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ORIGINAL RESEARCH

Role of Maternal Vitamin D_3 Levels in Shaping Adolescent Vascular Health: Evidence From a Spanish Population-Based Birth Cohort

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BACKGROUND: Low gestational vitamin D levels may increase offspring risk of cardiovascular disease from an early age. Studies investigating the impact on offspring macrovascular function have been inconsistent. Few included pulse wave velocity as an arterial stiffness indicator, and none included measures of microvascularization as an early marker of cardiovascular health. This study explored the association between gestational vitamin D levels and macro- and microvascular health across early adolescence.

METHODS AND RESULTS: We analyzed data from 430 mother–child pairs from a Spanish birth cohort. 25-hydroxyvitamin D_3 (vitamin D_3) levels were measured in serum at 13 weeks of pregnancy. At 11 and 15 years we assessed macrovascular parameters, including systolic and diastolic blood pressure (mm Hg) and pulse wave velocity (m/s), and microvascular parameters (central retinal artery/vein equivalent (μm)). We used continuous (in ng/mL) and categorical (deficient <20 ng/mL versus adequate >20 ng/mL) deseasonalized 25(OH)D_3 levels as exposure. Mixed effect and linear regression models were conducted. During their pregnancies, nearly 23% of the mothers had deficient vitamin D_3 levels. We did not find statistically significant associations between pregnancy vitamin D_3 levels and macro- and microvascular function markers across adolescence. However, subjects exposed to deficient vitamin D_3 levels showed a nonstatistically significant decrease in pulse wave velocity (β=−0.09 [95% CI, −0.19 to 0.01]) compared with those exposed to adequate levels. There was no evidence of a sex interaction.

CONCLUSIONS: Our findings show little evidence to support associations between low vitamin D levels during pregnancy and macro- or microvascular health parameters through early adolescence.

Key Words: adolescence ■ arterial stiffness ■ blood pressure ■ cardiovascular health ■ pregnancy ■ retinal microcirculation ■ vitamin D

Cardiovascular diseases represent a significant global health challenge, being the leading cause of death and disability worldwide.[1] The origins of cardiovascular diseases can be traced back to vascular or metabolic processes in childhood that may partially lie in fetal life.[2] For example, elevated blood pressure (BP) in children has been found to strongly predict hypertension in adulthood,[3] the primary modifiable risk factor for cardiovascular mortality.[1] Furthermore, children with obesity have increased pulse wave velocity

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CLINICAL PERSPECTIVE

What Is New?

- Our study is among the first to introduce retinal microcirculation measurements and to explore the association with pulse wave velocity as early markers of cardiovascular dysfunction in adolescents, expanding the understanding of prenatal vitamin D_3 impact beyond childhood.

What Are the Clinical Implications?

- Our findings provide little evidence to support a relationship between gestational vitamin D_3 levels and cardiovascular health markers in adolescents, which may reduce the immediate clinical urgency for vitamin D supplementation aimed at long-term cardiovascular outcomes.
- Future research on the impact of early life vitamin D on longer-term cardiovascular health requires longitudinal studies with larger sample sizes and consideration of postnatal vitamin D levels.

Nonstandard Abbreviations and Acronyms

25(OH)D_3	25-hydroxyvitamin D_3
AS	arterial stiffness
CRAE	central retinal artery equivalent
CRVE	central retinal vein equivalent
INMA	Infancia y Medio Ambiente
PWV	pulse wave velocity

(PWV), the gold standard technique for assessing arterial stiffness (AS), which has been linked with atherosclerosis in adulthood.[4,5] Additionally, structural changes in the retinal microvasculature during childhood, assessed through retinal vein and artery diameters, have been associated with later cardiovascular diseases.[6,7] Retinal vein widening is associated with inflammation and atherosclerosis and arterial narrowing with arterial damage.[7] These findings underscore the significance of these micro- and macrovascular markers measured in childhood as predictors of later-life vascular damage.

Early-life vitamin D levels have been identified as one of the risk factors for cardiovascular disease development,[8] a concern given the common prevalence of vitamin D deficiency in pregnant women and newborns.[9] Several studies have examined the relationship between early-life vitamin D and childhood BP,[8] but the evidence is inconclusive. Some studies support a protective effect of higher vitamin D levels in pregnancy or cord blood on childhood BP,[10–15] whereas others do not.[16–19] The effects of early-life vitamin D on other vascular markers such as PWV and retinal microcirculation have hardly been studied. Two small observational studies (<200 participants) suggest that prenatal vitamin D levels may not be linked to AS in children,[12,18] and no studies explored the effects of prenatal vitamin D levels on retinal microcirculation.

This study aims to investigate the relationship between maternal vitamin D levels during pregnancy and BP, PWV, and retinal microcirculation at ages 11 and 15.

METHODS

Data described in the article, code book, and analytic code will be made available upon request, subject to application and approval. External access procedures for INMA (Infancia y Medio Ambiente, Environment and Childhood) data are outlined in detail: https://www.proyectoinma.org/en/inma-project/inma-collaboration-policy/

Study Population

We used data from the Spanish population-based birth cohort INMA, specifically, from Sabadell, 1 of the 7 regions where the cohort is located. From July 2004 to July 2006, 657 women were recruited in the regional hospital between the 10 and 13 weeks of pregnancy. The inclusion criteria included at least 16 years old, singleton pregnancy, intention to deliver at reference hospital, and no assisted conception or communication issues.[20] Children and their families were followed up at 28 to 32 weeks gestation, birth, and child's aged 6 months and 1, 2, 4, 7, 9, 11, and 15 years. Information was obtained through medical registries, interview-based questionnaires with the mothers, and physical examinations of the children conducted by trained personnel.[20] The present analysis was limited to mother–child pairs with information on vitamin D levels during pregnancy and any cardiovascular measurement available either in the 11-year or the 15-year follow-up (N=259–423, depending on outcome and age) (Figure). The Ethics Review Board approved this study, and all participants signed a written consent (including adolescents at 15 years).

Vitamin D Assessment

Maternal vitamin D metabolic status was assessed using plasmatic levels of 25-hydroxyvitamin D_3 (25(OH)D_3, referred to as vitamin D_3 in this article) from a single maternal fasting blood sample. It was mainly collected in the second trimester of pregnancy (mean=13.6 [SD=1.8] weeks of gestation). Quantification of plasma levels of 25(OH)D_3 was performed by highperformance

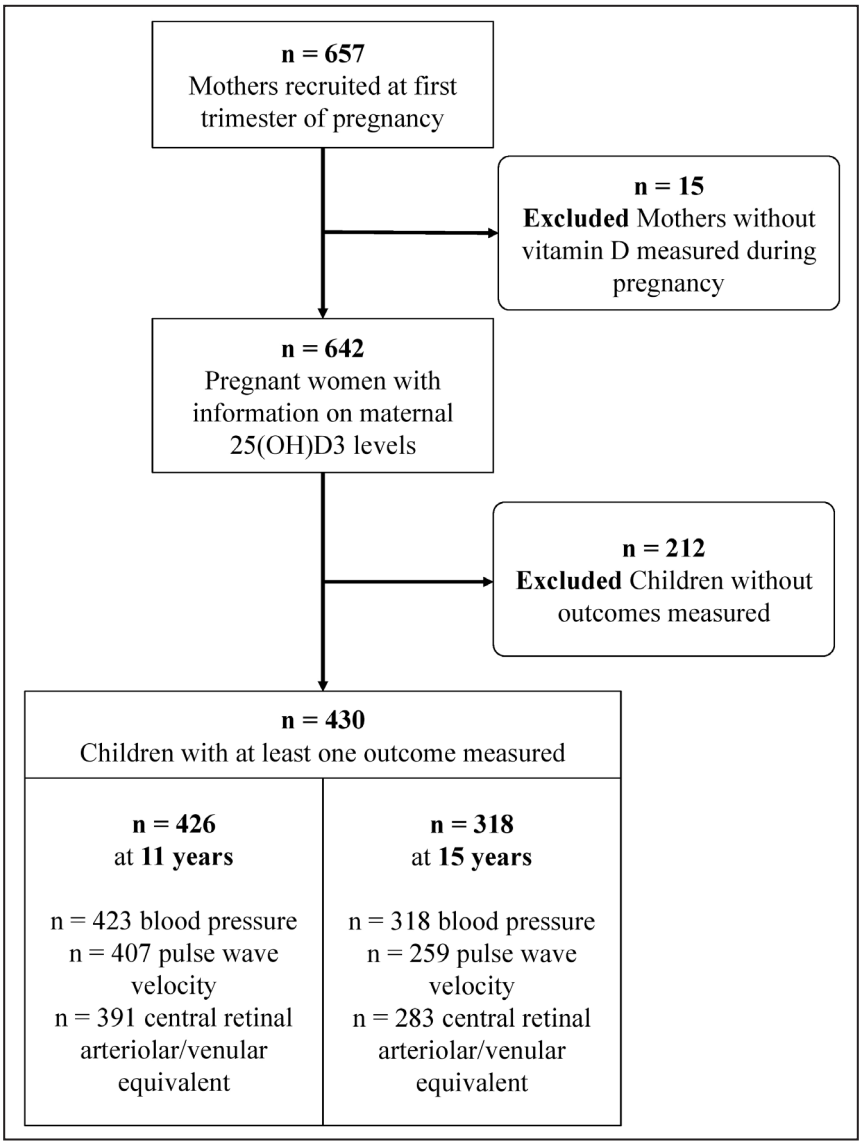

Figure. Flow chart of the study population.
25(OH)D_3 indicates 25-hydroxyvitamin D.

liquid chromatography using a BioRAD kit, according to Clinical and Laboratory Standard Institute protocols.[21] The detection limit was 5 ng/mL and the variation coefficient between assays was 4.5%. The assay was validated by German Programmes of External Evaluation of Quality, and results were satisfactory in 100% of the cases. To remove the effect of the season when the blood draw was obtained, we used deseasonalized vitamin D concentrations. The method was described in detail by Morales et al.[22]

Cardiovascular Measurements

Cardiovascular measurements were conducted by trained nurses from the INMA study during school hours at 11 years (mean 11.2 years) and 15 years (15.5 years). Children were primarily visited at their school place but, if not feasible, the visit occurred at a health care center.

Blood Pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed using an automated oscillometric device called OMROM 705IT (HEM-759-E, Omron Corporation, Kyoto, Japan), equipped with a brachial cuff. Following a 5-minute rest period, 3 consecutive measurements were taken at 1-minute intervals. The average of all measurements was computed, reported in millimeters of mercury (mm Hg) and used for the main analysis. For sensitivity analysis, age-, sex-, and height-adjusted *Z* scores were generated.[23]

Pulse Wave Velocity

Cardiofemoral PWV measurements were taken using the VICORDER Arterial Stiffness Testing System. To minimize measurement error, the 80% method was employed (multiplying the directly measured carotid-femoral distance by 0.8). Three waveform measurements were recorded, ensuring the child remained relaxed, and the nurse observed a stable wave pattern. PWV values were averaged from the 3 initial measurements and reported in m/sec.

Central Retinal Artery/Vein Equivalent

Retinal images were captured using Canon CR2-Plus Non-Mydriatic Retinal Camera for the 11-year follow-up and Canon CR2 Non-Mydriatic Retinal Camera for the 15-year follow-up. High-resolution images were taken for both eyes and analyzed using MONA-REVA software (version 2.1.1) developed by VITO (Mol, Belgium; http:\\mona.health). The central retinal vein equivalent (CRVE) and central retinal artery equivalent (CRAE) were calculated using the Parr–Hubbard–Knudtson formula 21.[24] To find the average CRVE and CRAE, we followed the "big 6."[25] For the 11-year follow-up, a trained researcher processed the measurements, and for the 15-year follow-up, the average of measurements by 2 trained researchers was calculated to minimize observer variability. The values obtained are presented in micrometers (μm). Finally, age- and sex-adjusted *Z* scores were created to facilitate the comparison of results between 11 and 15 years.

Covariates

Maternal and child characteristics were obtained through questionnaires administered to the mothers during pregnancy (race, age at delivery, prepregnancy body mass index, education, social class [coded according to the *International Standard Classification of Occupations-88* system], smoking during pregnancy, parity, cardiovascular history [defined as any cardiometabolic event], adherence to Mediterranean diet score,[26] and self-perception of physical activity) and at 1 year of the child (duration of breastfeeding [any]). Child's sex, birth weight, and gestational age were obtained from medical records.

Statistical Analysis

First, we compared the characteristics of children included and not included in the study. We assessed departures from linearity in the relationships between deseasonalized pregnancy vitamin D_3 levels and the cardiovascular outcomes at the 11- and 15-year follow-ups using general additive models. A few of the associations deviated from linearity (data not shown). Consequently, we decided to explore associations using vitamin D_3 levels as a continuous variable (per 5 ng/mL decrease) and as a categorical variable, categorized as deficient (≤20 ng/mL) and adequate (>20 ng/mL, reference category).[27] We also explored mean±SD or percentages of sociodemographic and anthropometric characteristics across the categories of maternal vitamin D. To mitigate bias due to missing data (missing values between 0% and 2.8%) and enhance statistical power, covariates were imputed by the multiple imputation by chained equations package in R.[28] Ten imputed data sets were generated, and no substantial differences in study population characteristics were found between the original and the pooled imputed data sets. The subsequent analyses are exclusively based on the pooled imputed data set.

Second, we estimated associations between prenatal vitamin D_3 levels, both continuous and categorical, and the different cardiovascular measurements across adolescence using linear mixed-effects regression models to examine the overall effect of vitamin D_3 levels across the 2 ages. We considered the identification number of each subject as a random effect to account for the correlation between repeated measures within the same individual across different ages. Then, we used separate linear regression models to estimate the associations at 11 years and 15 years. Estimates are presented as β-coefficients and their corresponding 95% CIs. Covariates were selected using directed acyclic graphs, and all models were adjusted for maternal race, prepregnancy body mass index, maternal age at delivery, maternal education, maternal social class, maternal smoking during pregnancy, parity, adherence to Mediterranean diet and physical activity during pregnancy, and maternal cardiometabolic history. SBP, DBP, and PWV models were adjusted for sex, height, and age at the time of measurement (Figure S1).

We conducted a series of sensitivity analyses to assess the robustness of our findings. First, we replicated all models without adjusting for covariates and then, using the complete case data set instead of the imputed. Second, we explored the association using raw vitamin D levels while adjusting models for the season of blood sampling. Third, to evaluate potential effect modification by sex, we tested for interaction and performed stratified models (*P* value for interaction <0.10). Fourth, we introduced an alternative approach to BP measurement using age-, sex-, and height-adjusted *Z* scores. Fifth, we also repeated models for PWV removing those children with incomplete measurements (<3 measurements), and then adjusting models for mean arterial pressure to assess AS independent of variations in BP. Finally, we repeated CRAE and CRVE models removing participants without "good quality" measures for both eyes (as assessed by a trained

researcher and computer software) and additionally, we excluded 5% of pictures with the largest between-operator difference from the 15-year follow-up. Statistical significance was set at P <0.05 for multivariate analyses. Statistical analyses were performed with R version 4.1.2 (The R Foundation, Vienna, Austria).

RESULTS

Characteristics of the Population

Characteristics of the study population are shown in Table 1. Almost all mothers were of White origin and were, on average, 32 years old. Twenty-eight percent of the mothers had overweight or obesity, more than a quarter smoked at the beginning of pregnancy (27%), and slightly less than a quarter had 1 or more cardiometabolic diagnoses (23%). Mean maternal vitamin D_3 levels during pregnancy were 29.6 ng/mL, and 22.6% of the mothers had deficient levels (<20 ng/mL). There were few differences in the population characteristics across categories of maternal vitamin D. Mothers with deficient vitamin D_3 levels were younger, lower educated, from higher social class, and more likely to have no previous pregnancy compared with mothers with adequate levels (Table 1). Differences in population characteristics between included and excluded populations are shown in Table S1.

Among the 426 participants included at 11 years, the average height was 147 cm and the mean body mass index was 19.5 kg/m^2. Average SBP and DBP were 103.1 mm Hg and 60.9 mm Hg, respectively (Table 2). The average PWV was 4.4 m/s, and mean CRAE and CRVE measures were 181.2 μm and 252.3 μm, respectively. The 15-year assessment was conducted in 318 participants. They were, on average, 167.1 cm tall and had a body mass index of 22.2 kg/m^2. Mean SBP and DBP were 103.8 mm Hg and 64.1 mm Hg, respectively (Table 2). Average PWV was 5.1 m/s, and for CRAE and CRVE measures were 131.8 μm and 205.9 μm, respectively. We found some sex-related differences in cardiovascular measures. At 11 years, boys had a slightly higher PWV and lower CRAE and CRVE than girls. At 15 years, boys were taller with higher SBP, lower DBP, and increased PWV compared with girls. Averages and SD for each cardiovascular measurement by sex can be found in Table 2. Correlation coefficients between cardiovascular measurements can be found in Figure S2. Briefly, all cardiovascular measures exhibited positive correlations when comparing their values between 11 and 15 years, with BP measures showing a weak to moderate correlation, PWV a moderate correlation, and retinal measurements a strong correlation. Furthermore, SBP and DBP exhibited moderate correlations across age periods. PWV showed moderate associations with BP measures. Finally, retinal measurements showed generally weak correlation with BP or PWV.

Vitamin D_3 and Cardiovascular Outcomes

Table 3 displays the associations between continuous and categorical vitamin D_3 levels during pregnancy and cardiovascular outcome measurements across both ages and at 11 and 15 years. There were no statistically significant associations between continuous or categorical vitamin D_3 levels during pregnancy and SBP, DBP, or PWV. We observed some associations that did not reach statistical significance: deficient pregnancy 25(OH)D_3 levels (<20 ng/mL) showed a tendency toward higher SBP and DBP at 15 years (β=1.91 [95% CI, −0.51 to 4.33] and β=1.21 [95% CI, −0.77 to 3.18], respectively), compared with adequate levels (>20 ng/mL). Also, there was a borderline nonsignificant association between lower vitamin D_3 levels during pregnancy and reduced PWV across adolescence (per 5 ng/mL decrease, β=−0.02 [95% CI, −0.04 to 0.00] and deficient versus adequate, β=−0.09 [95% CI, −0.19 to 0.01]). This borderline nonsignificant association was also observed at 15 years (per 5 ng/ml decrease, β=−0.02 [95% CI, −0.05 to 0.01] and deficient versus adequate, β=−0.14 [95% CI, −0.29 to 0.02]). Finally, we found no associations between pregnancy vitamin D_3 and retinal microvasculature across the 2 study visits.

Sensitivity Analysis

Results from crude and complete case analyses revealed similar results. In the unadjusted models, the previous borderline nonsignificant association with PWV was attenuated (β=−0.00 [95% CI, −0.03 to 0.03]) (Table S2). In addition, in the complete-case analyses, we observed a borderline nonsignificant association between deficient vitamin D_3 levels during pregnancy and higher SBP at 15 years (β=2.40 [95% CI, −0.12 to 4.91]) (Table S2). Moreover, the associations using raw vitamin D_3 levels as exposure variable revealed minor differences compared with deseasonalized vitamin D_3 levels, but no statistically significant associations or substantial changes were observed (data not shown). Regarding effect modification analysis, there was no evidence for sex interaction (P for interaction >0.1); therefore, stratified analysis by sex displayed similar results for boys and girls (data not shown). Similar findings were observed using BP Z scores instead of the raw BP variables (Table S3). No significant differences were observed between participants with complete and incomplete PWV measures, except at the 15-year follow-up, where the previously borderline nonsignificant association was attenuated (Table S3). Conversely, in models adjusting for mean arterial pressure, lower pregnancy vitamin D_3 levels significantly reduced PWV across adolescence and at the 15-year follow-up (Table S3). Finally, models restricted to participants

Table 1. Maternal and Child Characteristics of the Total Included Population (n=430), and Depending on the Vitamin D_3 Category During Pregnancy (<20 ng/mL) and Adequate (>20 ng/mL)

	Included population	Deficient	Adequate	
		n=97	N=333	*P* value‡
Maternal characteristics	†	†	†	
Maternal race, % White	98.4	99.0	98.2	0.943
Maternal age, y (SD)	31.9 (4.2)	31.2 (4.0)	32.1 (4.2)	0.044
Maternal prepregnancy body mass index, kg/m^2 (SD)	23.8 (4.6)	23.6 (4.3)	23.9 (4.7)	0.678
Maternal education, % (428)*				
Primary or les	24.3	34.4	21.4	0.015
Secondary	42.5	41.6	42.8	
High	33.2	24.0	35.8	
Maternal social class, %				
Semiskilled/unskilled	23.3	15.5	25.5	0.030
Skilled manual/nonmanual	34.0	30.9	34.8	
Professionals and managers	42.7	53.6	39.6	
Maternal smoking pregnancy, % (425)				
Never	72.9	66.0	75.0	0.190
Only early in pregnancy	14.8	17.5	14.0	
During whole pregnancy	12.3	16.5	11.0	
Parity, % (428)				
No previous pregnancies	57.7	67.7	54.8	0.033
25-hydroxyvitamin Dat 13 wks, ng/mL	29.6 (11.1)			
Deficient (<20 ng/mL)	22.6	…	…	…
Adequate (>20 ng/mL)	77.4			
Physical activity, % (428)				
Sedentary	6.3	6.2	6.3	0.646
Little active	23.6	26.0	22.9	
Moderately active	43.2	45.8	42.5	
Quite/very active	26.9	21.9	28.3	
Adherence to Mediterranean diet, % (428)				
Low	41.3	45.4	40.1	0.499
Medium	31.5	26.8	32.8	
High	27.3	27.8	27.1	
Maternal cardiometabolic history, % 1 or more diagnoses (418)	23.0	24.2	22.6	0.850
Child characteristics				
Sex, % male	50.7	52.6	50.2	0.760
Birth weight, gs (SD)	3263 (399)	3260 (427)	3263 (391)	0.941
Gestational age, wks (SD)	39.7 (1.3)	39.8 (1.4)	39.7 (1.3)	0.591
Preterm (<37 wks), %	2.1	4.1	1.5	0.236
Breastfeed, wks (SD) (429)	93.0	28.8 (19.9)	27.3 (20.1)	0.509

*N in brackets refer to total population where this deviates from 430 for a specific variable.
†Values are percentages for categorical variables and mean±SD for continuous variables. Nonimputed data.
‡*P* value was estimated by using 1-way ANOVA or chi-square tests.

with "good quality" measures for microvasculature at both eyes did not show substantial changes. Similarly, models excluding the 5% of pictures with the largest between-operator difference at the 15-year follow-up demonstrated consistent results.

DISCUSSION

In this prospective population-based study conducted in Spain there was insufficient evidence to support a relationship between prenatal vitamin D_3 levels during

Table 2. Child Outcomes Characteristics for the Total Population and Distributed by Sex

Outcomes	Total population	Boys	Girls	*P* value
11 y	N=426	n=218	n=208	
Age, y (SD)	11.2 (0.5)	11.1 (0.5)	11.2 (0.5)	0.307
Height, cm (SD)	146.7 (7.9)	146.3 (7.6)	147.2 (8.2)	0.237
BMI, kg/m^2 (SD)	19.5 (3.7)	19.7 (4.1)	19.3 (3.3)	0.176
SBP, mm Hg (SD)	103.1 (9.7)	103.8 (9.9)	102.3 (9.4)	0.114
DBP, mm Hg (SD)	60.9 (7.4)	60.9 (7.5)	60.8 (7.4)	0.913
PWV, m/s	4.4 (0.5)	4.5 (0.5)	4.3 (0.4)	<0.001
CRAE, µm	181.2 (12.8)	179.4 (12.4)	183.1 (13.1)	0.004
CRVE, µm	252.3 (17.4)	250.5 (17.5)	254.2 (17.0)	0.033
15 y	N=318	n=157	n=161	
Age, y (SD)	15.5 (0.6)	15.4 (0.7)	15.6 (0.6)	0.086
Height, cm (SD)	167.1 (8.1)	172.0 (7.0)	162.5 (6.0)	<0.001
BMI, kg/m^2 (SD)	22.2 (4.2)	22.4 (4.6)	22.0 (3.8)	0.487
SBP, mm Hg (SD)	103.8 (9.7)	107.4 (9.7)	100.2 (8.2)	<0.001
DBP, mm Hg (SD)	64.1 (7.3)	62.0 (7.3)	66.0 (6.8)	<0.001
PWV, m/s	5.1 (0.6)	5.3 (0.6)	5.0 (0.5)	<0.001
CRAE, µm	131.8 (11.0)	130.4 (10.7)	133.2 (11.1)	0.035
CRVE, µm	205.9 (15.0)	205.3 (15.6)	206.6 (14.3)	0.470

Values are mean±SD for continuous variables. *P* values (girls vs boys) were estimated by using *t* test. Nonimputed data. BMI indicates body mass index; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; DBP, diastolic blood pressure; PWV, pulse wave velocity; and SBP, systolic blood pressure.

pregnancy and offspring macro- and microvascular function measurements in adolescence.

Previous research has explored the potential link between pregnancy vitamin D levels and macrovascular function parameters like BP or PWV. Although several studies have examined the association with BP,[10–19] often yielding inconsistent results, fewer have explored the association with PWV. Consistent with our findings, other studies have also found no significant association between maternal vitamin D levels during pregnancy and BP in late childhood or adolescence.[10,11,17,18] For example, the ALSPAC (Avon Longitudinal Study of Parents and Children) cohort (England) investigated this association at ages 10 and 15 years, noting a borderline inverse association with SBP at 10 years (N=~4000), which disappeared by age 15 (N=~2000).[11] Similarly, a study in Denmark (N=410) found no links between pregnancy vitamin D levels and BP at 20 years.[10] Additionally, 2 studies assessing BP at 9 years, one in Southampton, England (N=178)[18] and another in India (N=225),[17] reported no significant associations. In contrast, a recent cohort study in Baltimore (USA) involving 590 mother–child pairs found that both low prenatal and childhood (1.2 years) vitamin D levels were independently associated with higher BP during school age and adolescence.[13] Notably, the study included participants aged 6 to 18 years old, with a mean age of 10.3 years, which may not precisely represent the adolescence period. However, evidence from other studies assessing younger age groups supports the association between prenatal vitamin D levels and BP,[12,14,15,19] although some conflicting findings exist.[16,17] Our results indicate a tendency for higher SBP and DBP in 15-year-old adolescents exposed to deficient prenatal vitamin D levels. This trend was nearly statistically significant when analyzing the complete case data set (β=2.40 [95% CI, −0.12 to 4.91]) or raw vitamin D levels (β=2.42 [95% CI, −0.09 to 4.94]). Additionally, we identified a potential association between deficient prenatal vitamin D_3 levels and decreased PWV, although its significance varied across sensitivity analyses. Previous studies examining the link between prenatal vitamin D levels and offspring AS yielded null results, similar to our findings.[12,18] These studies, like ours, were conducted in healthy populations and had smaller sample size (~150 participants). Limited sample size and the relatively young ages of the participants might have contributed to a reduced variability in AS or insufficient arterial damage, potentially explaining the lack of associations. Additionally, no previous studies have investigated the link between maternal vitamin D levels during pregnancy and retinal microvascular measurements (CRAE and CRVE). Our study is the first attempt to explore this association, but we did not find any evidence of an influence, regardless of whether vitamin D was treated as a continuous or a categorical variable. Similar to PWV measures, our young and healthy population displayed limited variability in retinal measures, which may explain the absence of an association.

Table 3. Associations of Maternal 25(OH)D_3 Levels, per 5 ng/mL Decrease and Categorical (Deficient vs Adequate), With Cardiovascular Outcomes Across Adolescence and at 11-y and 15-y Follow-Ups With Systolic and Diastolic Blood Pressure, Pulse Wave Velocity, Central Arteriolar, and Vein Equivalent

Outcome	Vitamin D_3	Across adolescence*		11 y†		15 y†	
		No.	β (95% CI)	No.	β (95% CI)	No.	β (95% CI)
Systolic blood pressure	Continuous (5 ng/mL decrease)	317	−0.05 (−0.38 to 0.28)	423	−0.30 (−0.70 to 0.10)	318	0.02 (−0.44 to 0.49)
	Deficient ‡	70	1.04 (−0.73 to 2.81)	95	0.12 (−1.98 to 2.21)	70	1.91 (−0.51 to 4.33)
Diastolic blood pressure	Continuous (5 ng/mL decrease)	313	−0.12 (−0.38 to 0.15)	422	−0.24 (−0.57 to 0.09)	318	0.04 (−0.33 to 0.42)
	Deficient	70	0.59 (−0.83 to 2.02)	95	0.33 (−1.39 to 2.05)	70	1.21 (−0.77 to 3.18)
Pulse wave velocity	Continuous (5 ng/mL decrease)	247	−0.02 (−0.04 to 0.00)	407	−0.01 (−0.03 to 0.01)	259	−0.02 (−0.05 to 0.01)
	Deficient	58	−0.09 (−0.19 to 0.01)	91	−0.05 (−0.15 to 0.05)	61	−0.14 (−0.29 to 0.02)
Age- and sex-adjusted *Z* score for central retinal arteriolar equivalent	Continuous (5 ng/mL decrease)	240	0.02 (−0.02 to 0.06)	383	0.01 (−0.04 to 0.05)	261	0.02 (−0.03 to 0.07)
	Deficient	57	0.05 (−0.15 to 0.26)	88	0.06 (−0.17 to 0.29)	60	−0.05 (−0.32–0.22)
Age- and sex-adjusted *Z* score for central retinal venular equivalent	Continuous (5 ng/mL decrease)	240	0.00 (−0.04 to 0.04)	383	−0.00 (−0.05 to 0.04)	261	0.01 (−0.04–0.06)
	Deficient	57	0.00 (−0.20 to 0.21)	88	−0.01 (−0.24 to 0.22)	60	−0.03 (−0.30 to 0.25)

All models were adjusted for race, maternal age at conception, prepregnancy body mass index, maternal education level and social class, adherence to Mediterranean diet and physical activity during pregnancy, and maternal cardiometabolic history. Systolic blood pressure, diastolic blood pressure, and pulse wave velocity were additionally adjusted for child's sex and child's age and height at cardiovascular assessment.

*Multivariable mixed effect models using imputed data.

†Multivariate linear regression models using imputed data.

‡Vitamin D_3 levels dichotomized: deficient: <20 ng/mL; adequate (reference category): ≥20 ng/mL.

Our results contrast with biological evidence suggesting potential long-term impacts of low prenatal vitamin D levels on the macro- and microvascular health of offspring. Pregnancy vitamin D levels have been linked to various aspects of development, including immune system development,[29] retinal maturation,[30] and nephrogenesis.[31] Animal studies indicate that vitamin D deficiency during pregnancy may lead to increased nephron and glomeruli numbers but reduced size and delayed maturation.[31] Moreover, it has been linked to hypermethylation of the *Panx1* gene promoter, which is associated with elevated SBP and DBP in the offspring due to impaired endothelial relaxation.[32] However, these adverse effects of deficient early-life vitamin D levels may be mitigated during childhood. Vitamin D plays a multifaceted role in influencing cardiovascular health,[8] influencing BP regulation via the renin-angiotensin-aldosterone system and parathyroid hormone regulation. It also affects inflammation, blood vessel regulation, endothelial and vascular smooth muscle cells, and atherosclerosis processes. Although cross-sectional observational studies suggest a connection between low serum vitamin D levels and higher BP or other cardiovascular parameters in children, adolescents, and adults, vitamin D supplementation has generally shown limited efficacy in reducing BP or improving other cardiometabolic markers.[33,34] However, randomized control trials often vary in parameters such as dosage, frequency of administration, trial duration, baseline vitamin D levels, and sample size, which may contribute to inconsistent results.

Our study possesses several strengths. First, its longitudinal design allowed us to explore the influence of prenatal exposure on offspring cardiovascular health across 2 time points in adolescence. This extended observation period enabled us to capture potential long-term effects, providing potential valuable insights into the impact of prenatal conditions on offspring health. Additionally, we introduced novel measures of vascular health, such as PWV and retinal microcirculation, which contribute to a more comprehensive understanding of cardiovascular risk based on both macro- and microvascular function. Investigating the association between pregnancy vitamin D levels and these specific vascular outcomes represents unexplored territory in the scientific literature.

The study also had limitations. First, our reliance on a single assessment of vitamin D during pregnancy may not fully capture the dynamic changes throughout gestation. However, it is noteworthy that our samples were collected at the end of the first trimester, coinciding with the initiation of nephrogenesis, which is particularly relevant as vitamin D deficiency has been shown to affect this process.[31] Moreover, a recent study examining vitamin D levels at different stages of pregnancy reported that early pregnancy showed the strongest associations with BP.[15] Additionally, the lack of data on vitamin D levels during adolescence is

especially relevant because vitamin D has been linked with cardiovascular markers and BP in some cross-sectional studies.[35] In our study, we primarily assessed vitamin D status using 25(OH)D_3 levels. Despite the fact that most of the 25(OH)D is in the 25(OH)D_3 form,[36] it does not reflect circulating levels of 25(OH)D_2 or other metabolites such as bioavailable 25(OH)D or the active form (1,25(OH)$_2$D), which have been associated to certain health outcomes. This limitation may have contributed to exposure misclassification. Furthermore, even though vitamin D levels were determined using high-performance liquid chromatography rather than mass spectrometry, which is considered the gold standard technique for assessing vitamin D levels, existing evidence suggests a strong concordance between both methods in terms of accuracy and precision.[37,38] Our study's outcome assessments, focusing on macro- and microvascular markers in a healthy population, may have limited our ability to detect associations due to reduced variability between individuals. However, previous research has linked these markers with future cardiovascular issues, even in asymptomatic children.[4,6] Nevertheless, it remains uncertain how responsive these cardiovascular outcomes are across adolescence. Also, these outcomes may be influenced by other factors, such as pubertal status, or child lifestyle factors, such as dietary habits, physical activity, and general health status. In our directed acyclic graphs, these factors were not considered to act as direct confounders and were not included in the models; the models were adjusted for maternal lifestyle factors. However, residual confounding by these are other unmeasured factors cannot be entirely ruled out. Also, factors such as pubertal status may act as effect modifiers, but the relatively small sample size in our study made it challenging to conduct a reliable effect modification analysis, which is a limitation that should be addressed in future studies.

Although many studies have investigated health effects related to BP, fewer have explored PWV, and our study represents the first to incorporate retinal imaging. Regarding retinal imaging, at 11 years 1 operator analyzed the images whereas at 15 years we used the mean between 2 new different operators, which could account for the disparities in measurements between the 2 time points. However, the measurements taken at both ages showed a strong positive correlation and to overcome this limitation, we created sex- and age-adjusted *Z* scores, generating comparable values between the 2 ages. Future studies with larger sample sizes could replicate our findings and explore the potential modifying influences of lifestyle factors. Additionally, some selection bias might be present due to loss-to-follow-up, but previous work suggests it is unlikely to bias associations between vitamin D and child health outcomes.[39]

CONCLUSIONS

There is little evidence for an association between pregnancy vitamin D3 levels and adolescent cardiovascular health, based on this prospective study that includes macro- and microvascular health measurements at the ages of 11 and 15 of the study participants. To enhance our understanding, replicating this study with a larger sample size, enabling the analysis of effect modification by other factors such as pubertal status, and incorporating measures of vitamin D during childhood and adolescence could be instrumental in broadening our insights into these relationships.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

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REFERENCES

1. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The global burden of cardiovascular diseases and risk. *J Am Coll Cardiol*. 2022;80:2361–2371. doi: 10.1016/j.jacc.2022.11.005
2. Ritz E, Amann K, Koleganova N, Benz K. Prenatal programming: effects on blood pressure and renal function. *Nat Rev Nephrol*. 2011;7:137–144. doi: 10.1038/nrneph.2011.1
3. Kelly RK, Thomson R, Smith KJ, Dwyer T, Venn A, Magnussen CG. Factors affecting tracking of blood pressure from childhood to adulthood: the childhood determinants of adult health study. *J Pediatr*. 2015;167:1422–1428. doi: 10.1016/j.jpeds.2015.07.055
4. Cote AT, Harris KC, Panagiotopoulos C, Sandor GGS, Devlin AM. Childhood obesity and cardiovascular dysfunction. *J Am Coll Cardiol*. 2013;62:1309–1319. doi: 10.1016/j.jacc.2013.07.042
5. Hudson LD, Rapala A, Khan T, Williams B, Viner RM. Evidence for contemporary arterial stiffening in obese children and adolescents using pulse wave velocity: a systematic review and meta-analysis. *Atherosclerosis*. 2015;241:376–386. doi: 10.1016/j.atherosclerosis.2015.05.014
6. Newman AR, Andrew NH, Casson RJ. Review of paediatric retinal microvascular changes as a predictor of cardiovascular disease. *Clin Experiment Ophthalmol*. 2017;45:33–44. doi: 10.1111/ceo.12773
7. Li LJ, Ikram MK, Wong TY. Retinal vascular imaging in early life: insights into processes and risk of cardiovascular disease. *J Physiol*. 2016;594:2175–2203. doi: 10.1113/JP270947
8. Savastio S, Pozzi E, Tagliaferri F, Degrandi R, Cinquatti R, Rabbone I, Bona G. Vitamin D and cardiovascular risk: which implications in children? *Int J Mol Sci*. 2020;21:3536. doi: 10.3390/ijms21103536
9. Saraf R, Morton SMB, Camargo CA, Grant CC. Global summary of maternal and newborn vitamin D status—a systematic review. *Matern Child Nutr*. 2016;12:647–668. doi: 10.1111/mcn.12210
10. Rytter D, Bech BH, Halldorsson TI, Henriksen TB, Grandström C, Cohen A, Olsen SF. Maternal vitamin D status at week 30 of gestation and offspring cardio-metabolic health at 20 years: a prospective cohort study over two decades. *PLoS One*. 2016;11:1–12. doi: 10.1371/journal.pone.0164758
11. Williams DM, Fraser A, Fraser WD, Hyppönen E, Davey Smith G, Deanfield J, Hingorani A, Sattar N, Lawlor DA. Associations of maternal 25-hydroxyvitamin D in pregnancy with offspring cardiovascular risk factors in childhood and adolescence: findings from the AVON longitudinal study of parents and children. *Heart*. 2013;99:1849–1856. doi: 10.1136/heartjnl-2013-303678
12. Sauder KA, Stamatoiu AV, Leshchinskaya E, Ringham BM, Glueck DH, Dabelea D. Cord blood vitamin D levels and early childhood blood pressure: the healthy start study. *J Am Heart Assoc*. 2019;8:1–12. doi: 10.1161/JAHA.118.011485
13. Wang G, Liu X, Bartell TR, Pearson C, Cheng TL, Wang X. Vitamin D trajectories from birth to early childhood and elevated systolic blood pressure during childhood and adolescence. *Hypertension*. 2019;74:421–430. doi: 10.1161/HYPERTENSIONAHA.119.13120
14. Larsen SD, Dalgård C, Christensen ME, Lykkedegn S, Andersen LB, Andersen M, Glintborg D, Christesen HT. Blood pressure in 3-year-old girls associates inversely with umbilical cord serum 25-hydroxyvitamin D: an Odense child cohort study. *Endocr Connect*. 2018;7:1236–1244. doi: 10.1530/EC-18-0308
15. Pedersen JN, Dalgård C, Möller S, Andersen LB, Birukov A, Andersen MS, Christesen HT. Early pregnancy vitamin D status is associated with blood pressure in children: an Odense child cohort study. *Am J Clin Nutr*. 2022;116:470–481. doi: 10.1093/ajcn/nqac118
16. Miliku K, Felix JF, Voortman T, Tiemeier H, Eyles DW, Burne TH, McGrath JJ, Jaddoe VWV. Associations of maternal and fetal vitamin D status with childhood body composition and cardiovascular risk factors. *Matern Child Nutr*. 2019;15:e12672. doi: 10.1111/mcn.12672
17. Krishnaveni GV, Veena SR, Winder NR, Hill JC, Noonan K, Boucher BJ, Karat SC, Fall CH. Maternal vitamin D status during pregnancy and body composition and cardiovascular risk markers in Indian children: the Mysore Parthenon study. *Am J Clin Nutr*. 2011;93:628–635. doi: 10.3945/ajcn.110.003921
18. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr*. 2008;62:68–77. doi: 10.1038/sj.ejcn.1602680
19. Hrudey EJ, Reynolds RM, Oostvogels AJJM, Brouwer IA, Vrijkotte TGM. The association between maternal 25-hydroxyvitamin D concentration during gestation and early childhood cardiometabolic outcomes: is there interaction with pre-pregnancy BMI? *PLoS One*. 2015;10:1–17. doi: 10.1371/journal.pone.0133313
20. Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardón A, Torrent M, et al. Cohort profile: the INMA - INfancia y medio Ambiente - (environment and childhood) project. *Int J Epidemiol*. 2012;41:930–940. doi: 10.1093/ije/dyr054
21. Instruction Manual BIO-RAD. *25(OH)-Vitamin D3 by HPLC*. Muchen, Germany: Laboratories GmbH; 2003.
22. Morales E, Guxens M, Llop S, Rodríguez-Bernal CL, Tardón A, Riaño I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. *Pediatrics*. 2012;130:913–920. doi: 10.1542/peds.2011-3289
23. Adolescents. National High Blood Pressure Education Program Working Group on high blood pressure in children and adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics*. 2004;114:555–576. doi: 10.1542/peds.114.2.S2.555
24. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143–149. doi: 10.1076/ceyr.27.3.143.16049
25. Khan A, De Boever P, Gerrits N, Akhtar N, Saqqur M, Ponirakis G, Gad H, Petropoulos IN, Shuaib A, Faber JE, et al. Retinal vessel multifractals predict pial collateral status in patients with acute ischemic stroke. *PLoS One*. 2022;17:e0267837. doi: 10.1371/journal.pone.0267837
26. Buckland G, González CA, Agudo A, Vilardell M, Berenguer A, Amiano P, Ardanaz E, Arriola L, Barricarte A, Basterretxea M, et al. Adherence to the mediterranean diet and risk of coronary heart disease in the Spanish EPIC cohort study. *Am J Epidemiol*. 2009;170:1518–1529. doi: 10.1093/aje/kwp282
27. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930. doi: 10.1210/jc.2011-0385
28. Van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med*. 1999;18:681–694. doi: 10.1002/(SICI)1097-0258(19990330)18:6<681::AID-SIM71>3.0.CO;2-R
29. Noriega DB, Savelkoul HFJ. Vitamin D and allergy susceptibility during gestation and early life. *Nutrients*. 2021;13:1015. doi: 10.3390/nu13031015
30. Alsalem JA, Patel D, Susarla R, Coca-Prados M, Bland R, Walker EA, Rauz S, Wallace GR. Characterization of vitamin D production by human ocular barrier cells. *Invest Ophthalmol Vis Sci*. 2014;55:2140–2147. doi: 10.1167/iovs.13-13019
31. Nascimento FAM, Ceciliano TC, Aguila MB, Mandarim de Lacerda CA. Maternal vitamin D deficiency delays glomerular maturity in F1 and F2 offspring. *PLoS One*. 2012;7:e41740. doi: 10.1371/journal.pone.0041740
32. Meems LM, Mahmud H, Buikema H, Tost J, Michel S, Takens J, Verkaik-Schakel RN, Vreeswijk-Baudoin I, Mateo-Leach IV, van der Harst P, et al. Parental vitamin D deficiency during pregnancy is associated with increased blood pressure in offspring via Panx1 hypermethylation. *Am J Physiol Heart Circ Physiol*. 2016;311:1459–1469. doi: 10.1152/ajpheart.00141.2016

33. Abboud M. Vitamin d supplementation and blood pressure in children and adolescents: a systematic review and meta-analysis. *Nutrients*. 2020;12:1163. doi: 10.3390/nu12041163
34. Hauger H, Laursen RP, Ritz C, Mølgaard C, Lind MV, Damsgaard CT. Effects of vitamin D supplementation on cardiometabolic outcomes in children and adolescents: a systematic review and meta-analysis of randomized controlled trials. *Eur J Nutr*. 2020;59:873–884. doi: 10.1007/s00394-019-02150-x
35. Dolinsky DH, Armstrong S, Mangarelli C, Kemper AR. The association between vitamin D and cardiometabolic risk factors in children: a systematic review. *Clin Pediatr*. 2013;52:210–223. doi: 10.1177/0009922812470742
36. Dirks NF, Ackermans MT, Lips P, de Jongh RT, Vervloet MG, de Jonge R, Heijboer AC. The when, what & how of measuring vitamin D metabolism in clinical medicine. *Nutrients*. 2018;10:482. doi: 10.3390/nu10040482
37. Roth HJ, Schmidt-Gayk H, Weber H, Niederau C. Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography-tandem mass spectrometry as a reference. *Ann Clin Biochem*. 2008;45:153–159. doi: 10.1258/acb.2007.007091
38. Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK. HPLC method for 25-hydroxyvitamin D measurement: comparison with contemporary assays. *Clin Chem*. 2006;52:1120–1126. doi: 10.1373/clinchem.2005.064956
39. Sangüesa J, Sunyer J, Garcia-Esteban R, Abellan A, Esplugues A, Garcia-Aymerich J, Guxens M, Irizar A, Júlvez J, Luque-García L, et al. Prenatal and child vitamin D levels and allergy and asthma in childhood. *Pediatr Res*. 2023;93:1745–1751. doi: 10.1038/s41390-022-02256-9
40. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol*. 2016;45:1887–1894.