OH)D concentration and offspring cerebellar volume, but this did not survive multiple comparison correction.

Research on gestational vitamin D status and brain morphology is scarce. In animal studies, rats born to vitamin D_3-deficient mothers showed smaller cortical volumes and larger lateral ventricle volumes than controls (Eyles et al., 2003; Feron et al., 2005). Similarly, in cross-sectional human studies, low vitamin D concentrations have been associated with a smaller total brain volume and a larger lateral ventricle volume in adults and the elderly, though these findings are inconsistent (Annweiler et al., 2012; Annweiler et al., 2013; Zivadinov et al., 2013). In our study, we found an association between gestational 25(OH)D concentration and the offspring total brain only if we studied persistent vitamin insufficiency. In another study no association between 25 (OH)D concentration and bilateral amygdala or hippocampus volume was found (Annweiler et al., 2010), which is in line with the current study. However, these studies are not comparable in terms of study design and subjects. Further, one study indicated that mothers with lower 25(OH)D concentration during pregnancy had offspring with smaller head circumference (as a marker for brain development) from the second trimester until birth (Miliku et al., 2016), while in a recent study no association was found between gestational 25(OH)D concentration and infant head

circumference at the age of 6 or 12 months (Hauta-Alus et al., 2019). Several explanations for the discrepancy with previous findings must be discussed.

First, as an important neurosteriod, vitamin D has important functions in the proliferation and differentiation of neurons, calcium signaling within the brain, neurotrophic and neuroprotective actions, and may alter neurotransmission and synaptic plasticity (Cui et al., 2017; Groves et al., 2014). An emerging concept suggests that vitamin deficiency may weaken the integrity of perineuronal nets (PNNs), thereby neural-circuit function is disturbed and cognitive processes such as learning and memory are impeded (Mayne and Burne, 2019). These mechanisms may function at such a micro level that brain morphological measures are not modalities with adequate sensitivity to capture any arising differences. Interestingly, we observed an association between maternal vitamin D concentration in mid-gestation and child cerebellar volume. This association remained when accounting for selection bias in the sensitivity analysis. Recent longitudinal studies suggested that deficient maternal vitamin D status in pregnancy is associated with adverse motor and social development in children in early childhood (Darling et al., 2017; Dhamayanti et al., 2019), which may be explained by the reduction in cerebellar volume because smaller cerebellum has been associated with worse motor and cognitive performance (D'Ambrosio et al., 2017). Moreover, emerging evidence shows that cerebellum involves in the complex neural underpinnings of autism spectrum disorder (Becker and Stoodley, 2013), suggesting a potential mediating role of cerebellum in the association of gestational vitamin D status and child autistic traits (Vinkhuyzen et al., 2018). Further studies exploring such a mediation pathway are warranted.

Second, it is feasible that more prolonged exposure to vitamin D in gestation may exert a cumulative effect on child brain development, which is not evident with more transient prenatal

exposures. Exposure to persistent vitamin D deficiency from mid-gestation to delivery has been associated with more severe autism-related traits in our previous study (Vinkhuyzen et al., 2018). Our analysis suggests that exposure to persistently deficient or insufficient 25(OH)D concentration from mid-gestation onwards is also associated with smaller brain volumes, which may be accounted for by reduced regional surface area rather than cortical thickness. Difference in brain volumes were reported in children with and without ASD at 10 years (Lange et al., 2015), but such unspecific neurological findings can also be indicative of a higher risk for other child problems such as early onset schizophrenia (Arango et al., 2012). In contrast to our finding from the mid-gestational assessment, these results suggest that the cerebrum and not the cerebellum is most sensitive to vitamin D, and that multiple assessments are needed to reliably identify vitamin deficiency. In addition, these findings were not found when we analyzed only European children. This could possibly be explained by a reduced ability to detect small differences in far fewer subjects (in particular the 'consistently deficient' group), but also an effect modification by ethnicity. Possibly, more pigmented children in the Netherlands are affected more by persistently low gestational vitamin D status (partly this could simply reflect less misclassification). Besides, interestingly, children exposed to persistent vitamin D deficiency and those with more autistic traits both showed decreased gyrification in the frontal, superior temporal and inferior parietal cortices in the left hemisphere when the same covariates were adjusted for (Blanken et al., 2015), suggesting that gyrification in these regions may play a role in the relationship between gestational vitamin D deficiency and autistic traits in childhood.

Third, gestational vitamin D concentration at both assessments was significantly associated with brain volumes and cortical metrics when only child age at the neuroimaging assessment and sex were adjusted for, while no significances remained when adjusting for all covariates. It has been

suggested that education and income are both important indicators of family social economic status (SES) that relates to child development (Ahmadi Doulabi et al., 2017). Moreover, SES has been suggested as a determinant of smoking, alcohol use and vitamin supplement use in pregnant women (Najman et al., 1998; Skagerström et al., 2011; Sullivan et al., 2009).Therefore it is possible that less rigorous control for confounding explains some of the previous findings.

The strengths of our study were the longitudinal study design to examine the temporal association of gestational vitamin D exposure with brain morphology, the inclusion of large sample size enabling us to detect small effects, and the implementation of IPW in the sensitivity analyses to reduce selection bias. Several limitations, however, should also be mentioned. First, vitamin D concentration in blood fluctuates with diet and sun exposure, and the half-life of vitamin D is relatively short, thus the assessed values at a specific time point may not be substantially representative for the average level over the target period. Additionally, as previously reported (Wegienka et al., 2016), vitamin D concentration measured in child cord blood was significantly lower than that of the mother in mid-gestation and no specific cut-offs have been established. Second, we measured 25(OH)D concentration only in mid-gestation and at delivery. The period of early pregnancy, which can be critical in terms of brain development, could not be investigated. Also, the possible influence of child 25(OH)D level at 10 years cannot be ruled out. Third, child brain morphology was only assessed once in preadolescence, thus whether any observed association is transient or lasting, and whether gestational vitamin D concentration is associated with brain morphology in other developmental phases cannot be determined.

To the best of our knowledge, this is the first longitudinal study to investigate the association between gestational vitamin D status and brain morphological development in children. We

found limited evidence for associations between gestational vitamin D level at single assessments and child brain morphology at 10 years, but observed differed brain volumes and cortical morphometry in children exposed to persistently low vitamin D levels from mid-gestation to delivery. Further studies are needed to ascertain the possible alterations in cerebellar volume and the more generalized gray and white matter changes, and explore how these findings are related to child neurodevelopment.

Disclosure

The authors report no conflicts of interest in this work.

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Table 1. Demographics of participants (n=2597)

Maternal characteristics	
Ethnicity, N (%)	
Dutch	1489 (57.3)
Non-Dutch Western	208 (8.0)
Turkish and Moroccan	307 (11.8)
African	278 (10.7)
Other	315 (12.1)
Age at intake, mean (SD), years	30.8 (4.8)
Marital status (with partner), N (%)	2292 (88.3)
Education level, N (%)	
Primary or low	189 (7.3)
Secondary	1084 (41.7)
Higher	1324 (51.0)
Household income per month, N (%)	
< €1200	429 (16.5)
€1200-2000	455 (17.5)
> €2000	1713 (66.0)
Smoking, N (%)	
Never smoked in pregnancy	1983 (76.4)
Smoked until pregnancy was known	243 (9.4)
Continued to smoke in pregnancy	371 (14.3)
Alcohol use, N (%)	
Never drank in pregnancy	1046 (40.3)
Drank until pregnancy was known	366 (14.1)
Continued to drink in pregnancy occasionally	958 (36.9)
Continued to drink in pregnancy frequently	227 (8.7)
Vitamin supplement use in pregnancy (Yes), N (%)	883 (34.0)
25(OH)D concentration (n=2427), median (95% range), nmol/L	53.8 (11.2, 110.7)
Season of blood sampling (n=2427), N (%)	
Spring	669 (27.6)
Summer	468 (19.3)
Autumn	621 (25.6)
Winter	669 (27.6)
Child characteristics	
Age at the neuroimaging assessment, mean (SD), years	10.1 (0.6)
Gender (boys), N (%)	1286 (49.5)
25(OH)D concentration in cold blood (n=1706), median (95% range), nmol/L	31.0 (7.3, 72.5)
Season of cord blood sampling (n=1706), N (%)	
Spring	500 (29.3)
Summer	496 (29.1)
Autumn	317 (18.6)
Winter	393 (23.0)

Imputed data were shown (except for 25(OH)D concentration and season of blood sample collection).

Table 2. Gestational 25(OH)D concentration and child brain volume at 10 years

25(OH)D concentration in mid-gestation (N=2427)	Cerebral Gray Matter (cm^3)			Cerebral White Matter (cm^3)			Cerebellar Volume (cm^3)		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Model 1									
Continuous	0.27	0.20, 0.33	<0.001	0.16	0.10, 0.21	<0.001	0.06	0.05, 0.08	<0.001
Categorical									
Sufficient (n=1316)	reference	-	-	reference	-	-	reference	-	-
Insufficient (n=630)	-9.99	-14.66, -5.31	<0.001	-6.08	-10.11, -2.06	0.003	-2.64	-3.77, -1.51	<0.001
Deficient (n=481)	-23.58	-28.71, -18.45	<0.001	-15.67	-20.09, -11.26	<0.001	-5.07	-6.31, -3.82	<0.001
Model 2									
Continuous	0.02	-0.06, 0.10	0.65	0.03	-0.04, 0.10	0.45	0.02	0.001, 0.04	0.04
Categorical									
Sufficient (n=1316)	reference	-	-	reference	-	-	reference	-	-
Insufficient (n=630)	-1.35	-6.17, 3.48	0.58	-1.97	-6.22, 2.28	0.36	-1.11	-2.31, 0.08	0.07
Deficient (n=481)	-3.24	-9.81, 3.34	0.33	-5.98	-11.72, -0.23	0.04	-1.34	-2.95, 0.27	0.10
25(OH)D concentration at delivery (N=1706)	**Cerebral Gray Matter (cm^3)**			**Cerebral White Matter (cm^3)**			**Cerebellar Volume (cm^3)**		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Model 1									
Continuous	0.39	0.28, 0.50	<0.001	0.24	0.14, 0.33	<0.001	0.10	0.08, 0.13	<0.001
Categorical									
Sufficient (n=376)	reference	-	-	reference	-	-	reference	-	-
Insufficient (n=656)	-9.88	-16.15, -3.60	0.002	-8.98	-14.33, -3.63	0.001	-2.10	-3.59, -0.61	0.006
Deficient (n=674)	-22.78	-29.03, -16.54	<0.001	-14.98	-20.31, -9.66	<0.001	-5.64	-7.12, -4.16	<0.001
Model 2									
Continuous	0.01	-0.12, 0.15	0.83	0.02	-0.10, 0.14	0.79	0.03	-0.004, 0.06	0.08
Categorical									
Sufficient (n=376)	reference	-	-	reference	-	-	reference	-	-
Insufficient (n=656)	-3.95	-10.27, 2.38	0.22	-5.35	-10.87, 0.17	0.06	-0.96	-2.49, 0.56	0.22
Deficient (n=674)	-3.24	-10.88, 4.39	0.40	-3.65	-10.31, 3.00	0.28	-1.75	-3.59, 0.09	0.06

Model 1 was adjusted for child age at time of the neuroimaging assessment and sex; Model 2 was additionally adjusted for maternal ethnicity, marital status, education, age at intake, household income, smoking and alcohol use in pregnancy, vitamin supplement use in pregnancy, and season of blood sampling. Deficient is 25(OH)D concentration < 25 nmol/L; insufficient is 25(OH)D concentration 25 to < 50 nmol/L; sufficient is 25(OH)D concentration ≥ 50 nmol/L.

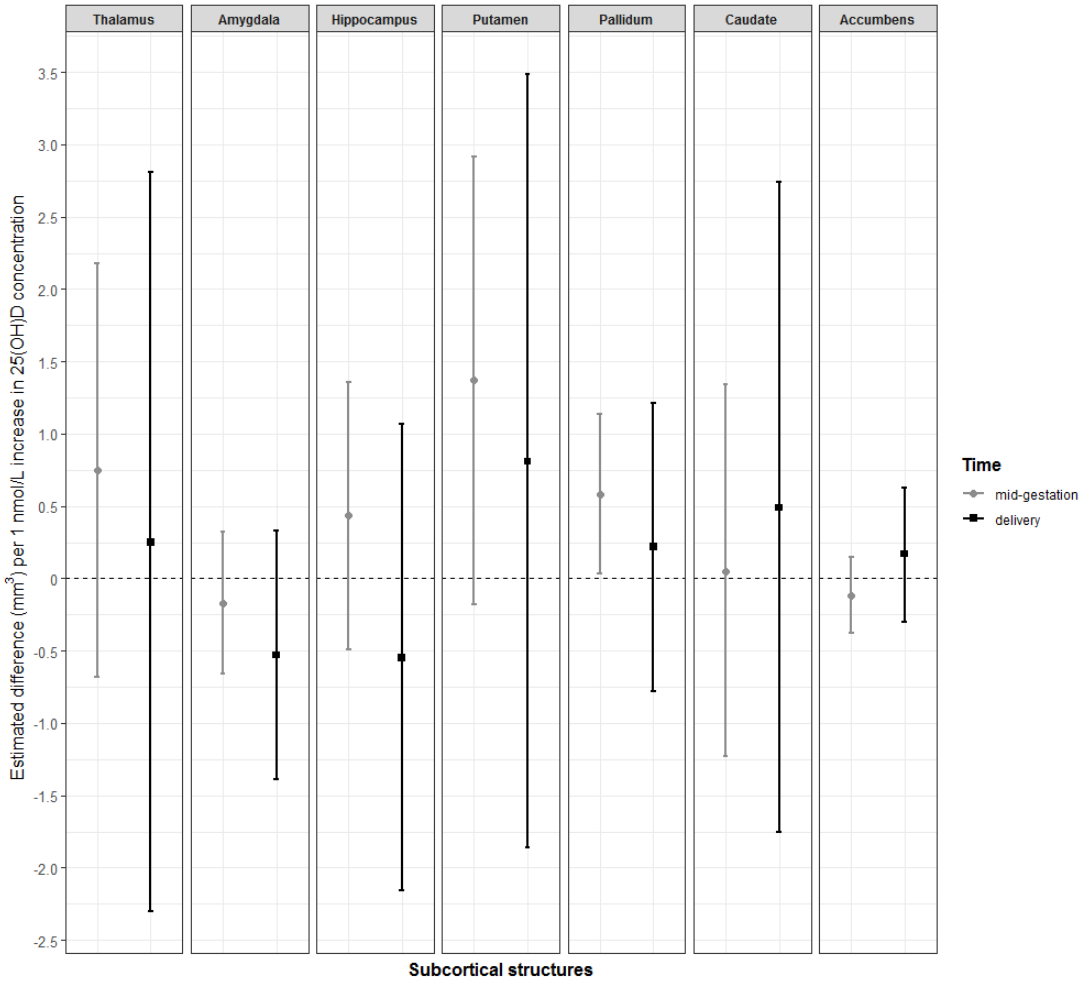
Table 3. 25(OH)D status from mid-gestation to delivery in relation to child brain volume at 10 years (n=689)

25(OH)D status from mid-gestation to delivery	Cerebral Gray Matter (cm^3)			Cerebral White Matter (cm^3)			Cerebellar Volume (cm^3)		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Model 1									
Consistently sufficient	reference	-	-	reference	-	-	reference	-	-
Consistently insufficient	-10.73	-19.69, -1.76	0.02	-10.47	-18.25, -2.68	0.008	-2.17	-4.32, -0.02	0.05
Consistently deficient	-29.48	-37.64, -21.32	<0.001	-18.53	-25.61, -11.44	<0.001	-7.24	-9.20, -5.28	<0.001
Model 2									
Consistently sufficient	reference	-	-	reference	-	-	reference	-	-
Consistently insufficient	-7.02	-16.42, 2.38	0.14	-10.09	-18.43, -1.75	0.02[a]	-1.09	-3.38, 1.21	0.35
Consistently deficient	-18.77	-32.90, -4.64	0.009[a]	-14.77	-27.23, -2.32	0.02[a]	-2.84	-6.25, 0.56	0.10

Model 1 was adjusted for child age at time of the neuroimaging assessment and sex; Model 2 was additionally adjusted for maternal ethnicity, marital status, education, age at intake, household income, smoking and alcohol use in pregnancy, vitamin supplement use in pregnancy, season of blood sampling in mid-gestation and season of blood sampling at delivery; n =230 for 'consistently deficient' group; n=168 for 'consistently insufficient' group; and n=291 for 'consistently sufficient' group. Deficient is 25(OH)D concentration < 25 nmol/L; insufficient is 25(OH)D concentration 25 to < 50 nmol/L; sufficient is 25(OH)D concentration $\geqslant$ 50 nmol/L.

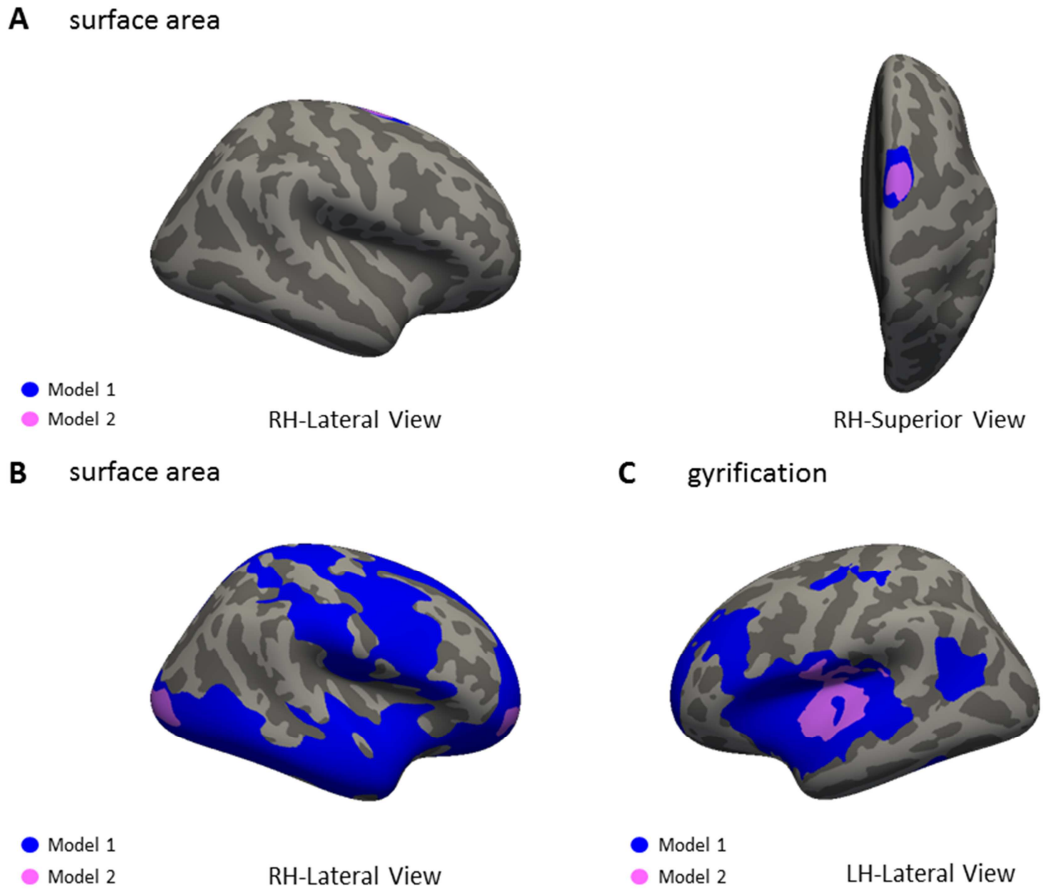
a These p-values survived FDR correction for multiple testing.

Figure 1. Gestational 25(OH)D concentration and volume of subcortical structures in children at 10 years



Models were adjusted for child age at time of the neuroimaging assessment and sex, maternal ethnicity, age at intake, marital status, education, smoking and alcohol use in pregnancy, vitamin supplement use in pregnancy, household income, season of blood sampling, and intracranial volume. n=2427 for 25(OH)D concentration in mid-gestation, and n=1706 for 25(OH)D concentration at delivery.

Figure 2. 25(OH)D status from mid-gestation to delivery in relation to surface-based cortical metrics in children at 10 years



Compared to children with 'consistently sufficient' 25(OH)D concentration (as assessed in maternal blood in mid-gestation and in umbilical cord blood, n=288), those exposed to 'consistently insufficient' 25 (OH)D concentration (n=165) had significantly smaller surface area in the colored regions (section A); those exposed to 'consistently deficient' 25(OH)D concentration (n=229) showed significantly smaller surface area and less gyrification in the colored regions (section B, C). These associations remained if corrected for multiple comparisons. Model 1 was adjusted for child age at time of the neuroimaging assessment and sex; Model 2 was additionally adjusted for maternal ethnicity, marital status, education, age at intake, household income, smoking and alcohol use in pregnancy, vitamin supplement use in pregnancy, season of blood sampling in mid-gestation and season of blood sampling at delivery. Deficient is 25(OH)D concentration < 25 nmol/L; insufficient is 25(OH)D concentration 25 to < 50 nmol/L; sufficient is 25(OH)D concentration ≥ 50 nmol/L. LH=left hemisphere; RH=right hemisphere.