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Original Article

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Circulating levels of maternal vitamin D and risk of ADHD in offspring: results from the Vitamin D Antenatal Asthma Reduction Trial

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Abstract

Background: Low levels of circulating 25-hydroxy-vitamin D [25(OH)D] have been shown to associate with prevalent attention-deficit/hyperactivity disorder (ADHD), but few studies have examined the association between 25(OH)D during fetal development and risk of childhood ADHD.

Methods: Maternal plasma 25(OH)D was measured at 10–18 and 32–38 weeks of gestation, with sufficiency defined as 25(OH)D $\geq$ 30 ng/ml. Offspring ADHD status between ages 6–9 years was measured by parent report of clinical ADHD diagnosis among 680 mother-child pairs from the Vitamin D Antenatal Asthma Reduction Trial. Association between maternal 25(OH)D and child ADHD was assessed using logistic regression, adjusting for maternal age, race and ethnicity. Effect modification by offspring sex was also assessed.

Results: No associations between maternal 25(OH)D at 10–18 weeks of gestation and offspring ADHD were observed. In the third trimester, we observed associations between maternal vitamin D sufficiency and offspring ADHD [odds ratio (OR) 0.47, 95% confidence interval (CI) 0.26–0.84], in addition to maternal 25(OH)D sufficiency category, comparing the deficient (OR 0.34, 95% CI 0.12–0.94), insufficient (OR 0.41, 95% CI 0.15–1.10) and sufficient (OR 0.20, 95% CI 0.08–0.54) categories against highly deficient 25(OH)D, respectively. Stratified analyses revealed a protective association for sufficient maternal 25(OH)D and child ADHD among males (OR 0.47, 95% CI 0.23–0.94); the synergy index for additive effect modification of risk was 1.78 (95% CI 0.62–5.08).

Conclusions: Higher levels of maternal vitamin D in the third trimester are associated with lower risk of ADHD in offspring, with modest evidence for a stronger effect among male offspring. However, larger studies will be necessary to confirm these findings.

Key words: Vitamin D, ADHD, pregnancy, 25-hydroxy-vitamin D, sufficiency

Key Messages

- Sufficient levels of maternal vitamin D during pregnancy are associated with clinically meaningful reductions in risk of offspring attention-deficit/hyperactivity disorder (ADHD).
- A slightly stronger protective association between higher levels of maternal vitamin D and child ADHD risk was observed among male offspring than female, but requires validation with larger studies.
- These associations were identified in the third trimester, but not the first; the third trimester may reflect a sensitive period for intervening to reduce offspring ADHD risk.

Introduction

Vitamin D deficiency is highly prevalent worldwide, and represents a major public health concern.[1,2] Acting as both a nutrient and a hormone, vitamin D has been found to play a critical role in neurodevelopment across sensitive periods *in utero*, infancy and early childhood.[3] Given the accumulating evidence from epidemiological studies supporting associations between maternal vitamin D deficiency during pregnancy and decreased offspring cognitive, motor and behavioural outcomes,[4] the global prevalence of vitamin D deficiency of 67% among pregnant women is troublingly high.[5]

Among neurodevelopmental and behavioural disorders in early life, attention-deficit/hyperactivity disorder (ADHD) is the most common among children worldwide.[6] ADHD manifests in two primary clinical domains, affecting either sustained attention and executive function, or hyperactivity/impulsivity or a combination of both. Low levels of circulating 25-hydroxy-vitamin D [25(OH)D] have been shown to associate with prevalent ADHD,[7,8] but few studies have investigated the role of maternal 25(OH)D levels during pregnancy, a period during which vitamin D plays an essential role in neurodevelopment and when fetal development is highly sensitive to insult.[3,9] Among the limited number of previous investigations to date, findings have been largely inconclusive, likely due in part to small sample sizes and the use of ADHD-like characteristics (e.g. via teacher/parent report)[10] rather than directly diagnosed ADHD.[11–14] However, a more recent, large case-control study in a Finnish Biobank reported inverse associations between maternal 25(OH)D levels in early pregnancy and offspring ADHD, operationalized via the presence of at least one ICD-10 code for hyperkinetic disorders.[15] The timing of gestational 25(OH)D measurements has also varied by study; most have assessed maternal 25(OH)D in the first and second trimesters, though two studies obtained 25(OH)D measures in either the third trimester or at delivery.[11,12] Importantly, none have surveyed 25(OH)D at multiple stages of pregnancy within the same group of women.

In this study, we sought to: (i) determine the association between maternal vitamin D levels in the first and third trimesters of pregnancy and the risk of offspring ADHD by age 6 years or later; and (ii) to identify potential sensitive periods *in utero* during which vitamin D levels might be most important for reducing risk of ADHD. Thus, we conducted an ancillary study to investigate these associations in a diverse sample of mothers participating in the Vitamin D Antenatal Asthma Reduction Trial (VDAART).

Methods

Study sample

The VDAART was a randomized, double-blinded, multicentre, clinical trial in which 876 participating mothers were recruited between 10–18 weeks of gestation and assigned to receive either 4400 or 400 IU/day of vitamin D throughout pregnancy. VDAART was designed to investigate the effect of prenatal vitamin D supplementation on risk of childhood asthma in the offspring, and has been described previously.[16] Briefly, enrolment occurred between October 2009 and July 2011 at three clinical sites (Boston, San Diego and St Louis) among women aged 18–39 years with a history of asthma, eczema or allergic rhinitis, or whose partner (biological father of child) had a history of the aforementioned conditions. Follow-up of the children, via annual questionnaires and clinical visits, is still ongoing.

All mother-child pairs from VDAART where maternal vitamin D levels during pregnancy and child ADHD status between ages 6–9 were measured and available were eligible for inclusion in the study. The final analytical sample

of 680 unique mother-child pairs (676 mother-child pairs at 10–18 weeks and 651 mother-child pairs at 32–38 weeks) in this study was highly representative of the parent study participants. A flow diagram of the study sample selection for these analyses can be seen in Figure 1.

This study is a secondary analysis on the data from the VDAART. The protocols for VDAART were approved by the institutional review boards at each participating institution and the Brigham and Women's Hospital. Clinical trial registry information for VDAART are as follows: ClinicalTrials.gov identifier: NCT00920621; URL: [https://clinicaltrials.gov/ct2/show/NCT00920621]. All women provided written informed consent.

Collection of maternal and child covariates

Measures of circulating level of 25(OH)D were assessed in maternal plasma samples taken before randomization at 10–18 weeks (baseline) and post-randomization at 32–38 weeks (third trimester) of gestation using the DiaSorin Liaison (DiaSorin) chemiluminescence immunoassay.[16] Maternal vitamin D levels were classified with respect to: (i) sufficiency, defined as 25(OH)D $\geq$ 30 ng/mL; and (ii) sufficiency category, using previously established clinical cut-points as follows: highly deficient, 25(OH)D $<$12 ng/ml; deficient, 12 ng/mL to 19.9 ng/mL; insufficient, 20 ng/mL to 29.9 ng/mL; and sufficient, $\geq$30 ng/mL.[15–17] Offspring ADHD status, a secondary endpoint in the VDAART study, was assessed from annual surveys via parental report of clinical diagnosis of ADHD between ages 6–9 years. The following potential confounding variables for these analyses were identified a priori based on existing literature: clinical site, and maternal characteristics [age, race (White, Black, or other) and ethnicity (Hispanic or not Hispanic)].

Statistical analysis

We conducted logistic regressions to investigate the association between maternal vitamin D levels in pregnancy and offspring ADHD. Minimally adjusted models for vitamin D at 10–18 weeks of gestation were univariate, and third trimester models further adjusted for clinical site to account for conditional randomization in the clinical trial design. Fully adjusted models for both the baseline and third trimester included their respective minimally adjusted model covariates, as well as maternal age, race and ethnicity; the mechanistic assumptions underlying the selection of these covariates for potential confounding control is depicted in the directed acyclic graph (DAG) in Figure 2. We note that we did not adjust for treatment assignment

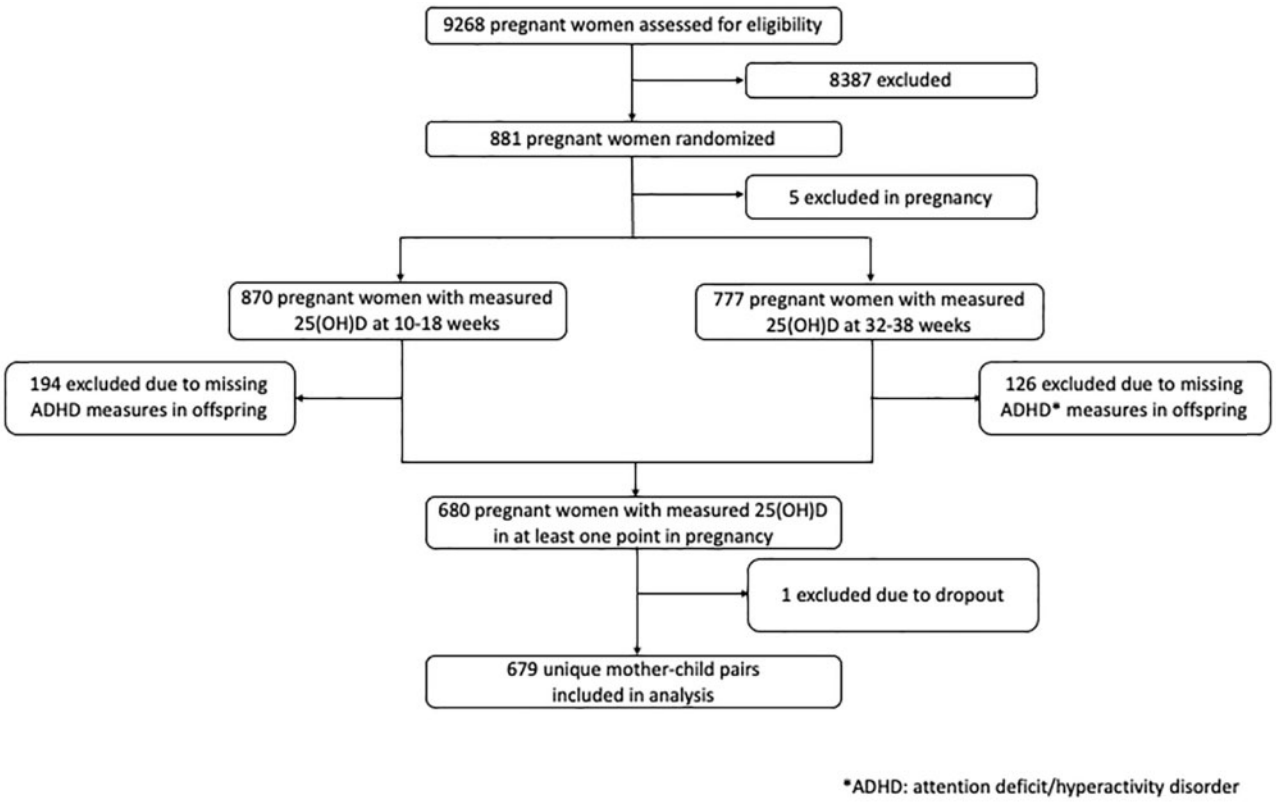

Figure 1 Overview of the nested study sample from the Vitamin D Antenatal Asthma Reduction Trial

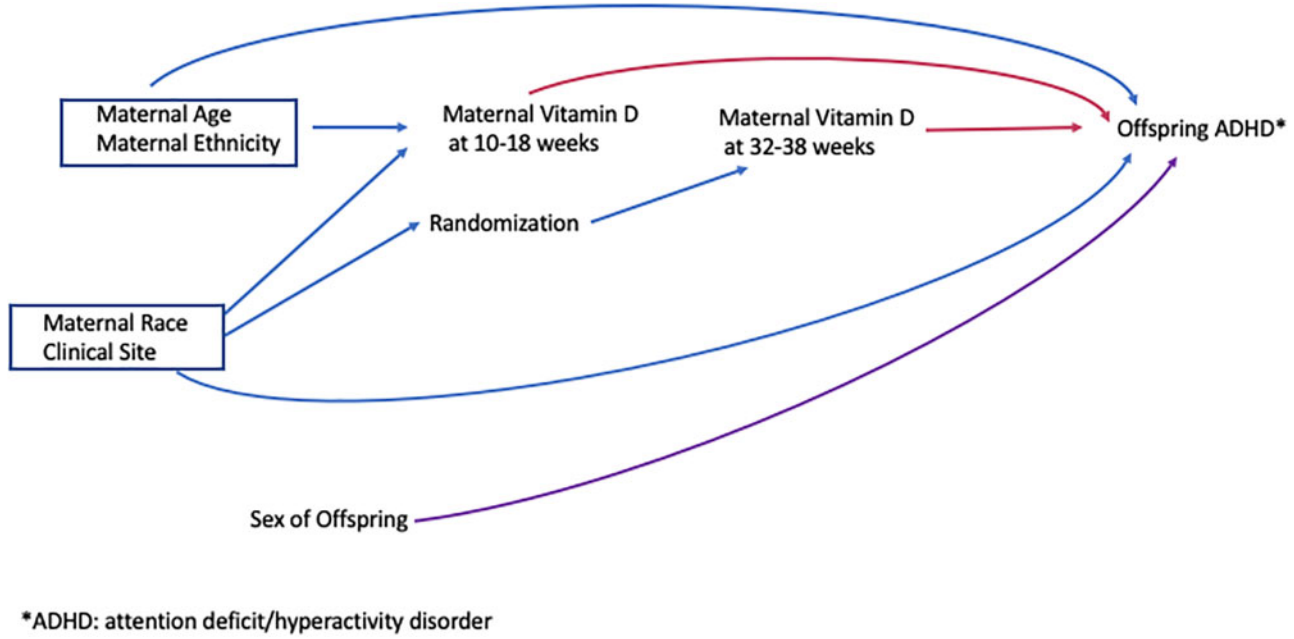

Figure 2 Underlying directed acyclic graph of the assumed causal structures relevant for testing the association between maternal vitamin D and child attention-deficit/hyperactivity disorder in the Vitamin D Antenatal Asthma Reduction Trial. Red arrows denote the associations of interest, blue boxes represent adjustment covariates and the purple arrow denotes assumed effect modification

because it did not meet the definition of a confounder, i.e. as a common cause of the exposure and outcome. We reasoned that there was no plausible biological mechanism by which randomization to treatment at baseline could affect offspring ADHD risk independent of its effect on vitamin D levels throughout the remainder of pregnancy. Robustness of the third trimester model specifications were assessed via sensitivity analyses further adjusting for baseline maternal 25(OH)D.

To investigate potential effect modification of association between gestational vitamin D levels and child ADHD status by sex, we conducted: (i) logistic regressions further including an interaction term between vitamin D level (binary: sufficiency vs insufficiency, or categorical vitamin D dosage level) and sex of offspring; and (ii) stratified analyses, to assess potential effect measure modification on both the multiplicative and the additive scales, respectively. Effect modification on the additive scale was estimated via the synergy index (S),[18] which can be interpreted as excess risk of an outcome given the presence of both the exposure and the effect modifier as compared with risk given presence of only the exposure. The variance for the synergy index was estimated via the delta method.[19] Because our primary hypotheses sought to determine the existence of a protective association between maternal vitamin D and child ADHD, and protective odds ratios are constrained between 0 and 1, calculation of S necessarily proceeded by recoding the reference group for maternal vitamin D to the lowest risk group (i.e. maternal vitamin D sufficient) as recommended by Knol *et al.*[20] Therefore, in the effect modification analyses, the interpretation of the synergy index is framed with respect to the excess risk of ADHD with increasing deficiency of maternal vitamin D.

Results

Study sample

In the full sample, mean maternal age was approximately 27.5 years, mean gestational age was 14.1 weeks and 77.8% of the mothers were vitamin D deficient or insufficient at baseline. There were no observable differences with respect to maternal age at enrolment, race or ethnicity, and gestational age at enrolment, after stratifying by treatment arm. Maternal race, 25(OH)D sufficiency status at 10–18 weeks of gestation, and clinical site also did not vary by treatment arm assignment; however, maternal asthma was slightly higher in the treatment group (43.7% vs 35.8%). The maternal study sample characteristics stratified by vitamin D sufficiency status at 32–38 weeks of gestation and by treatment arm can be seen in, respectively, Table 1 and Supplementary Table S1, available as Supplementary data at *IJE* online.

Vitamin D sufficiency status and ADHD

We did not identify baseline associations between 25(OH)D sufficiency and offspring ADHD status in the

Table 1 Characteristics of the Vitamin D Antenatal Asthma Reduction Trial (VDAART) participants by vitamin D supplementation treatment group

	Vitamin D supplementation group		
	400 IU/day $n = 332$	4400 IU/day $n = 348$	All $n = 680$
Maternal characteristics			
Mean age at enrolment (SD)	27.4 (5.5)	27.5 (5.4)	27.5 (5.5)
Mean gestation age at enrolment in weeks (SD)	14.1 (2.68)	14.2 (2.81)	14.1 (2.75)
Race, *n* (%)			
White	133 (40.1)	138 (39.7)	271 (39.9)
African American	147 (44.3)	149 (42.8)	296 (43.5)
Other	52 (15.7)	61 (17.5)	113 (16.6)
Ethnicity, *n* (%)			
Hispanic	83 (25.0)	89 (25.6)	172 (25.3)
1st trimester vitamin D (baseline)			
Mean ng/mL at 10–18 weeks (SD)	22.8 (10.6)	22.9 (10.3)	22.8 (10.4)
Deficient or insufficient [25(OH)D <30 ng/mL], *n* (%)	259 (78.0)	270 (77.6)	529 (77.8)
Missing, *n* (%)	1 (0.3)	3 (0.9)	4 (0.6)
3rd trimester vitamin D			
Mean ng/mL at 32–38 weeks (SD)	26.7 (10.8)	39.4 (15.0)	33.1 (14.6)
Deficient or insufficient [25(OH)D <30 ng/mL], *n* (%)	213 (64.2)	78 (22.4)	291 (42.8)
Missing, N (%)	13 (3.9)	16 (4.6)	29 (4.3)
Asthma, *n* (%)	119 (35.8)	152 (43.7)	271 (39.9)
Clinical site			
San Diego	112 (33.7)	112 (32.2)	224 (32.9)
Boston	89 (26.8)	96 (27.6)	185 (27.2)
St. Louis	131 (39.5)	140 (40.2)	271 (39.9)
Offspring characteristics			
Sex, *n* (%)			
Male	181 (54.5)	175 (50.3)	356 (52.4)
Clinical diagnosis of ADHD between ages 6–9 years, *n* (%)	33 (9.9)	29 (8.3)	62 (9.1)

IU, international unit; SD, standard deviation.

maternal samples collected at 10–18 weeks of gestation (OR 1.06, 95% CI 0.51–2.19; $P = 0.871$). However, we observed an association between maternal vitamin D sufficiency at 32–38 weeks and offspring ADHD (OR 0.47, 95% CI 0.26–0.84; $P = 0.011$) (Table 2, and Supplementary Figure S1, available as Supplementary data at *IJE* online). The interpretation of these estimates are as follows: the odds of having children with ADHD at age 6 years or later among mothers with 25(OH)D levels of ≥30 ng/mL at 32–38 weeks of gestation were 0.47 times those of mothers with 25(OH)D levels of <30 ng/mL during the same stage of pregnancy. The unadjusted and fully adjusted third trimester models were highly consistent, and demonstrated virtually no attenuation of effect estimates.

Vitamin D sufficiency category and ADHD

No associations between vitamin D sufficiency category and offspring ADHD were observed at 10–18 weeks of gestation. However, analyses of vitamin D concentration at 32–28 weeks of gestation revealed a protective association between higher prenatal 25(OH)D and reduced risk of offspring ADHD, and the unadjusted Cochran-Armitage test yielded evidence to support a linear trend ($P = 2.6 \times 10^{-4}$) (Table 2; Supplementary Figure S1). The fully adjusted analyses also identified protective associations, comparing the deficient (OR 0.34, 95% CI 0.12–0.94; $P = 0.039$), the insufficient (OR 0.41, 95% CI 0.15–1.10; $P = 0.076$) and the sufficient (OR 0.20, 95% CI 0.08–0.54; $P = 0.001$) categories against the highly deficient vitamin D category, respectively. Minimally adjusted models produced highly similar results to the adjusted analyses.

Sensitivity analyses

Sensitivity analyses further adjusting for baseline maternal vitamin D levels were also consistent with the findings reported above, but produced slightly more protective effect estimates (Supplementary Table S2, available as Supplementary data at *IJE* online). Sex-stratified analyses

Table 2 Association between maternal 25(OH)D during pregnancy and offspring attention-deficit/hyperactivity disorder status between ages 6–9 years

			Unadjusted[a]				Adjusted[b]		
	n_M	$n_{Ca/Co}$	OR	95% CI	P	P_{Trend}	OR	95% CI	P
1st trimester (10–18 weeks), $n = 670$									
Vitamin D sufficiency									
25(OH)D ≥30 ng/mL	147	12/135	0.89	(0.46–1.73)	0.731		1.06	(0.51–2.19)	0.871
Vitamin D sufficiency category						0.303			
Highly deficient, <12 ng/mL	91	10/81		Ref					
Deficient, 12–19.9 ng/mL	197	20/177	0.92	(0.41–2.04)	0.829		1.11	(0.48–2.56)	0.807
Insufficient, 20–29.9 ng/mL	241	18/223	0.65	(0.29–1.48)	0.306		0.89	(0.36–2.19)	0.797
Sufficient, ≥30 ng/mL	147	12/135	0.72	(0.30–1.74)	0.466		1.03	(0.37–2.86)	0.956
3rd trimester (32–38 weeks), $n = 646$									
Vitamin D sufficiency									
25(OH)D >30 ng/mL	360	21/339	0.48	(0.27–0.84)	**0.010**		0.47	(0.26–0.84)	**0.011**
Vitamin D sufficiency category						**2.6E–4**			
Highly deficient, <12 ng/mL	34	9/25		Ref					
Deficient, 12–19.9 ng/mL	100	10/90	0.34	(0.12–0.94)	**0.036**		0.34	(0.12–0.94)	**0.038**
Insufficient, 20–29.9 ng/mL	157	17/140	0.42	(0.17–1.08)	0.071		0.41	(0.15–1.10)	0.076
Sufficient, ≥30 ng/mL	360	21/339	0.22	(0.09–0.53)	**8.9E–4**		0.20	(0.08–0.54)	**0.001**

N_M: number of mothers with the given vitamin D status; $N_{Ca/Co}$: number of cases (ca) vs number of controls (co); P_{Trend}: P-value for unadjusted linear trend test for ordinal variables.
The bolded numbers are those P-values which met a significance threshold of P-value<0.05.
[a]Minimally adjusted models: 1st trimester models are univariate models, but 3rd trimester models were additionally adjusted for clinical site.
[b]Fully adjusted models: 1st and 3rd trimester models were equivalent to their respective Model 1, but additionally adjusted for maternal race and age.

Table 3 Effect modification of the association between sufficient maternal vitamin D and child Attention-deficit/hyperactivity disorder by sex

	Maternal vitamin D insufficient			Maternal vitamin D sufficient			OR (95% CI) for vitamin D sufficiency within strata of sex of offspring	
	25(OH)D < 30 ng/mL			25(OH)D ≥ 30 ng/mL				
Sex of offspring	$n_{Ca/Co}$	OR (95%CI)	*P*	$n_{Ca/Co}$	OR (95%CI)	*P*	OR (95% CI)	*P*
1st trimester (10–18 weeks),[a] $n = 670$								
Females	14/236	Ref	–	1/72	0.29 (0.04–2.34)	0.248	0.26 (0.03–2.28)	0.225
Males	34/245	2.32 (1.20–4.45)	**0.012**	11/63	4.81 (0.54–42.84)	0.159	1.44 (0.64–3.34)	0.380
3rd trimester (32–38 weeks),[a,b] $n = 646$								
Females	9/125	Ref	–	6/172	0.52 (0.18–1.52)	0.229	0.52 (0.17–1.60)	0.252
Males	27/130	2.78 (1.24–6.22)	**0.013**	15/167	0.89 (0.25–3.17)	0.861	0.47 (0.23–0.94)	**0.033**

Measure of effect modification on the additive scale, synergy index S (95% CI); *P*: 1st trimester: 0.549 (0.119, 2.537); P = 0.241; 3rd trimester: 1.810 (0.630, 5.196); *P* = 0.211.

$n_{Ca/Co}$: number of cases (ca) vs number of controls (co).

The bolded numbers are those *P*-values which met a significance threshold of *P*-value<0.05.

[a]Adjusted for maternal age, race and ethnicity.

[b]Also adjusted for clinical site.

for both maternal vitamin D sufficiency and sufficiency category were highly underpowered due to limited case counts in our study sample; these are reported in Supplementary Tables S3 and S4, available as Supplementary data at *IJE* online. Because the thresholds used in the current analyses are consistent with the thresholding used in previous VDAART studies of vitamin D, we also conducted sensitivity analyses applying 25(OH)D thresholds established by the National Academy of Medicine.[21] These findings of these analyses were also consistent with the results reported above (Supplementary Table S5, available as Supplementary data at *IJE* online).

Effect modification of the association between maternal vitamin D on ADHD risk by offspring sex

At 10–18 weeks of gestation, insufficient maternal vitamin D was associated with higher odds of ADHD (OR 2.32, 95% CI 1.20–4.45; $P = 0.012$) among male offspring as compared with female offspring (Table 3). There was insufficient evidence to suggest effect measure modification on the multiplicative scale (OR 4.87, 95%CI 0.55–43.38; $P = 0.156$). Sex-specific stratified analyses produced null findings for the association between maternal 25(OH)D at 10–18 weeks of gestation and child ADHD within each sex; and there was insufficient evidence to suggest additive effect modification at 10–18 weeks of gestation (S = 0.549, 95% CI 0.119–2.537; $P = 0.241$).

In the third trimester interaction analyses, insufficient maternal vitamin D was again associated with higher odds of ADHD (OR 2.78, 95% CI 1.24–6.22; $P = 0.013$) among male offspring as compared with female offspring. As in the baseline analyses at 10–18 weeks of gestation, we failed to find sufficient evidence to support effect modification on the multiplicative scale (OR 0.89, 95% CI 0.25–3.17; $P = 0.861$). However, sex-specific stratified analyses revealed a protective association for third trimester maternal 25(OH)D and child ADHD within males (OR 0.47, 95% CI 0.23–0.94; $P = 0.033$). The synergy index for effect modification on the additive scale for the third trimester analyses was 1.81 (95% CI 0.63–5.20; $P = 0.211$). The S value can be interpreted as follows: the excess estimated risk of ADHD among male offspring is 81% higher than female offspring, among those whose mothers were 25(OH)D insufficient in the third trimester.

Discussion

We identified protective associations between maternal 25(OH)D sufficiency in the third trimester and child ADHD, but not at baseline. Our lack of evidence for association between maternal vitamin D at 10–18 weeks and child ADHD

may be due in part to low observed variability in measured 25(OH)D—with 77.8% of mothers classified as vitamin D insufficient at the start of trial—and hence, power. Given our interest in identifying specific sensitive periods for 25(OH)D in pregnancy, our analyses suggest that the period between 10 and 18 of weeks gestation may not reflect a unique, developmental period affecting risk of offspring ADHD which is independent of periods later in pregnancy. Our results in the third trimester were associated with protective effects for maternal 25(OH)D, but in the randomized clinical trial context (assuming moderate to high levels of adherence), the 25(OH)D measurements at 32–38 weeks of gestation might also reflect the overall maternal vitamin D exposure levels throughout pregnancy and thus an average cumulative effect. In other words, it is possible that the joint profile of vitamin D throughout the second and third trimesters of pregnancy is the meaningful effector of ADHD risk. Although studies examining the association between maternal 25(OH)D and child ADHD are still few in number, gestational vitamin D has also been implicated in other neurodevelopmental and neuropsychiatric disorders including autism spectrum disorders and schizophrenia.[3,22,23] Additional studies will be necessary to better understand the patterns of association between maternal vitamin D and child ADHD risk.

At both baseline and in the third trimester, we observed higher odds of ADHD in male offspring as compared with female offspring with 25(OH)D insufficient mothers, but these analyses of effect modification were limited by small sample sizes and thus reduced power. Whereas much evidence exists for the biological basis of sex differences in neurocognitive, behavioural and immune phenotypes arising due to insults in early life/developmental periods,[4,24,25] it is possible that our findings may also partly reflect biases in ADHD diagnosis: male children falling into the hyperactive/impulsive domain are more likely to be diagnosed with ADHD than their primarily inattentive domain female counterparts. Whether the modest effect measure modification by sex reported in the present study is attributable to biological mechanisms versus differential clinical diagnosis cannot be directly determined from these analyses. However, in this case, our findings nonetheless may provide some insight into the associations between maternal vitamin D and ADHD which is more easily clinically detected according to domain-specific profiles, i.e. the hyperactive/impulsive domain.[26] Future studies in larger samples, ideally with more refined ADHD and molecular phenotyping, will be necessary to properly decompose these effects.

Strengths of our study include the use of a prospective randomized clinical trial of maternal vitamin D supplementation with multiple measures of circulating 25(OH)D throughout pregnancy, and the direct report of previously clinically diagnosed ADHD in children, rather than proxy measures such as parent or teacher report of ADHD-like symptoms. Importantly, VDAART was designed from the outset with maternal vitamin D levels as an exposure of interest, and the randomization of mothers to vitamin D treatment arms facilitated the homogeneous distribution of both baseline maternal vitamin D levels and crucially, also potential confounders, across the treatment arms. Leveraging this randomization, we were able to estimate the association between circulating 25(OH)D in early and late pregnancy and offspring ADHD independently, within a fixed set of mother-child pairs. Finally, our sample also includes a large proportion of minorities, including 43.4% African Americans and 24.7% identifying as of Hispanic ethnicity, which improves the generalizability of our results across multiple populations.

Limitations of our study include that while we categorized vitamin D levels using previously established clinical thresholds,[2,15,16] these cutoffs are largely artificial, as there are likely subtle gradients of effects that exist on either side of the various thresholds imposed which we were unable to identify given limited power. Although the most commonly collected biospecimen for metabolomic analyses is blood, it is also worth noting that there are likely differences in the vitamin D levels collected from circulating serum as compared with levels that prevail globally and regionally within the brain, which is the primary organ involved in the behavioural manifestation of ADHD. Confounding by non-compliance is a threat to unbiased estimation when failure to comply with assigned treatment occurs for reasons that are related to the outcome; one such variable might be maternal ADHD, which was not measured in our study. However, given the similar compliance rates in both arms (treatment group: 70%, 95% CI: 67.3–72.7%; control group: 71.3%, 95% CI: 68.6–74%) of VDAART, we anticipate that despite not directly measuring parental ADHD status, the distribution of these covariates would be roughly exchangeable after randomization, and that non-compliance due to this particular trait would also be similar between the two groups. Other forms of residual confounding may remain.

In conclusion, this study explores the association between prenatal vitamin D levels and childhood ADHD risk, leveraging the powerful, randomized, clinical trial design of VDAART, which was specifically designed to investigate the effects of maternal vitamin D supplementation throughout pregnancy on asthma risk in children. Our findings suggest that higher levels of maternal vitamin D during pregnancy may play a protective role against risk of ADHD in offspring, but further studies will be necessary to confirm this association and any therapeutic potential therein.

Supplementary Data

Supplementary data are available at *IJE* online.

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Author Contributions

S.H.C. and J.L.S. conceived of the study; A.A.L., S.T.W. and J.L.S. conducted the research; S.H.C. performed statistical analysis and wrote the paper; M.H., R.S.K., P.K., A.A.L., S.T.W., J.L.S. contributed to the statistical methodology, interpretation of the analysis results and the writing of the manuscript; S.H.C. and J.L.S. hold primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of Interest

S.T.W. and A.A.L. receive author royalties from UpToDate. S.H.C. has previously provided consulting to Verge Genomics. All other authors have declared that they have no conflict of interest .

References

1. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? *J Steroid Biochem Mol Biol* 2014;**144**(**Pt A**):138–45.
2. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;**357**: 266–81.
3. Eyles D, Burne T, McGrath J. Vitamin D in fetal brain development. *Semin Cell Dev Biol* 2011;**22**:629–36.
4. Janbek J, Specht IO, Heitmann BL. Associations between vitamin D status in pregnancy and offspring neurodevelopment: a systematic literature review. *Nutr Rev* 2019;**77**:330–49.
5. Saraf R, Morton SM, Camargo CA Jr, Grant CC. Global summary of maternal and newborn vitamin D status - a systematic review. *Matern Child Nutr* 2016;**12**:647–68.
6. Faraone SV, Asherson P, Banaschewski T *et al.* Attention-deficit/hyperactivity disorder. *Nat Rev Dis Primers* 2015;**1**:15020.
7. Kotsi E, Kotsi E, Perrea DN. Vitamin D levels in children and adolescents with attention-deficit hyperactivity disorder (ADHD): a meta-analysis. *Atten Defic Hyperact Disord* 2019;**11**:221–32.
8. Goksugur SB, Tufan AE, Semiz M *et al.* Vitamin D status in children with attention-deficit-hyperactivity disorder. *Pediatr Int* 2014;**56**:515–19.
9. Harms LR, Burne TH, Eyles DW, McGrath JJ. Vitamin D and the brain. *Best Pract Res Clin Endocrinol Metab* 2011;**25**: 657–69.
10. Daraki V, Roumeliotaki T, Koutra K *et al.* High maternal vitamin D levels in early pregnancy may protect against behavioral difficulties at preschool age: the Rhea mother-child cohort, Crete, Greece. *Eur Child Adolesc Psychiatry* 2018;**27**:79–88.
11. Strom M, Halldorsson TI, Hansen S *et al.* Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. *Ann Nutr Metab* 2014;**64**:254–61.
12. Gustafsson P, Rylander L, Lindh CH *et al.* Vitamin D status at birth and future risk of Attention Deficit/Hyperactivity Disorder (ADHD). *PLoS One* 2015;**10**:e0140164.
13. Morales E, Julvez J, Torrent M *et al.* Vitamin D in pregnancy and attention deficit hyperactivity disorder-like symptoms in childhood. *Epidemiology* 2015;**26**:458–65.
14. Lopez-Vicente M, Sunyer J, Lertxundi N *et al.* Maternal circulating Vitamin D3 levels during pregnancy and behaviour across childhood. *Sci Rep* 2019;**9**:14792.
15. Sucksdorff M, Brown AS, Chudal R *et al.* Maternal vitamin D levels and the risk of offspring attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2021;**60**: 142–51.
16. Litonjua AA, Carey VJ, Laranjo N *et al.* Effect of prenatal supplementation with vitamin D on asthma or recurrent wheezing in offspring by age 3 years: The VDAART Randomized Clinical Trial. *JAMA* 2016;**315**:362–70.
17. Hart PH, Lucas RM, Walsh JP *et al.* Vitamin D in fetal development: findings from a birth cohort study. *Pediatrics* 2015;**135**: e167–73.
18. Skrondal A. Interaction as departure from additivity in case-control studies: a cautionary note. *Am J Epidemiol* 2003;**158**: 251–58.
19. Richardson DB, Kaufman JS. Estimation of the relative excess risk due to interaction and associated confidence bounds. *Am J Epidemiol* 2009;**169**:756–60.
20. Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. *Eur J Epidemiol* 2011;**26**:433–38.
21. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academies Press (US), 2011.
22. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. *Front Neuroendocrinol* 2013;**34**:47–64.
23. Ali A, Cui X, Eyles D. Developmental vitamin D deficiency and autism: Putative pathogenic mechanisms. *J Steroid Biochem Mol Biol* 2018;**175**:108–18.
24. Pet MA, Brouwer-Brolsma EM. The impact of maternal vitamin D status on offspring brain development and function: a systematic review. *Adv Nutr* 2016;**7**:665–78.
25. Loke H, Harley V, Lee J. Biological factors underlying sex differences in neurological disorders. *Int J Biochem Cell Biol* 2015;**65**:139–50.
26. Mowlem FD, Rosenqvist MA, Martin J, Lichtenstein P, Asherson P, Larsson H. Sex differences in predicting ADHD clinical diagnosis and pharmacological treatment. *Eur Child Adolesc Psychiatry* 2019;**28**:481–89.