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Language Impairment

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# Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

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**KEY WORDS**
vitamin D, neurocognitive, language impairment, behavioral problems, emotional problems, Raine study

**ABBREVIATIONS**
95% CI—95% confidence interval
CBCL—Child Behavior Checklist
OR—odds ratio
PPVT-R—Peabody Picture Vocabulary Test—Revised

Ms Kusel and Dr Hart contributed equally to this work.

Drs Whitehouse, Kusel, and Hart developed the hypotheses; Ms Holt, Mr Serralha, Dr Holt, and Dr Hart analyzed serum samples for 25(OH)-vitamin D concentrations; and Dr Whitehouse conducted the statistical analyses and wrote the main drafts of the manuscript. All authors contributed to the interpretation and discussion of the results and other sections of the manuscript.

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**WHAT'S KNOWN ON THIS SUBJECT:** Vitamin D levels in the general population have decreased considerably over the past decade. The implications of maternal vitamin D insufficiency during pregnancy for offspring neurocognitive development remain unclear.

**WHAT THIS STUDY ADDS:** Studying a large sample and using a prospective longitudinal design, this study demonstrates a link between maternal vitamin D insufficiency during pregnancy and offspring language impairment. There was no association with childhood behavioral or emotional problems.

## abstract

**OBJECTIVE:** To determine the association between maternal serum 25(OH)-vitamin D concentrations during a critical window of fetal neurodevelopment and behavioral, emotional, and language outcomes of offspring.

**METHODS:** Serum 25(OH)-vitamin D concentrations of 743 Caucasian women in Perth, Western Australia (32°S) were measured at 18 weeks pregnancy and grouped into quartiles. Offspring behavior was measured with the Child Behavior Checklist at 2, 5, 8, 10, 14, and 17 years of age ($n$ range = 412–652). Receptive language was assessed with the Peabody Picture Vocabulary Test—Revised at ages 5 ($n$ = 534) and 10 ($n$ = 474) years. Raw scores were converted to standardized scores, incorporating cutoffs for clinically significant levels of difficulty.

**RESULTS:** $\chi^2$ analyses revealed no significant associations between maternal 25(OH)-vitamin D serum quartiles and offspring behavioral/emotional problems at any age. In contrast, there were significant linear trends between quartiles of maternal vitamin D levels and language impairment at 5 and 10 years of age. Multivariate regression analyses, incorporating a range of confounding variables, found that the risk of women with vitamin D insufficiency (≤46 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with vitamin D levels >70 nmol/L.

**CONCLUSIONS:** Maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment. Maternal vitamin D supplementation during pregnancy may reduce the risk of developmental language difficulties among their children. *Pediatrics* 2012;129:485–493

Maternal vitamin D insufficiency during pregnancy is associated with adverse health outcomes in offspring, including intrauterine growth-restriction,[1] reduced bone-mineral accrual[2] and recurrent wheeze.[3] To date, the majority of evidence for an effect of maternal vitamin D levels on fetal brain development has come from studies investigating the timing of pregnancies, which have identified an increased risk for multiple sclerosis among the offspring of pregnancies in which the first and second trimesters coincided with the winter and spring months.[4] Direct sunlight is a main source of vitamin D, and it has been hypothesized that maternal vitamin D insufficiency (most common in winter and spring) during the first and second trimesters, may underpin the association between the timing of pregnancy and offspring morbidity.[4,5] However, it is often difficult in these observational studies to investigate sunlight exposure and consequent vitamin D levels in isolation from other seasonal factors that may also influence fetal neurodevelopment, such as changes in temperature, and maternal nutrition and infection.[6]

Vitamin D from the skin and diet is metabolized in the liver to 25(OH)-vitamin D, which can then be measured in blood samples as a more direct investigation of vitamin D status.[5] Although rodent studies have linked low maternal 25(OH)-vitamin D during pregnancy with atypical behavior among pups,[7,8] only 1 study has examined this association in humans. Gale and colleagues[9] measured the circulating 25 (OH)-vitamin D levels of 466 pregnant women and reported no statistically significant association with offspring behavior and verbal IQ at 9 years of age. However, the study was limited by considerable sample attrition, with only 178 (38.2%) of the original cohort contributing data at the 9-year-old follow-up. Consequently, there were small numbers of children identified with "problem behaviors" at follow-up ($n$ range: 24–33), which limited the capacity of the study to identify statistically significant effects. Furthermore, maternal vitamin D concentrations were obtained during the third trimester of pregnancy (median: 32.6 weeks; range: 28–42 weeks), which may not reflect circulating levels earlier in gestation, when cortical structures critical to behavioral regulation and language acquisition are first formed.[10]

Given that vitamin D insufficiency is observed in up to 60% of Caucasian women[11] and that the rate among women with dark skin is estimated to be even higher,[12–14] understanding the implications for offspring neurodevelopment is of urgent importance. Here we report a large-scale longitudinal study of the association between maternal 25(OH)-vitamin D concentrations measured during the second trimester and behavioral and language development of offspring to age 17 years. Participants were from the Western Australian Pregnancy Cohort (Raine) Study, a sample of pregnant women and their offspring from Perth, Western Australia, which is at latitude 32°S. Given the relatively few number of non-Caucasian participants in the Raine Study and recent findings that ethnicity may confound both 25(OH)-vitamin D serum concentrations and cognitive test scores,[15,16] the current study investigated Caucasian mothers and their offspring only.

## METHODS

### Participants

The Western Australian Pregnancy Cohort (Raine) Study recruited pregnant women from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics in Perth (Australia) between May 1989 and November 1991 ($n$ = 2900). The inclusion criteria were a gestational age between 16 and 20 weeks, English language skills sufficient to understand the study demands, an expectation to deliver at King Edward Memorial Hospital, and an intention to remain in Western Australia to enable future follow-up of their child.[17] Participant recruitment and all follow-ups of the study families were approved by the Human Ethics Committees at King Edward Memorial Hospital and/or Princess Margaret Hospital for Children in Perth, Western Australia. Parents provided written informed consent to participate at each follow-up. The current study included only those mother-child dyads in which the mother self-identified as Caucasian and where maternal blood was collected at 18 weeks' gestation and behavioral data were available for the offspring.

### Maternal 25(OH)-Vitamin D

From 1989 to 1991, venous blood was obtained at 18 weeks' pregnancy in 929 randomly selected women, centrifuged, and serum collected and stored at −80°C. In June 2011, Serum 25(OH)-vitamin D levels were measured using an enzyme immunoassay kit from Immunodiagnostic Systems Ltd (Scottsdale, AZ). Vitamin D concentrations in stored sera have been shown to remain stable for >3 decades.[18,19] Twenty-eight samples were also measured by using isotope-dilution liquid chromatography-tandem mass spectrometry by RMIT Drug Discovery Technologies (Melbourne, Australia) according to published methodology.[20] A correlation of 25(OH)-vitamin D concentrations for samples assayed by both techniques was strong ($r^2$ = .87) and confirmed that there were no molecules (vitamin D metabolites or otherwise) in sera of 18-week pregnant women that interfered with the immunoassay of 25(OH)-vitamin D. The assay of 25(OH)-vitamin D by isotope-dilution liquid chromatography-tandem

mass spectrometry gave concentrations of 25(OH)-vitamin D that were slightly higher than those measured by immunoassay (slope 0.95 ± 0.07). Overestimation of 25(OH)-vitamin D by the former assay has recently been reported.[21] For these reasons, the 25(OH)-vitamin D levels in the serum from pregnant women have been divided into quartiles, which because of the strong correlative value was not influenced by the assay used for the measure of serum 25(OH)-vitamin D concentration.

### Behavioral Development

The Child Behavior Checklist (CBCL), an empirically validated measure of child behavior by parent report, was used to measure child and adolescent behavior. The CBCL for Ages 2–3 (CBCL/2–3)[22] was used at the 2-year follow-up, and the CBCL for Ages 4–18 (CBCL/4–18)[23] was administered at the 5-, 8-, 10-, 14-, and 17-year follow-ups. These measures contain a list behavioral/emotional problem items (CBCL/2–3: $n = 99$; CBCL/4–18: $n = 118$) that parents rate as not true (score of 0), somewhat or sometimes true (score of 1), or very or often true (score of 2) of their children. Both measures are widely used in the research literature and show good internal reliability and validity in a number of population settings.[23] A clinical calibration with Australian children demonstrated moderately high sensitivity (83% overall) and specificity (67% overall) to a clinical diagnosis, and good test-retest reliability.[24] The 3-year predictive validity of the CBCL/2–3 for CBCL/4–18 outcomes across both genders is $r = .49$, indicating moderate predictive power.[19]

The raw scores produced by the CBCL/2–3 and CBCL/4–18 were converted into $t$ scores (standardized by age and gender) for total, internalizing, and externalizing behavior. The recommended clinical cutoff score ($t \geq 60$) was applied to the CBCL $t$ scores, to obtain 3 binary variables indicative of clinically significant total, internalizing, and externalizing problems.[23] The term "clinically significant" refers here to maladaptive behavior that falls within a defined clinical range for behavioral problems.[22]

### Language Development

The Peabody Picture Vocabulary Test—Revised (PPVT-R)[25] was administered to the children at the 5- and 10-year follow-ups, providing a widely used measure of receptive vocabulary. Because of a lack of Australian norms, raw scores were converted to $z$ scores, which were then used to identify children with mild ($z$ score between $-1$ and $-1.5$) or moderate-severe ($z$ score $<1.5$) language impairment.[26,27] The PPVT-R correlates well with the vocabulary subtests of the Stanford-Binet Intelligence Scales ($r = .72$) and the Wechsler Intelligence Scale for Children ($r = .69$).[25]

### Sample Characteristics

A range of variables were investigated to determine whether the participants in the current study were representative of the broader Raine cohort. These included sociodemographic factors recorded at 18 weeks' pregnancy (maternal race/ethnicity; maternal age at conception, maternal education, family income, presence of biological father in the family home); antenatal variables recorded at 34 weeks' pregnancy (maternal smoking and alcohol consumption during pregnancy); and obstetric variables recorded at birth (gestational age, offspring gender, parity, Apgar scores 5 minutes after birth).

### Statistical Analyses

Because there is currently no standard definition of optimal levels of vitamin D,[5] we divided maternal 25(OH)-vitamin D concentrations into quartiles, which is a well-established practice within the field.[5,9,16,28] The current study was primarily interested in developmental problems among offspring, and thus all outcome variables were examined categorically, incorporating thresholds for clinical levels of difficulty on the CBCL ($t \geq 60$) and PPVT-R (mild difficulty: $z$ score between $-1$ and $-1.5$; moderate-severe difficulty $<1.5$). $\chi^2$ linear-by-linear trends were examined to determine the effect of in utero exposure to increasing levels of maternal 25(OH)-vitamin D. Significant effects were followed up by using generalized estimating equations, which enabled a longitudinal investigation of the influence of maternal 25(OH)-vitamin D concentrations on offspring performance on that particular scale across multiple ages. For generalized estimating equations modeling, we adopted a 3-stage procedure: model 1 investigated the effect of maternal 25(OH)-vitamin D concentration on the longitudinal outcome variable; model 2 included any confounder in which $\chi^2$ analyses revealed a main effect on the independent variable at the conservative $\alpha$ level of $P < .20$[29]; and model 3 included the same confounders as model 2 in addition to a variable denoting the season in which maternal blood was collected to determine whether any effect was specific to maternal 25(OH)-vitamin D levels rather than other seasonal factors. For each model, odds ratios (OR) and 95% confidence intervals (95% CI) are reported. The $\alpha$ level for all analyses was $P < .05$. The analyses were conducted separately for each gender, and highly similar patterns and magnitudes of findings were observed.

## RESULTS

### Sample Characteristics

Among the 929 women who had maternal blood obtained at 18 weeks' pregnancy and later analyzed for 25(OH)-vitamin D levels, 815 (87.7%) self-identified as Caucasian. The 114

**TABLE 1** Frequency Characteristics of the Sample in the Current Study According to Quartiles of Maternal Serum 25(OH)-Vitamin D Concentration Obtained at 18 Weeks' Pregnancy

| | | Maternal 25(OH)-Vitamin D Concentration | | | | *P* Value |
|---|---|---|---|---|---|---|
| | | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| 25(OH)-vitamin D concentration (nmol/L) | | | | | | |
| *n* | | 187 | 189 | 182 | 185 | |
| M (SD) | | 36.81 (7.14) | 53.12 (3.89) | 65.17 (3.37) | 83.54 (12.08) | <.01 |
| Range | | 15–46 | 47–59 | 60–71 | 72–154 | |
| Covariates | *N* | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *P* Value |
| Maternal age at conception, y | 723 | | | | | .19 |
| <20 | | 12 (6.6) | 13 (9.5) | 21 (11.7) | 15 (8.4) | |
| 20–24 | | 49 (26.9) | 41 (22.4) | 37 (20.6) | 27 (15.5) | |
| 25–29 | | 58 (31.9) | 58 (31.7) | 43 (23.9) | 56 (31.5) | |
| 30–34 | | 43 (23.6) | 44 (24.0) | 60 (33.3) | 60(33.7) | |
| ≥35 | | 20 (11.0) | 27 (14.8) | 19 (10.6) | 20 (11.2) | |
| Mother completed secondary school | 741 | | | | | .90 |
| Yes | | 67 (36.0) | 64 (33.9) | 67 (36.8) | 69 (37.5) | |
| No | | 119 (64.0) | 1265 (66.1) | 115 (63.2) | 115 (62.5 | |
| Family income above poverty line | 706 | | | | | .11 |
| Yes | | 95 (52.5) | 108 (59.7) | 99 (58.6) | 108 (61.7) | |
| No | | 86 (47.5) | 73 (40.3) | 70 (41.4) | 67 (38.3) | |
| Biological father living in family home | 724 | | | | | .21 |
| Yes | | 163 (89.6) | 163 (88.6) | 151 (83.9) | 154 (88.5) | |
| No | | 19 (10.4) | 21 (11.4) | 29 (16.1) | 24 (13.5) | |
| Season in which maternal blood was collected at 18 wk pregnancy | 743 | | | | | <.01 |
| Summer | | 9 (4.8) | 31 (16.4) | 47 (25.8) | 54 (29.2) | |
| Autumn | | 22 (11.8) | 40 (21.2) | 37 (20.3) | 58 (31.4) | |
| Winter | | 65 (34.8) | 55 (29.1) | 29 (15.9) | 29 (15.7) | |
| Spring | | 91 (48.7) | 63 (33.3) | 69 (37.9) | 44 (23.8) | |
| Smoking during pregnancy | 724 | | | | | <.05 |
| None | | 115 (63.2) | 131 (71.2) | 127 (70.6) | 137 (77.0) | |
| 1–5 cigarettes daily | | 23 (12.6) | 18 (9.8) | 16 (8.9) | 15 (8.4) | |
| 6–10 cigarettes daily | | 17 (9.3) | 12 (6.5) | 13 (7.2) | 13 (7.3) | |
| 11–15 cigarettes daily | | 14 (7.7) | 11 (6.0) | 9 (5.0) | 7 (3.9) | |
| 16–20 cigarettes daily | | 10 (5.5) | 9 (4.9) | 9 (5.0) | 3 (1.7) | |
| 21+ cigarettes daily | | 3 (1.6) | 3 (1.6) | 6 (3.3) | 3 (1.7) | |
| Alcohol intake during pregnancy | 724 | | | | | .37 |
| None | | 99 (54.4) | 105 (57.1) | 85 (47.2) | 85 (47.8) | |
| Once a week or less | | 42 (23.1) | 42 (22.8) | 52 (28.9) | 45 (25.3) | |
| 2–6 drinks per week | | 30 (16.5) | 39 (16.3) | 37 (20.6) | 41 (3.0) | |
| 7–10 drinks per week | | 4 (2.2) | 2 (1.1) | 3 (1.7) | 6 (3.4) | |
| 11+ drinks per week | | 7 (3.8) | 5 (2.7) | 3 (1.7) | 1 (0.6) | |
| Offspring gender | 743 | | | | | |
| Female | | 112 (59.9) | 118 (62.4) | 105 (57.7) | 106 (57.3) | .44 |
| Male | | 75 (40.1) | 71 (37.6) | 77 (42.3) | 79 (42.7) | |
| Gestational age at birth of offspring | 739 | | | | | .07 |
| <32 wk | | 4 (2.2) | 4 (2.2) | 3 (1.7) | 2 (1.1) | |
| 32–37 wk | | 37 (20.3) | 37 (20.2) | 27 (15.0) | 26 (14.7) | |
| 38–40 wk | | 106 (58.2) | 116 (63.4) | 121 (67.2) | 110 (62.1) | |
| >41 wk | | 35 (19.2) | 26 (14.2) | 29 (16.1) | 39 (22.0 | |
| Offspring parity | 724 | | | | | <.05 |
| 1 | | 101 (55.5) | 95 (51.6) | 87 (48.3) | 68 (38.2) | |
| ≥2 | | 81 (44.5) | 89 (48.4) | 93 (51.7) | 110 (61.8) | |
| Offspring Apgar scores 5 min after birth | 722 | | | | | .83 |
| Generally normal | | 175 (96.2) | 174 (95.1) | 176 (98.3) | 170 (95.5) | |
| Fairly low | | 7 (3.8) | 9 (4.9) | 3 (1.7) | 8 (4.5) | |
| Critically low | | 0 (0) | 0 (0) | 0 (0) | 0 (0) | |

*P* values are for between-quartile comparisons.

offspring who had a non-Caucasian mother and another 2 Caucasian offspring with Down syndrome were excluded from the study. Seventy of the remaining 813 offspring did not contribute any behavioral data, which left 743 offspring who were investigated in the current study.

Participants in the current study were representative of the wider Raine cohort in terms of sociodemographic and obstetric characteristics ($P > .05$), except that female offspring were proportionately overrepresented in the current study (current study: 59.4%; remainder of Raine cohort: 43.9%; $\chi^2 = 49.72$, $P < .01$). See Supplemental Table 4 for additional details.

Table 1 presents the sample characteristics of the mother-offspring pairs within each 25(OH)-vitamin D concentration quartile. The quartile markers were ≤46 nmol/L, 47–59 nmol/L, 60–71 nmol/L, and ≥72 nmol/L for quartiles 1 to 4, respectively. The quartile markers were similar to those reported in other Western countries.[30–32] Importantly, the lower quartile threshold (≤46 nmol/L) corresponded well with the most widely used definition of vitamin D insufficiency as a 25(OH)-vitamin D concentration <50 nmol/L.[5]

There was an effect of season of maternal blood collection on 25(OH)-vitamin D quartiles ($P < .01$), in which women in quartiles 1 and 2 were more likely to have had blood samples obtained during the winter and spring months. Women in quartile 1 were also more likely to have smoked cigarettes during pregnancy and less likely to have given birth previously.

### Bivariate Analyses

There were no significant linear-by-linear associations in the proportion of children scoring above the clinical cutoff for CBCL morbidity (see Table 2). In contrast, there was a significant linear-by-linear association between vitamin D quartiles and the proportion of offspring with language difficulties at age 5, $\chi^2 = 5.27$, $df = 1$, $P < .05$, and age 10, $\chi^2 = 5.64$, $df = 1$, $P < .05$. Figure 1 shows that as maternal 25(OH)-vitamin D levels during pregnancy increased the proportion of mothers who had offspring with mild or moderate-severe language difficulties decreased.

**TABLE 2** Number (%) of Children Exceeding the CBCL Clinical Thresholds ($t$ score ≥60) at Each Follow-Up According to Quartiles of Maternal Serum 25(OH)-Vitamin D Concentration at 18 Weeks' Pregnancy

| | Maternal 25(OH)-Vitamin D Concentration | | | | $P$ |
|---|---|---|---|---|---|
| | Quartile 1[a] (Lowest) | Quartile 2[b] | Quartile 3[c] | Quartile 4[d] (Highest) | |
| CBCL year 2 | | | | | |
| Total | 16 (10.7) | 23 (15.1) | 14 (9.9) | 12 (8.1) | .26 |
| Internalizing | 13 (8.7) | 12 (7.9) | 14 (9.9) | 11 (7.4) | .85 |
| Externalizing | 18 (12.8) | 28 (18.4) | 19 (13.5) | 20 (13.4) | .96 |
| CBCL year 5 | | | | | |
| Total | 33 (19.8) | 33 (21.0) | 36 (22.2) | 37 (22.3) | .54 |
| Internalizing | 21 (12.6) | 26 (16.6) | 28 (17.3) | 29 (17.5) | .22 |
| Externalizing | 27 (16.2) | 32 (20.4) | 32 (19.8) | 34 (20.5) | .36 |
| CBCL year 8 | | | | | |
| Total | 36 (23.1) | 32 (21.1) | 32 (20.3) | 30 (19.1) | .85 |
| Internalizing | 34 (21.8) | 36 (23.7) | 37 (23.4) | 27 (13.2) | .34 |
| Externalizing | 35 (22.4) | 27 (17.8) | 28 (17.7) | 28 (17.8) | .32 |
| CBCL year 10 | | | | | |
| Total | 25 (16.3) | 29 (18.4) | 22 (14.9) | 21 (13.5) | .35 |
| Internalizing | 26 (17.0) | 31 (19.6) | 31 (20.9) | 23 (14.7) | .68 |
| Externalizing | 24 (15.7) | 21 (13.3) | 19 (12.8) | 18 (11.5) | .29 |
| CBCL year 14 | | | | | |
| Total | 22 (16.2) | 21 (15.1) | 17 (12.7) | 17 (12.6) | .33 |
| Internalizing | 17 (12.5) | 17 (12.5) | 18 (13.4) | 17 (12.6) | .91 |
| Externalizing | 23 (16.9) | 22 (15.8) | 22 (16.4) | 15 (11.1) | .22 |
| CBCL year 17 | | | | | |
| Total | 9 (8.8) | 10 (9.7) | 9 (8.4) | 5 (5.0) | .29 |
| Internalizing | 12 (11.8) | 11 (10.7) | 8 (7.5) | 9 (9.0) | .74 |
| Externalizing | 13 (12.7) | 14 (13.6) | 12 (11.2) | 6 (6.0) | .31 |

$P$ values are for $\chi^2$ linear-by-linear association tests.
[a] $N$: age 2 = 150, age 5 = 167, age 8 = 156, age 10 = 153, age 14 = 136, age 17 = 102.
[b] $N$: age 2 = 152, age 5 = 157, age 8 = 152; age 10 = 158, age 14 = 139, age 17 = 103.
[c] $N$: age 2 = 141, age 5 = 162, age 8 = 158, age 10 = 148, age 14 = 134, age 17 = 107.
[d] $N$: age 2 = 149, age 5 = 166, age 8 = 157, age 10 = 156, age 14 = 135, age 17 = 100.

### Multivariate Analyses

Table 3 presents the findings from the multivariate analyses. Offspring of quartile 1 women were more than twice as likely to be categorized in a more severe language impairment category (ie, "typical" to mild impairment or mild impairment to moderate/severe impairment) relative to offspring of quartile 4 women (model 1), OR = 2.04, 95% CI = 1.14 to 3.68, $P < .05$. Model 2, which adjusted for maternal age at conception, family income, maternal smoking during pregnancy, and offspring parity (Table 1 for $P$ values < .20), identified a slightly weakened but still significant effect of "low" maternal 25(OH)-vitamin D levels (ie, quartile 1) on offspring language impairment, OR = 1.92, 95% CI = 1.01 to 3.62, $P < .05$. This effect remained after additional adjustment for season of maternal blood collection (model 3), OR = 1.97, 95% CI = 1.00 to 3.92, $P < .05$.

A final, post hoc analysis found that, within quartile 1, there were no significant correlations between the continuous measures of maternal 25(OH)-vitamin D concentration and PPVT-R $z$ scores at 5 ($n = 130$, $r = -.03$, $P = .78$) or 10 years ($n = 121$, $r = -.01$, $P = .99$) of age.

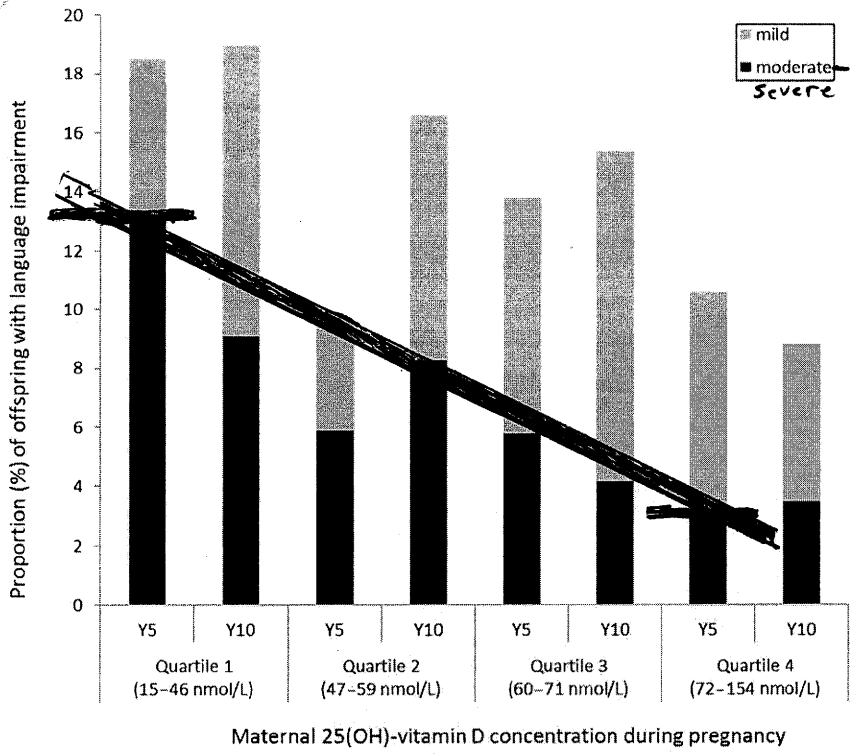


**FIGURE 1**

The proportion of offspring with mild or moderate-severe language impairment at 5 (Y5)[a] and 10 years (Y10)[b] of age according to maternal serum 25(OH)-vitamin D levels at 18 weeks' pregnancy.

[a] Quartile 1, $n = 130$; quartile 2, $n = 136$; quartile 3, $n = 136$; quartile 4, $n = 132$.

[b] Quartile 1, $n = 121$; quartile 2, $n = 121$; quartile 3, $n = 119$; quartile 4, $n = 113$.

**TABLE 3** Generalized Estimating Equations (GEE) Models Showing the Association Between Maternal 25(OH)-Vitamin D Concentration at 18 Weeks' Pregnancy and Offspring Language Impairment During Childhood

| Maternal 25(OH)-Vitamin D Concentration | Model 1[a] | | Model 2[b] | | Model 3[c] | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | *P* | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Quartile 4 (highest) | 1.00 (reference) | — | 1.00 (reference) | — | 1.00 (reference) | — |
| Quartile 3 | 1.47 (0.80–2.70) | .21 | 1.43 (0.75–2.73) | .29 | 1.44 (0.74–2.80) | .28 |
| Quartile 2 | 1.28 (0.68–2.40) | .45 | 1.33 (0.70–2.54) | .39 | 1.35 (0.71–2.57) | .36 |
| Quartile 1 (lowest) | 2.04 (1.14–3.68) | <.05 | 1.92 (1.01–3.62) | <.05 | 1.97 (1.00–3.93) | <.05 |

[a] Unadjusted model.

[b] GEE adjusted for maternal age at conception, family income, maternal smoking during pregnancy, and offspring parity.

[c] GEE adjusted for same confounders as for Model 2, as well as the season of maternal blood collection.

## DISCUSSION

This study reports the largest investigation to date of the association between maternal 25(OH)-vitamin D status during pregnancy and offspring neurocognitive development. There was no statistically significant association between maternal 25(OH)-vitamin D concentrations during pregnancy and offspring behavioral and emotional difficulties as measured by the CBCL. This finding replicates the only previous investigation in this area,[9] which revealed no effect of maternal 25(OH)-vitamin D levels on offspring behavior to middle childhood, and extends this null association to adolescence. However, significant linear associations were observed with language impairment at 5 and 10 years of age. The risk of women with serum 25(OH)-vitamin D insufficiency (≤46 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with 25(OH)-vitamin D levels >70 nmol/L, even after taking into account a range of confounding variables. Importantly, the statistically significant effect was still present after adjusting for the season at which maternal blood was collected, indicating that the association was primarily driven by maternal 25(OH)-vitamin D levels, rather than other seasonal factors.

The developing fetus is completely reliant on maternal vitamin D stores, and thus the concentration of maternal circulating levels at 18 weeks' pregnancy provides an accurate measure of fetal exposure during the second trimester.[31,32] In vitro[33–39] and in vivo[7,40,41] studies have found that vitamin D performs a number of biological functions that are fundamental to neurodevelopment, including a signaling role in neuronal differentiation, a regulation role in the metabolism of neurotrophic factors and neurotoxins, and a protective role during brain inflammation. Vitamin D may also be indirectly involved in fetal brain growth through its role in a number of endocrine functions.[6] Reduced levels of vitamin D may disrupt 1 or more of these functions during critical phases of neurodevelopment. Intriguingly, the current study found evidence for a threshold effect, in that offspring of quartile 1 women were the only group of children at significantly increased risk of language impairment, and within quartile 1, there was no correlation between the continuous 25(OH)-vitamin D and PPVT-R variables. Similar findings have been reported by cross-sectional studies for a range of health outcomes in postnatal life, including bone density, dental health, and colorectal cancers,[42] and together these findings suggest

that a circulating 25(OH)-vitamin D concentration of <50 nmol/L may provide a valid cutpoint to identify vitamin D insufficiency.

The significant association with language ability observed in the current investigation contrasts with the study by Gale and colleagues,[9] which found no effect of maternal vitamin D concentrations on verbal IQ measured by the Wechsler Abbreviated Scale of Intelligence. One possible explanation for this discrepancy is that the current sample (*n* range: 412–652 mother-offspring pairs) was considerably larger than that investigated by Gale et al (178 mother-offspring pairs) and thus provided greater statistical power to identify significant effects. A second possibility relates to the timing of maternal serum sampling, which occurred during the 18th week of pregnancy in the current study but over a later and broader period in the study by Gale et al (median: 32.6 weeks; range: 28–42 weeks). Vitamin D serum concentrations are known to fluctuate according to sunlight exposure and, to a lesser extent, diet.[43,44] Perisylvian structures that subserve much of the neural architecture responsible for language[45] and are anomalous in children with developmental language difficulties,[46,47] including the planum temporal, the pars triangularis and the inferior frontal gyrus, are known to develop during the second and third prenatal trimesters.[48] Although the sampling regimen used in our study was temporally sensitive to second trimester fetal growth, it is possible that 25(OH)-vitamin D serum concentrations measured during the third trimester do not reflect circulating levels during the early stages of perisylvian development. Studies that sample maternal serum at several stages throughout pregnancy will reveal important information about the biological mechanisms underpinning the association between maternal vitamin D levels and fetal neurodevelopment.

Strengths of the study design include the large participant sample, a follow-up period that spanned 2 decades, the assessment of the behavioral and language phenotypes with the same measures at multiple time points, and the direct measurement of maternal 25 (OH)-vitamin D serum levels using enzyme immunoassay and validated by liquid chromatography-tandem mass spectrometry. However, similar to most longitudinal studies, the Raine cohort has experienced a degree of sample attrition over time, such that CBCL data at age 17 was available for just over 50% (*n* = 412) of the original 743 participants eligible for the current study. It is possible that a reduction in the number of participants with age may have influenced the current pattern of findings. Importantly, however, the majority of eligible participants contributed CBCL (82.8%) and PPVT (63.8%) data at age 10, and there is evidence that both behavioral[49,50] and language[51,52] ability in the general population is relatively stable from middle childhood onward. It is also important to highlight that our study included Caucasian participants only, and we caution against extrapolating the findings to non-Caucasian populations. Future studies that investigate large non-Caucasian populations will build on the findings presented here.

## CONCLUSIONS

This study found that vitamin D insufficiency among Caucasian women during pregnancy was associated with an increased rate of language impairment among offspring. The findings suggest that the trend over the past decade of a reduction in vitamin D levels among women of reproductive age[11–14] has significant implications for offspring neurodevelopment and public health more generally. Randomized controlled trials of vitamin D supplementation are required to verify these observational data that suggest that an adequate maternal vitamin D status during pregnancy is necessary for optimal language development in offspring.

## ACKNOWLEDGMENTS

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***FAIR FARES:*** *I have a lot of flights scheduled during the next several months. Not only will I be flying to cities along the Atlantic seaboard but to cities in Europe and Asia as well. Trying to determine the best fare is quite challenging. I fly to Philadelphia often enough to know the "sweet spot" for getting the best deal. However, I am not so familiar with the flights to the west coast or Italy. Scrolling through the scores of travel sites only contributes to my confusion. Some quote a fare and will allow me to book a flight directly on their website. Others quote fares but re-direct me to another website to purchase tickets. Some airlines are not listed on the travel sites, so to check on routes and fares I need to go directly to that airline's homepage. More confusing yet is comparing the fares between travel sites, which take different approaches to posting fares. Some post the final cost while others only the baseline fare. The final cost, e.g. including fuel surcharges and landing fees, is not discovered until later stages of the search process. Most sites do not add their own fees to the final cost of the ticket until late in the purchase process. What appears at first glance to be the cheapest ticket, may wind up costing far more than expected. According to an article in* The Wall Street Journal *(The Middle Seat: January 12, 2011), there are a myriad of reasons for the confusion. First, airlines publish fares for every conceivable route and connection. So a travel site looking for a flight between Burlington and Philadelphia can route me through Detroit and Chicago. In a further wrinkle, some sites won't publish fares for routes if it seems unlikely than anyone would actually purchase a ticket for that itinerary. For example, I can usually get a cheaper flight than I would normally take to Philadelphia but I would have to make three stops. Some travel sites publish this itinerary while others do not. So what is the bottom line for travelers? Scan multiple sites. No single site offers the best fares all the time. And as always, if the price is too good to be true, it usually is.*

*Noted by WVR, MD*

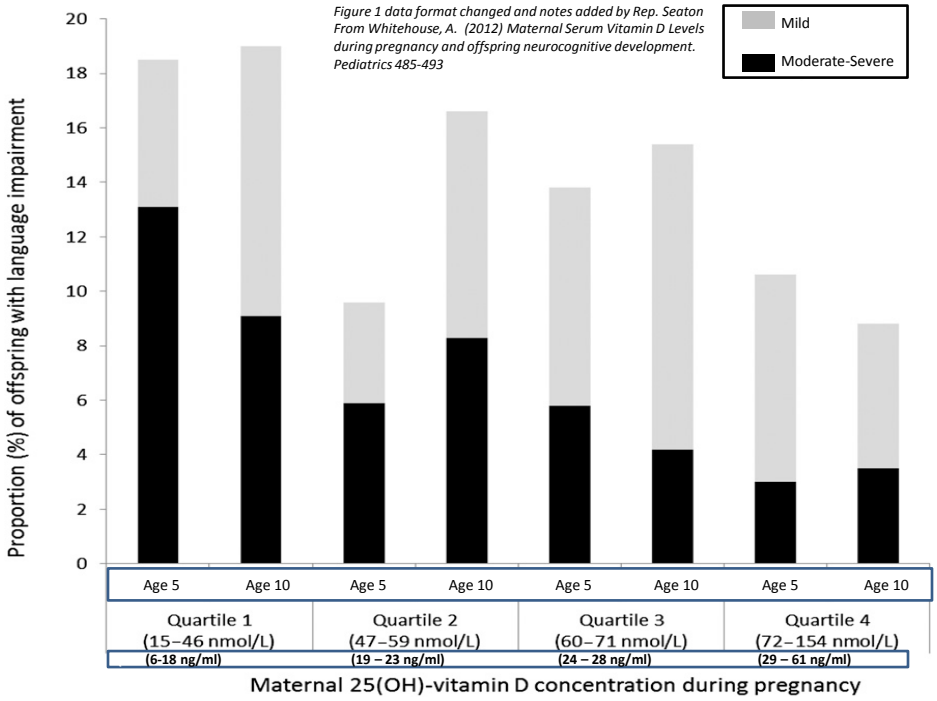
Figure 1 data format changed and notes added by Rep. Seaton
From Whitehouse, A. (2012) Maternal Serum Vitamin D Levels during pregnancy and offspring neurocognitive development. Pediatrics 485-493
Mild
Moderate-Severe
Proportion (%) of offspring with language impairment
20
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16
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Age 10
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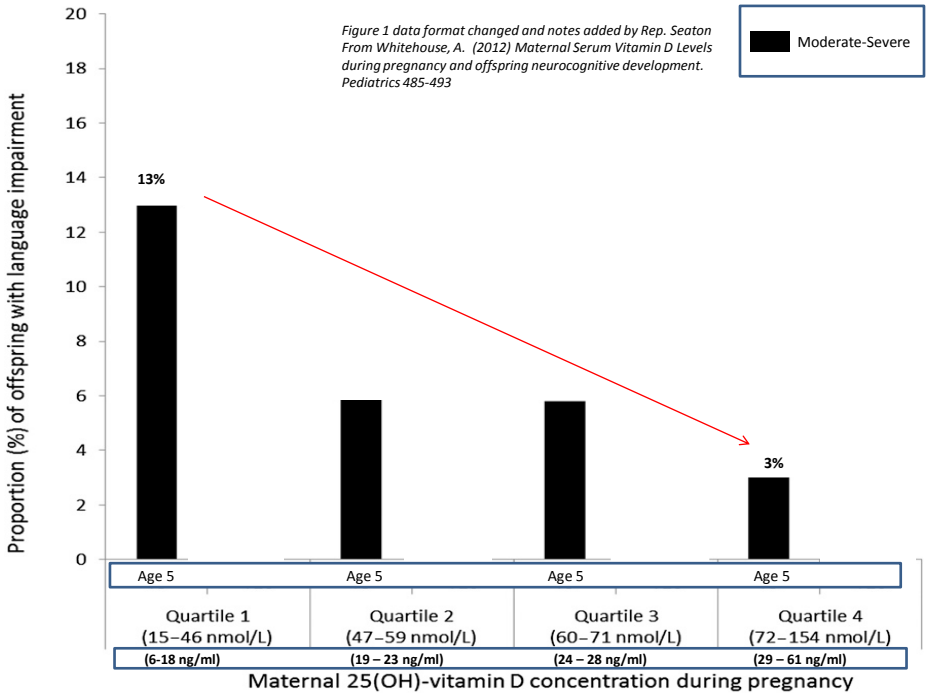
Age 10
Quartile 1 (15–46 nmol/L) (6-18 ng/ml)
Quartile 2 (47–59 nmol/L) (19 – 23 ng/ml)
Quartile 3 (60–71 nmol/L) (24 – 28 ng/ml)
Quartile 4 (72–154 nmol/L) (29 – 61 ng/ml)
Maternal 25(OH)-vitamin D concentration during pregnancy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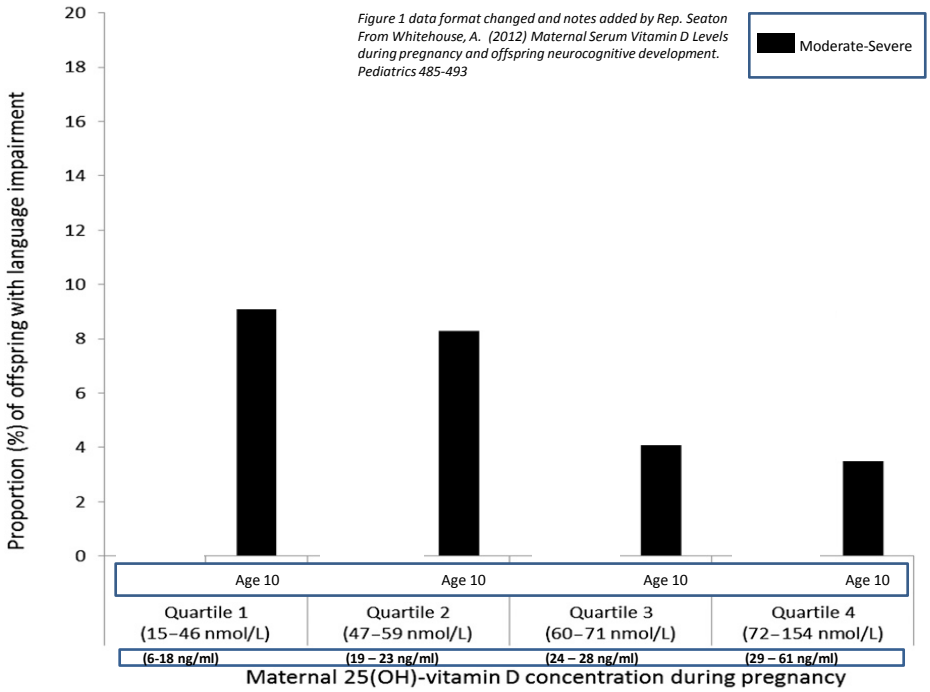
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