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Vitamin D Deficiency in Middle Childhood Is Related to Behavior Problems in Adolescence

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ABSTRACT

Background: Vitamin D deficiency (VDD) is associated with depression and schizophrenia in adults. The effect of VDD in childhood on behavioral development is unknown.

Objectives: We aimed to study the associations of VDD and vitamin D binding protein (DBP) in middle childhood with behavior problems in adolescence.

Methods: We quantified plasma total 25-hydroxyvitamin D [25(OH)D] and DBP in 273 schoolchildren aged 5–12 y at recruitment into a cohort study in Bogota, Colombia. Externalizing and internalizing behavior problems were assessed after a median 6-y follow-up by parental report [Child Behavior Checklist (CBCL)] and self-report [Youth Self-Report (YSR)]. We estimated mean problem score differences with 95% CIs between exposure categories using multivariable linear regression. We also compared the prevalence of clinical behavior problems (score >63) between exposure groups. We assessed whether the associations between DBP and behavior problems were mediated through VDD.

Results: Mean ± SD CBCL and YSR externalizing problems scores were 56.5 ± 9.3 and 53.2 ± 9.5, respectively. Internalizing problems scores averaged 57.1 ± 9.8 and 53.7 ± 9.8, respectively. VDD [25(OH)D <50 nmol/L] prevalence was 10.3%. VDD was associated with an adjusted 6.0 (95% CI: 3.0, 9.0) and 3.4 (95% CI: 0.1, 6.6) units higher CBCL and YSR externalizing problems scores, respectively, and an adjusted 3.6 (95% CI: 0.3, 6.9) units higher CBCL internalizing problems scores. The prevalence of clinical total externalizing problems was 1.8 (95% CI: 1.1, 3.1) times higher in children with VDD than that in children without VDD. DBP concentration below the population median was related to higher YSR aggressive behavior and anxious/depressed subscale scores and to higher prevalence of clinical total externalizing problems. The associations between DBP and behavior problems were not mediated through VDD.

Conclusions: VDD and low DBP in middle childhood are related to behavior problems in adolescence. *J Nutr* 2019;00:1–9.

Keywords: vitamin D deficiency, vitamin D binding protein, behavior problems, middle childhood, adolescence, Bogota School Children Cohort

Introduction

Mental health disorders are the leading cause of years lived with disability in adults worldwide (1). The development of these disorders likely begins in childhood. For example, clinical externalizing behavior problems (e.g., conduct or oppositional defiant disorder) or internalizing behavior problems (e.g., anxiety or depressive disorders) in adolescence often track into adulthood and predict the development of mental illness (2, 3). Clinical externalizing problems predict the incidence of disruptive behavior and major depressive disorders (MDDs) with high specificity, whereas clinical internalizing problems predict the development of anxiety disorders (4). Because of this ability to predict adverse mental health clinical outcomes, investigating the causes of behavioral problems in adolescence is a relevant research priority.

Vitamin D deficiency (VDD) in adults is associated with depression and schizophrenia (5–7), but little is known about the role of VDD earlier in life on mental health. In

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Address correspondence to EV (Email: villamor@umich.edu).
Abbreviations used: CBCL, Child Behavior Checklist; DBP, vitamin D binding protein; MDD, major depressive disorder; VDD, vitamin D deficiency; YSR, Youth Self-Report; 25(OH)D, 25-hydroxyvitamin D.

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animal models, gestational VDD results in altered dopamine metabolism in the offspring (8, 9), a neurotransmitter involved in the regulation of behavioral responses. Correspondingly, most research in children has focused on the effect of vitamin D status in pregnancy on the development of behavior problems in childhood, but the results have been inconsistent (10–16) and only a few studies (11, 13) have extended into adolescence, when behavior problems often arise. Although areas of the central nervous system develop throughout childhood, the relation of children's vitamin D status with the incidence of behavior problems later in life has not been thoroughly investigated. Among British children aged 9.9 y of whom 27% had VDD, serum 25-hydroxyvitamin D [25(OH)D] was inversely associated with prosocial problems at age 11.7 y (17) and depressive symptoms at age 13.8 y (18). However, no studies have addressed the role of vitamin D in middle childhood (ages 5–12 y) on the development of adolescent externalizing or internalizing behavior problems using multiple informants.

Vitamin D binding protein (DBP), the primary transporter of vitamin D to target tissues, may influence the circulating concentration (19) and bioavailability (20) of 25(OH)D and thus may be associated with behavior problems through its regulation of 25(OH)D. In young adults, DBP is positively correlated with total 25(OH)D (21, 22) and with free vitamin D (21, 23), the fraction of the vitamin that circulates unbound and may more accurately represent its bioavailable form. In an animal study, extrahepatic synthesis of DBP was demonstrated in hypothalamic regions that produce neurotransmitters involved in the stress response (24), which suggests a potential direct role of DBP on behavior. However, the association between DBP and behavior problems has not been investigated.

The objectives of this study were to examine the associations of VDD and circulating DBP in middle childhood with externalizing and internalizing behavior problems in adolescence in a cohort of schoolchildren from Bogotá, Colombia. We hypothesized that VDD and low DBP concentration would be positively associated with externalizing and internalizing problems. Because DBP could affect 25(OH)D concentration (19), we also explored whether any associations between DBP and behavior problems were mediated through VDD.

Methods

Study design and population

We conducted a prospective study as part of the Bogota School Children Cohort, a longitudinal investigation of nutrition and health in Bogota, Colombia. Details on the cohort design have been previously reported (25). In brief, in February 2006, we recruited 3202 children aged 5–12 y through random selection from primary public schools in Bogota, Colombia. As most children enrolled in the public school system in Bogota are from low- and middle-income families, our sample pertains to these groups.

Baseline information

At the time of enrollment, we obtained information on sociodemographic characteristics and health habits of children with the use of a parental self-administered survey. This survey included questions on children's daily habits; maternal education level, height, and weight; and household level of food insecurity and socioeconomic status. Household food insecurity was ascertained with a modified Spanish-language version of the US Department of Agriculture Household Food Security Survey module (26). Household socioeconomic status was categorized according to the municipal government's classification for tax and planning purposes.

During the weeks following enrollment, trained research assistants scheduled school visits at which the children's height was measured without shoes to the nearest 1 mm using a wall-mounted portable Seca 202 stadiometer, and weight was measured in light clothing to the nearest 0.1 kg using Tanita H5301 electronic scales. Height and weight were also measured among the children's mothers who were present at schools. Research assistants obtained fasting blood samples through antecubital venipuncture in 88% of the children. The samples were protected from sunlight and transported in refrigerated coolers on the day of collection to the Colombian National Institute of Health, where they were processed and cryostored for future analyses. All blood samples were obtained in the month of February, within a 3-wk period.

Follow-up assessment

Between 2011 and 2015, we conducted an in-person follow-up assessment in a random subsample of 1139 participants. We assessed the children's behavior with use of questionnaires administered to the parents [Child Behavior Checklist (CBCL)] and the children [Youth Self-Report (YSR)]. The Spanish-language versions of these questionnaires have been widely used in other Latin American settings (27). The CBCL may be used to assess behavior of children aged 5–18 y, whereas the YSR has been validated for use in adolescents aged 11–18 y (28). Correlations between parent and self-report of behavior problems are generally moderate ($r \approx 0.5$) (29). Each instrument consists of 112 statements that describe behaviors or feelings. Respondents rate these statements as false, sometimes true, or very/often true. From the responses to the questionnaires, we computed sex- and age-standardized continuous scores for 8 behavior problems subscales (aggressive and rule-breaking behavior, anxious/depressed, withdrawn/depressed, somatic complaints, and attention, social, and thought problems) using software provided by the test developer (30). The software then computed age- and sex-standardized scores for the total externalizing problems composite scale, which comprises the aggressive and rule-breaking behavior subscales, and for the total internalizing problems scale, comprising the anxious/depressed, withdrawn/depressed, and somatic complaints subscales.

The parents or primary caregivers of all children gave written informed consent prior to enrollment in the study and before participation in the follow-up assessment. Youth gave written assent to participate. The Ethics Committee of the National University of Colombia Medical School approved the study protocol. The use of data from the study was approved by the Institutional Review Board at the University of Michigan.

Laboratory methods

Total plasma 25(OH)D was quantified in a random subset of the samples collected at recruitment ($n = 544$) at Children's Hospital Boston (Boston, MA) by an enzyme immunoassay (Immunodiagnostic Systems, Inc.) with a competitive binding technique (31). This validated method has a sensitivity of 2.5 ng/mL and an inter- and intra-assay CV of 11.2% and 8.1%, respectively. Plasma DBP was measured with a Quantikine ELISA kit (R&D Systems, Inc.) that uses a monoclonal antibody specific to DBP at the Center for Chemical Genomics, University of Michigan (Ann Arbor). The mean CV for replicate measures was 13.21%; individual sample CVs ranged from 0.02% to 33.05%. Some investigators have highlighted the importance of considering free 25(OH)D as an exposure (23) since <1% of 25(OH)D is bioavailable. Although financial constraints prevented us from quantifying free 25(OH)D in all children, we measured it in a subsample of 19 participants to estimate correlations with total 25(OH)D. We quantified free 25(OH)D using the DIASource ELISA at the Heartland Assays laboratory (Ames, IA). In this 2-step immunoassay procedure, free 25(OH)D is first bound to an anti–vitamin D antibody. Then, a chromogenic substrate is added and free 25(OH)D is measured using a plate spectrophotometer; inter- and intra-assay CVs range from 1.9–5.5% and 4.0–6.1%, respectively. Plasma vitamin B-12 and ferritin were quantified at the Colombian National Institute of Health with the use of a competitive chemiluminescent immunoassay in an ADVIA Centaur analyzer (Bayer Diagnostics). Hemoglobin concentrations were

TABLE 1 Behavior problems scores at 11–18 y of age according to plasma 25(OH)D concentrations in middle childhood among children from Bogota, Colombia[1]

	Child Behavior Checklist				Youth Self-Report			
	Plasma 25(OH)D			Adjusted difference (95% CI)[3]	Plasma 25(OH)D			Adjusted difference (95% CI)[3]
Behavior problems	≥50 nmol/L ($n = 203$)	<50 nmol/L ($n = 27$)	P^2		≥50 nmol/L ($n = 245$)	<50 nmol/L ($n = 28$)	P^2	
Externalizing problems								
Total	55.7 ± 9.3	62.3 ± 7.5	0.0003*	6.0 (3.0, 9.0)*	52.7 ± 9.6	57.4 ± 7.7	0.009*	3.4 (0.1, 6.6)*
Aggressive behavior	59.0 ± 8.2	64.9 ± 7.6	0.0004*	5.5 (2.6, 8.4)*	56.1 ± 7.5	58.7 ± 7.9	0.03*	1.6 (–1.5, 4.8)
Rule-breaking behavior	55.1 ± 5.2	58.0 ± 6.6	0.02*	2.6 (0.1, 5.2)*	54.8 ± 5.4	56.6 ± 5.4	0.03*	1.5 (–0.7, 3.7)
Internalizing problems								
Total	56.7 ± 9.9	60.2 ± 8.6	0.09	3.6 (0.3, 6.9)*	53.4 ± 9.9	56.0 ± 8.3	0.22	2.0 (–1.4, 5.4)
Anxious/depressed	56.4 ± 7.3	57.3 ± 6.3	0.18	0.3 (–2.4, 3.0)	55.5 ± 6.5	55.9 ± 7.1	0.80	0.1 (–2.7, 2.9)
Withdrawn/depressed	56.1 ± 6.4	58.9 ± 8.2	0.11	2.9 (–0.3, 6.2)	55.3 ± 6.0	56.5 ± 7.1	0.32	0.9 (–1.9, 3.7)
Somatic complaints	61.3 ± 8.1	63.3 ± 8.9	0.28	1.9 (–1.4, 5.3)	56.9 ± 7.6	58.5 ± 8.7	0.14	1.4 (–1.9, 4.6)
Other problems								
Attention problems	54.1 ± 5.3	56.3 ± 5.5	0.01*	2.0 (–0.1, 4.1)	52.4 ± 4.1	51.9 ± 2.6	0.83	–1.2 (–2.3, 0.0)*
Social problems	57.8 ± 6.4	59.4 ± 5.4	0.15	1.1 (–1.2, 3.4)	56.4 ± 6.9	56.2 ± 7.0	0.81	–0.6 (–3.2, 2.0)
Thought problems	55.7 ± 6.5	58.7 ± 8.1	0.08	3.0 (–0.3, 6.2)	54.7 ± 5.8	55.5 ± 5.6	0.32	0.4 (–1.9, 2.7)

[1] Mean ± SD unless noted otherwise. *$P < 0.05$. 25(OH)D, 25-hydroxyvitamin D.
[2] Wilcoxon rank-sum test.
[3] From linear regression models with the behavior problems score as the continuous outcome. Adjustment variables included child's sex, age, low plasma vitamin B-12, and low plasma vitamin D binding protein. Total externalizing problems and externalizing problems subscales per the Child Behavior Checklist were also adjusted for screen time >30 h/wk and mother's education as continuous. Total internalizing problems per the Child Behavior Checklist, total externalizing problems per the Youth Self-Report, and their subscales were also adjusted for screen time >30 h/wk. Empirical variances were specified in all models.

determined using the hemiglobincyanide method. Serum C-reactive protein concentration was measured with a turbidimetric immunoassay on an ACS180 analyzer (Bayer Diagnostics).

Data analysis

Of the 1139 participants followed in adolescence, 1097 completed the behavioral assessment tests. After excluding 13 forms with incomplete data and 42 forms outside the 11- to 18-y age range, there were 1042 valid tests. In total, 273 of these participants were in the subset with baseline vitamin D measurements; this constituted the analytic sample (**Supplemental Figure 1**). Parental report (CBCL) was available in 230 of these participants. Children included in the analysis had a higher BMI-for-age *z* score, more food insecurity, and a higher prevalence of anemia compared with those not included in the analysis (**Supplemental Table 1**).

The primary outcomes were the continuous scores for total externalizing and internalizing behavior problems as computed from responses to the CBCL and the YSR. Secondary outcomes were scores in the subscales that comprise total externalizing and internalizing problems. In supplemental analyses, we also considered scores in the 3 additional subscales reflecting other problems (attention, social, and thought problems).

Primary exposures were plasma 25(OH)D and DBP concentrations. Covariates were selected based on prior knowledge of the predictors of the outcomes in this population that are not consequences of the exposures (32). They included child, parental, and household characteristics. Low plasma vitamin B-12 concentration was considered a covariate because we previously observed an association between low plasma vitamin B-12 and externalizing behavior problems among boys in this cohort (32). Low vitamin B-12 was defined as concentrations below the lowest quartile of the sex-specific distribution: <247.5 pmol/L for boys and <261.5 pmol/L for girls, as previously reported (32). Maternal BMI was calculated as kg/m^2 from objectively measured height and weight in 39.2% of mothers and from self-reported data in the rest. Correlations between objectively measured and self-reported height and BMI were high (Pearson $r = 0.81$ and 0.85, respectively). Covariates were categorized as presented in Table 1. We did not consider season as a covariate since there was no seasonal variation in the collection of blood samples.

We first compared the continuous distributions of total externalizing and internalizing problems scores per the CBCL and YSR between categories of baseline covariates with the use of means and SDs. Values in the results section are means ± SDs unless otherwise indicated. For ordinal exposures, we conducted tests for linear trend by fitting a linear regression model with the behavior problems score as the continuous outcome and a variable representing ordinal categories of each covariate as a continuous predictor. For sex, we used the Wald statistic. Nonlinear associations with covariates were examined by estimating differences in the outcome between exposure categories and a reference level.

Next, we examined the continuous distributions of behavior problems scores in relation to plasma concentrations of 25(OH)D and DBP as continuous exposures with use of restricted cubic splines to identify potential nonlinear relations. Restricted cubic splines included nonlinear terms for 25(OH)D or DBP, which allowed for the smoothing of the exposure-outcome association, with knots placed at the 5th, 35th, 65th, and 95th percentiles of the 25(OH)D or DBP distributions (33). Because the associations were nonlinear, we compared the distributions of behavior outcomes between categories of the exposures according to conventional cut points. VDD was defined as 25(OH)D <50 nmol/L (34), whereas low DBP was defined as plasma concentrations below the population median (2497 nmol/L) since there are no conventionally accepted cut points for DBP. We estimated mean differences and 95% CIs in behavior problems scores between exposure categories with the use of multivariable linear regression adjusted for sex, age at baseline, and low plasma vitamin B-12. Models were also adjusted for baseline characteristics that were associated with total externalizing or internalizing problems scores in linear or nonlinear manners in bivariate analysis ($P < 0.05$). Only adjustment variables that remained significantly associated with the outcome were retained in multivariable models. Child's BMI- or height-for-age *z* scores (35) were not considered covariates as they may be consequences of VDD (36). Estimates by VDD categories were also adjusted for low DBP, since DBP may influence 25(OH)D concentrations (19).

We also considered a lower cut point for 25(OH)D, defined as the median 25(OH)D value among children with VDD (46.5 nmol/L) and redefined exposure categories as <46.5 nmol/L, 46.5 to <50.0 nmol/L, or ≥50.0 nmol/L. In supplemental analysis, we repeated the models excluding children with iron deficiency (plasma ferritin <15 μg/L when C-reactive protein was ≤10 mg/L, $n = 11$) or anemia

(hemoglobin <12.7 g/dL after adjustment for altitude, $n = 6$), since these conditions have been associated with VDD in other populations (37, 38) and predict behavior problems in adolescence in our cohort (32); their numbers in the vitamin D subsample were too small to warrant inclusion in adjusted models as covariates. Empirical estimates of the variance were specified in all models.

We then ascertained to what extent the associations of low DBP with the behavior problems outcomes were mediated through VDD, relying on the assumptions of a counterfactual frame. This analysis was undertaken when both the exposure (low DBP) and potential mediator (VDD) were related to the specific behavior problem outcome. We estimated the direct association of DBP and behavior problems and the indirect association of DBP mediated through VDD using Valeri and VanderWeele's formulas (39). This method assumes no unmeasured confounding of the exposure-mediator, exposure-outcome, or mediator-outcome relations and no effect of the exposure on confounders of the mediator-outcome relation. The exposure-mediator and exposure-outcome relations were modeled with logistic and linear regression, respectively. The percentage of the associations between low DBP and the outcomes mediated through VDD were estimated with use of the %mediation macro (39) for SAS.

CBCL and YSR scores have been used to identify adolescents with clinical behavior problems that may require intervention, since these problems predict mental illness later in life with high specificity (4). Thus, we conducted supplemental analyses to investigate the associations of 25(OH)D and DBP with clinical externalizing and internalizing behavior problems defined dichotomously as recommended by the test developer, when scale-specific scores were >63 (30). We first compared the prevalence of clinical behavior problems in adolescence across categories of baseline covariates using prevalence ratios and 95% CIs. Next, we estimated the associations of 25(OH)D and DBP concentrations in middle childhood with the prevalence of these problems using multivariable Poisson regression with the log-link, following analogous modeling approaches to those implemented in the analyses of behavior problems scores as continuous outcomes. All analyses were conducted with SAS version 9.4 (SAS Institute, Inc.).

Results

Age at enrollment was 8.6 ± 1.6 y; 53.5% of children were girls. Plasma 25(OH)D was 74.1 ± 25.8 nmol/L; VDD prevalence was 10.3%. Only 3 children had 25(OH)D concentrations <40 nmol/L, and none of the children had concentrations <30 nmol/L. The correlation between total and free 25(OH)D ($n = 19$) was high ($r = 0.74$, $P = 0.0003$). DBP concentration was 2660 ± 1131 nmol/L. DBP was weakly correlated with total 25(OH)D among all participants ($r = 0.18$, $P = 0.003$). Similarly, low DBP and VDD were weakly associated; the prevalence of VDD in low and high DBP categories was 11.0% and 9.6%, respectively. DBP was positively correlated with free 25(OH)D in the 19 children with these measurements ($r = 0.54$, $P = 0.02$).

Age at the follow-up assessment was 14.7 ± 1.7 y. Compared with baseline, at follow-up, fewer children were obese (BMI-for-age z score >2 SD) or food insecure, and children spent more time watching television/playing video games (**Supplemental Table 2**).

Continuous behavior problems scores

Total externalizing problems scores were 56.5 ± 9.3 and 53.2 ± 9.5 according to the CBCL and the YSR, respectively; total internalizing problems scores were 57.1 ± 9.8 and 53.7 ± 9.8, respectively. Correlations between scores from the CBCL and the YSR were 0.49 for total externalizing and 0.41 for total internalizing problems; correlations for subscales were weaker (**Supplemental Table 3**). In bivariate analysis, child's age at baseline was positively related to total externalizing problems scores according to the YSR while time spent watching television/playing video games was positively associated with this outcome according to both the CBCL and the YSR (**Supplemental Table 4**). Mother's education was inversely related to total externalizing problems scores per the CBCL. Child's height-for-age z score at baseline was positively associated with total internalizing problems scores according to the CBCL (**Supplemental Table 5**).

Associations with vitamin D.

There was a nonlinear inverse association between 25(OH)D concentrations and total externalizing problems (**Figure 1**). In bivariate analysis, VDD was positively associated with total externalizing problems, aggressive behavior, and rule-breaking behavior scores according to the CBCL and the YSR (Table 1). In addition, VDD was positively related to attention problems scores per the CBCL. After multivariable adjustment, children with VDD had 6.0 (95% CI: 3.0, 9.0; $P < 0.0001$) and 3.4 (95% CI: 0.1, 6.6; $P = 0.04$) units higher total externalizing problems scores per the CBCL and YSR, respectively, compared with children without VDD (Table 1). Aggressive and rule-breaking behavior scores from the CBCL were 5.5 (95% CI: 2.6, 8.4; $P = 0.0002$) and 2.6 (95% CI: 0.1, 5.2; $P = 0.04$) units higher, respectively, in children with VDD compared with those without VDD. Differences in externalizing problems subscales from the YSR were not statistically significant after adjustment (Table 1). Children with the lowest 25(OH)D concentrations (<46.5 nmol/L) had higher total externalizing problems scores compared with children with 25(OH)D ≥50 nmol/L according to both tests (**Supplemental Table 6**).

Total internalizing problems scores per the CBCL were an adjusted 3.6 (95% CI: 0.3, 6.9; $P = 0.03$) units higher in children with VDD compared with those without VDD (Table 1). Exclusion of children with iron deficiency or anemia did not change the results (**Supplemental Table 7**).

Associations with DBP.

There were inverse associations between DBP concentrations and total externalizing and internalizing problems (**Figure 2**), but they were not statistically significant after adjustment (**Table 2**). Low DBP concentration was associated with an adjusted 2.2 (95% CI: 0.4, 3.9; $P = 0.02$) and 2.2 (95% CI: 0.6, 3.7; $P = 0.008$) units higher aggressive behavior and anxious/depressed scores per the YSR, respectively (Table 2). The results did not change after exclusion of children with iron deficiency or anemia (**Supplemental Table 8**).

Mediation.

VDD did not mediate the association between low DBP and total externalizing problems (percent mediated CBCL = 2.5, YSR = 0.4) or aggressive behavior (percent mediated CBCL = 2.3, YSR = 0.1) scores. We did not conduct mediation analyses for total internalizing behavior or the internalizing subscales because the exposure (low DBP) was not related to these outcomes.

Clinical behavior problems

According to the CBCL and YSR, respectively, prevalence of clinical externalizing problems was 23.0% and 13.9%, and prevalence of clinical internalizing problems was 25.2% and 12.8%. The associations of baseline characteristics with clinical externalizing (**Supplemental Table 9**) and internalizing

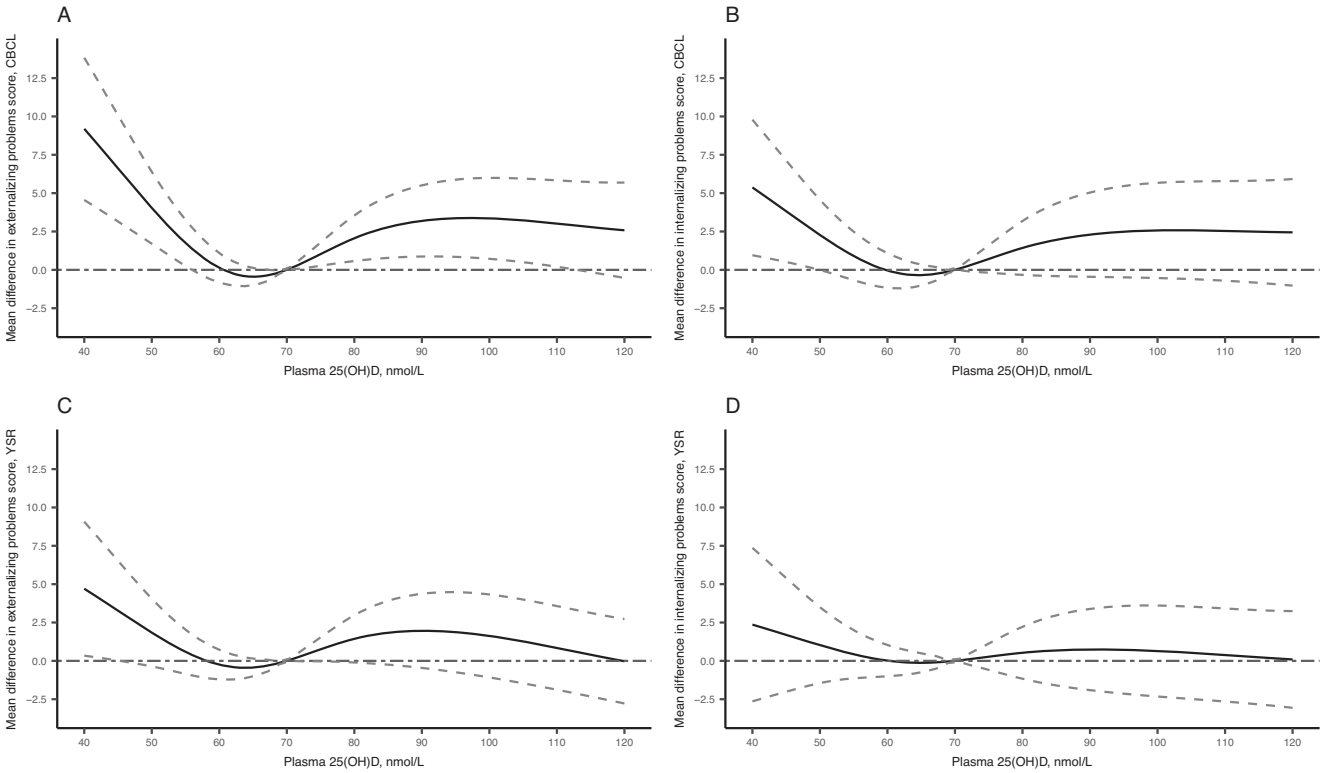

FIGURE 1 Mean adjusted differences (continuous line) with 95% CIs (dotted lines) in Child Behavior Checklist (CBCL) (A, B) and Youth Self-Report (YSR) (C, D) total behavior problems scores in adolescence according to plasma 25-hydroxyvitamin D (25[OH]D) concentrations in middle childhood. Estimates are from linear regression models; total externalizing or internalizing problems were the continuous outcomes. Predictors for all models included linear and spline terms for 25(OH)D and child's sex, age (continuous), low plasma vitamin B-12, and low plasma vitamin D binding protein. Estimates of total externalizing problems per the CBCL were also adjusted for screen time >30 h/wk and mother's education as continuous. Estimates of total internalizing problems per the CBCL and total externalizing problems per the YSR were also adjusted for screen time >30 h/wk. Empirical variances were specified in all models.

(**Supplemental Table 10**) problems were similar to those with the continuous behavior problems scores.

Associations with vitamin D.

There was a nonlinear inverse association between 25(OH)D concentrations and prevalence of clinical total externalizing and internalizing problems per the CBCL (**Supplemental Figure 2**). After multivariable adjustment, the prevalence of clinical total externalizing problems per the CBCL was 1.8 (95% CI: 1.1, 3.1; $P = 0.02$) times higher in children with VDD compared with that of children without VDD (**Supplemental Table 11**). VDD was not significantly related to clinical total internalizing problems.

Associations with DBP.

DBP was inversely related to clinical total externalizing problems per the CBCL (**Supplemental Figure 3**). Low DBP was related to an adjusted 1.8 (95% CI: 1.1, 2.9; $P = 0.02$) times higher prevalence of clinical total externalizing problems per the CBCL in multivariable analysis (**Supplemental Table 12**).

Mediation.

VDD did not mediate the association of DBP with clinical total externalizing problems according to the CBCL (percent mediated: 0.7).

Discussion

In this longitudinal study of schoolchildren, VDD in middle childhood was associated with higher parent- and self-reported total externalizing problems scores in adolescence, potentially due to higher aggressive and rule-breaking behavior. VDD was related to a 2-fold higher prevalence of clinical externalizing problems per parental report, which predict disruptive behavior disorders and MDD (4). In addition, middle-childhood VDD was related to higher parent-reported total internalizing problems scores in adolescence. Low middle-childhood DBP concentration was associated with higher self-reported aggressive behavior and anxious/depressed symptoms as well as higher prevalence of parent-reported clinical externalizing problems in adolescence. These associations were independent of child, parental, and household characteristics.

Most previous longitudinal studies focused on the potential effects of vitamin D status in pregnancy on behavior problems of the offspring. Early pregnancy 25(OH)D concentrations were inversely associated with externalizing problems in childhood (16), whereas late pregnancy 25(OH)D concentrations were not associated with this behavior problem outcome (10–15). VDD in pregnancy has not been associated with later internalizing behavior problems (10–12, 14–16) or a diagnosis of depression (13). The potential effects of early pregnancy VDD on behavior could relate to altered brain morphology and neurotransmitter metabolism, which may affect inhibitory and social responses, according to studies in rodents (40).

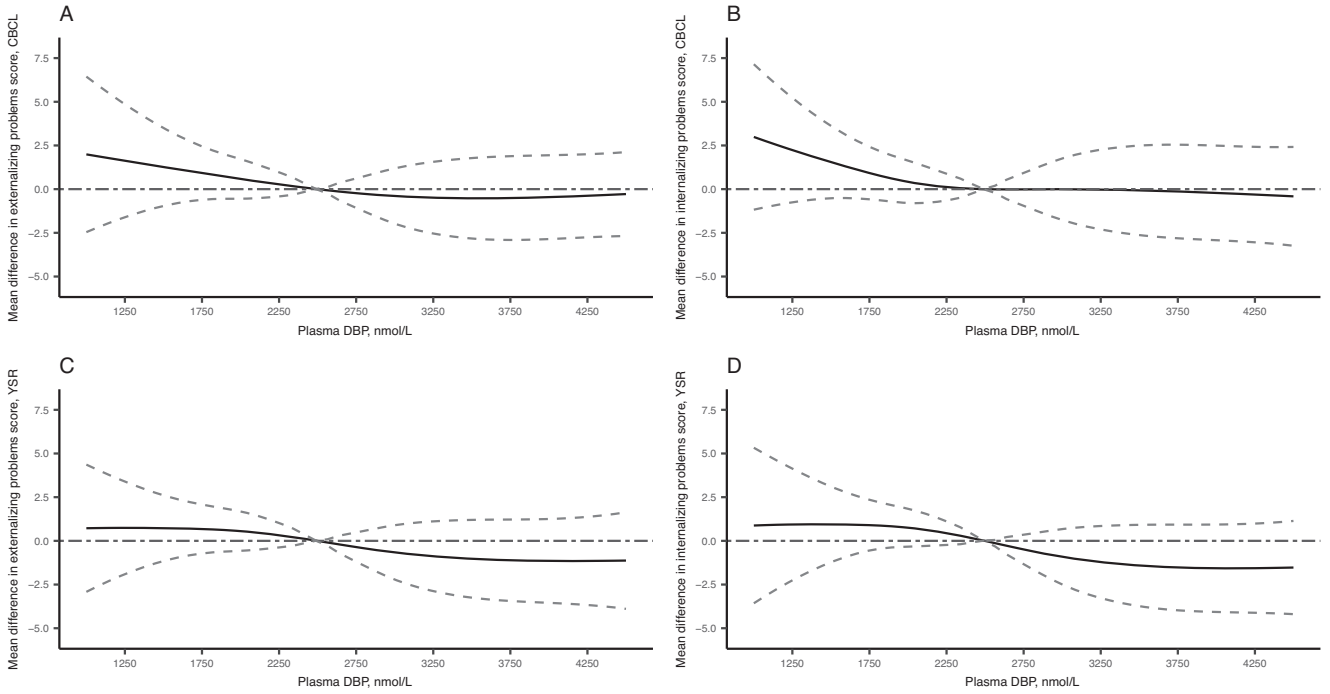

FIGURE 2 Mean adjusted differences (continuous line) with 95% CIs (dotted lines) in Child Behavior Checklist (CBCL) (A, B) and Youth Self-Report (YSR) (C, D) total behavior problems scores in adolescence according to plasma vitamin D binding protein (DBP) concentrations in middle childhood. Estimates are from linear regression models; total externalizing or internalizing problems were the continuous outcomes. Predictors for all models included linear and spline terms for DBP and child's sex, age (continuous), and low plasma vitamin B-12. Estimates of total externalizing problems per the CBCL were also adjusted for screen time >30 h/wk and mother's education as continuous. Estimates of total internalizing problems per the CBCL and total externalizing problems per the YSR were also adjusted for screen time >30 h/wk. Empirical variances were specified in all models.

Only 1 previous investigation has addressed the relation of vitamin D status in middle childhood and later behavior problems. Among English children, serum 25(OH)D concentration at age 9.9 y was inversely associated with parent-reported prosocial problems at age 11.7 y (17) and self-reported depressive symptoms, a type of internalizing problem, at age 13.8 y (18). Serum 25(OH)D concentration was not associated with externalizing problems at age 11.7 y (17);

TABLE 2 Behavior problems scores at 11–18 y of age according to plasma DBP concentrations in middle childhood among children from Bogota, Colombia[1]

	Child Behavior Checklist				Youth Self-Report			
	Plasma DBP			Adjusted difference (95% CI)[3]	Plasma DBP			Adjusted difference (95% CI)[3]
Behavior problems	≥2497 nmol/L ($n = 114$)	<2497 nmol/L ($n = 116$)	P^2		≥2497 nmol/L ($n = 136$)	<2497 nmol/L ($n = 137$)	P^2	
Externalizing problems								
Total	55.6 ± 9.1	57.3 ± 9.5	0.18	1.8 (–0.7, 4.2)	52.3 ± 9.1	54.0 ± 10.0	0.10	1.8 (–0.4, 4.0)
Aggressive behavior	58.8 ± 7.7	60.5 ± 8.8	0.19	1.8 (–0.5, 4.0)	55.3 ± 6.9	57.3 ± 8.0	0.02*	2.2 (0.4, 3.9)*
Rule-breaking behavior	54.9 ± 5.4	55.9 ± 5.6	0.12	1.1 (–0.3, 2.6)	54.8 ± 5.3	55.2 ± 5.5	0.67	0.5 (–0.8, 1.8)
Internalizing problems								
Total	56.7 ± 9.6	57.5 ± 10.0	0.42	1.3 (–1.3, 3.9)	53.1 ± 8.6	54.3 ± 10.9	0.16	1.4 (–1.0, 3.7)
Anxious/depressed	56.0 ± 7.0	56.9 ± 7.4	0.25	1.1 (–0.8, 3.0)	54.6 ± 5.6	56.5 ± 7.2	0.07	2.2 (0.6, 3.7)*
Withdrawn/depressed	56.5 ± 7.0	56.4 ± 6.4	0.78	0.4 (–1.4, 2.2)	55.4 ± 5.8	55.5 ± 6.5	0.91	0.0 (–1.5, 1.5)
Somatic complaints	61.2 ± 8.1	61.8 ± 8.3	0.56	0.9 (–1.3, 3.1)	56.4 ± 7.5	57.6 ± 7.9	0.21	1.3 (–0.5, 3.1)
Other problems								
Attention problems	53.9 ± 5.0	54.7 ± 5.7	0.41	0.7 (–0.7, 2.2)	52.2 ± 3.9	52.6 ± 3.9	0.15	0.5 (–0.5, 1.4)
Social problems	57.6 ± 6.2	58.4 ± 6.4	0.37	0.8 (–0.9, 2.5)	55.8 ± 6.0	57.0 ± 7.7	0.49	1.3 (–0.3, 3.0)
Thought problems	55.8 ± 6.8	56.2 ± 6.7	0.56	0.4 (–1.4, 2.3)	54.2 ± 4.9	55.4 ± 6.5	0.23	1.4 (0.0, 2.8)*

[1] Mean ± SD unless noted otherwise. *$P < 0.05$. DBP, vitamin D binding protein.
[2] Wilcoxon rank-sum test.
[3] From linear regression models with the behavior problems score as the continuous outcome. Adjustment variables included child's sex, age, and low plasma vitamin B-12. Total externalizing problems and externalizing problems subscales per the Child Behavior Checklist were also adjusted for screen time >30 h/wk and mother's education as continuous. Total internalizing problems per the Child Behavior Checklist, total externalizing problems per the Youth Self-Report, and their subscales were also adjusted for screen time >30 h/wk. Empirical variances were specified in all models.

however, this may be due to short follow-up, as externalizing behavior problems may develop later in adolescence (41, 42). Mechanisms through which VDD in middle childhood may influence behavioral development are speculative and may differ from those related to intrauterine exposure. During middle childhood, dopamine receptor density decreases in humans (43), which may indicate synaptic pruning. Lack of vitamin D could influence this process as 1,25(OH)2D enhances the expression of glial cell line–derived neurotrophic factor in animal models (44), which promotes the survival of dopaminergic neurons (45). Dopamine regulates emotions and motivation, and thus alterations in the dopaminergic system may have a long-lasting impact on behavior.

In our study, low DBP concentration was related to higher self-reported aggressive behavior and anxious/depressed symptoms. These associations were independent of middle-childhood VDD. This inverse relation is contrary to results from 2 case-control studies in adults in which DBP concentration was positively related to MDD (46, 47). Our results may not be comparable to those from the case-control studies due to reverse causation in the latter; MDD alters the inflammatory response, which may be associated with higher concentrations of DBP (48). Furthermore, exposure in adulthood could have different effects than it does in middle childhood. Mechanisms that might explain an inverse association between DBP concentration and behavior problems are uncertain. Although one might anticipate that low DBP concentrations result in increased free 25(OH)D, previous studies (21, 22) and our own analyses indicate that DBP and total 25(OH)D are positively correlated. Thus, the directions of the associations between total 25(OH)D and DBP with behavior outcomes are consistent. However, VDD did not mediate the associations between DBP and behavior problems; thus, a potential role of DBP might be independent of vitamin D. DBP binds extracellular actin in the central nervous system (49) and thus may prevent the tissue damage induced by this protein. Alleles that regulate actin cytoskeletal dynamics have been associated with several mental health disorders (50), but the role of extracellular actin on the development of mental health disorders is unclear.

This study has several strengths. Its prospective nature minimizes the possibility that results are due to reverse causation. In addition, the vitamin D biomarker we used integrates dietary and sunlight sources of the vitamin. We tested a novel hypothesis on the potential role of DBP on behavioral development. We were able to examine parent- and self-reported behavior problems with validated questionnaires (28) that have been used in populations similar to ours (27). The use of multiple informants allows the capture of information on behavior problems from different sources (51). The associations between VDD with externalizing and internalizing problems and their subscales were in the same direction across multiple informants. Adolescents may better report behaviors or feelings that are covert or unexpressed (51), whereas parents may be more able to objectively evaluate their child's behavior as parent-reported behavior problems of children are more closely correlated with diagnoses from the *Diagnostic and Statistical Manual of Mental Disorders* (52, 53). Weaker associations between VDD and the YSR measures may reflect more measurement error in self-reporting behavior problems among adolescents. Associations were also consistent when using clinically relevant cut points. Our study took place in a setting close to the equator, where seasonality does not have a strong effect on circulating concentrations of vitamin D (54). Finally, we were able to control for relevant potential confounders of the relation between vitamin D status or DBP concentration and behavior problems.

There are limitations as well. First, we lacked a baseline measurement of behavior; thus, reverse causation cannot be disregarded as an explanation for some of these results if the behavioral problems were already present in middle childhood and affected 25(OH)D or DBP status. Second, 25(OH)D and DBP concentrations may reflect long-term exposure, rather than middle-childhood exposure specifically. Third, there were a few children who were older at baseline than the youngest children were at follow-up and may have entered adolescence at the time of exposure assessment. This suggests that middle childhood may not be the only relevant exposure life stage but that exposure during early adolescence could also be important in relation to the development of behavioral problems later in life. We had limited statistical power to examine the associations stratified by age at recruitment. Fourth, we used a monoclonal assay to quantify DBP, and plasma concentrations of DBP vary by isoform (55). Among Hispanics, the predominant isoform is 1f/1f (34–35%), followed by 1s/2 (16–18%), 1s/1s (14–18%), 1f/2 (15–16%), 1f/1f (13–14%), and 2/2 (3–4%) (19, 56). If isoform distribution is related to behavior outcomes, our results may be confounded by genetics. In addition, the CVs for DBP were >10%, which suggests random measurement error. This error should be nondifferential with respect to the outcomes and would therefore bias the associations toward the null. Up to 88% of plasma 25(OH)D binds to DBP with high affinity and is not bioavailable (57). In addition, 25(OH)D concentration may vary with liver and kidney functions, sex hormones, and genetic polymorphisms. Thus, some investigators have advocated for the assessment of free vitamin D in studies of the vitamin's health effects (23). We lacked free vitamin D measurements in all participants due to financial constraints, and this is a limitation of the study. Nevertheless, in the small subsample of 19 participants in whom we quantified free 25(OH)D, the correlation between total and free 25(OH)D was high, suggesting that total 25(OH)D may appropriately represent bioavailable vitamin D in this population. Fifth, the analytic sample may not be representative of the broader cohort. Sixth, we lacked measurements of other potential confounders, including the family home environment, which are independent predictors of behavior and could influence vitamin D status by affecting time spent outdoors. Seventh, although associations of behavioral outcomes with environmental factors, including exposure to prenatal stress (58, 59), bisphenol A (60), and phthalates (61), have differed by sex, we lacked statistical power to examine the associations separately among boys and girls. In addition, statistical power for analyses of dichotomous endpoints may have been reduced compared with that for analyses of behavior problems as continuous outcomes. Finally, type I error may be enhanced by analyzing a large number of outcomes; thus, we cannot rule out chance as an explanation for the associations observed.

In conclusion, VDD in middle childhood was positively related to total externalizing and internalizing problems in adolescence. The associations with total externalizing problems were consistent with the use of 2 outcome assessment methods. VDD was also associated with a higher prevalence of clinically relevant parent-reported externalizing problems scores. Low DBP concentration in middle childhood was associated with self-reported aggressive behavior and anxious/depressed symptoms in adolescence. Additional studies involving different neurobehavioral outcomes in populations with varying distributions of vitamin D status and DBP are warranted.

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