ORIGINAL RESEARCH

Cord Blood Vitamin D Levels and Early Childhood Blood Pressure: The Healthy Start Study

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***Background*—**Vitamin D deficiency is associated with cardiovascular events among adults, but it is unclear whether early-life vitamin D deficiency influences cardiovascular risk factors in children.

***Methods and Results*—**We measured total and bioavailable 25-dihydroxyvitamin D (25OHD) in cord blood and in blood from 4- to 6-year-old children, and we assessed cardiovascular risk factors (blood pressure, arterial stiffness, body size, and adiposity) at 4 to 6 years. We tested for racial/ethnic differences in total and bioavailable 25OHD (n=715) and modeled the adjusted association between cord blood 25OHD and childhood cardiovascular risk factors (n=171). We observed racial/ethnic differences in total and bioavailable 25OHD levels in both cord and child blood samples (all $P<0.05$). Each 25-nmol/L increase in cord blood total 25OHD was associated with a 2.5–mm Hg (SE 0.8) decrease in systolic blood pressure ($P=0.002$) and a 1.7–mm Hg (SE 0.6) decrease in diastolic blood pressure ($P=0.01$), independent of childhood 25OHD levels, race/ethnicity, and other covariates. There was no association between cord blood total 25OHD and any other cardiovascular risk factors. Cord blood levels of bioavailable and free 25OHD were not associated with any cardiovascular risk factor in childhood.

***Conclusions*—**In this diverse prebirth cohort, we observed lower systolic and diastolic blood pressure among children with higher total 25OHD levels at birth. Our findings suggest that intrauterine exposure to vitamin D may contribute to early-life programming of offspring blood pressure. Intervention studies are needed to determine whether increasing fetal vitamin D exposure can reduce the risk of elevated blood pressure in childhood. **(*J Am Heart Assoc*. 2019;8:e011485. DOI: 10.1161/JAHA.118.011485.)**

Key Words: blood pressure • developmental origins • vitamin D

Cardiovascular disease risk factors, such as overweight/obesity, dyslipidemia, and elevated blood pressure, are increasingly being observed in children.[1–6] The manifestation of these previously adult concerns in pediatric populations suggests that exposures very early in life, even before birth, may be contributing to cardiovascular risk factor development.[7] Intrauterine exposures, including micronutrient deficiencies, have been shown to alter fetal development and trigger changes in organ structure, physiology, and metabolism that increase risk for chronic disease later in life.[8,9] Vitamin D is relevant to cardiovascular health because of its role in regulating vascular smooth muscle cell proliferation and the renin-angiotensin system.[10,11] Numerous longitudinal studies have reported a significantly increased risk of future cardiovascular events among adults with low vitamin D levels.[12–15] However, the degree to which intrauterine vitamin D deficiency may increase cardiovascular risk in children is not well understood.

Intrauterine vitamin D deficiency results from inadequate maternal intake of vitamin D from foods or exposure to ultraviolet B (sunshine) during pregnancy.[16] Maternal vitamin D is metabolized into 25-dihydroxyvitamin D (25OHD), freely crosses the placental barrier, and is the only source of vitamin D available to the developing fetus.[17] One-third of pregnant women in the United States are vitamin D deficient, despite high use of vitamin D–containing prenatal supplements,[18] and thus a high proportion of infants are vitamin D deficient at birth.[19,20] Experimental animal studies have shown that low maternal vitamin D intake in pregnancy is associated with increased blood pressure and endothelial dysfunction in adolescent-aged offspring.[21] Observational human studies have reported that higher maternal 25OHD in mid-late pregnancy is associated with lower offspring systolic blood

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Clinical Perspective

What Is New?

- Higher vitamin D levels in cord blood are associated with lower systolic and diastolic blood pressure at 4 to 6 years of age.

What Are the Clinical Implications?

- Intrauterine exposure to vitamin D may contribute to early-life programming of offspring blood pressure.
- Increasing fetal vitamin D exposure may reduce the risk of elevated blood pressure in childhood.

pressure[22] and fat mass[23] in childhood, although other studies reported no association of maternal 25OHD with offspring blood pressure, adiposity, or arterial stiffness in childhood.[24–26] The null studies did not account for childhood 25OHD, which may modify the association between early-life vitamin D intake and cardiovascular risk. Moreover, none of these studies measured bioavailable 25OHD, which is the amount of circulating vitamin D that is not bound tightly to vitamin D binding protein (VDBP).[27] Bioavailable 25OHD is more strongly related to several health outcomes than total circulating 25OHD,[27–30] including endothelial dysfunction in adolescent females.[31] However, the relative significance of total versus bioavailable 25OHD levels at birth and in early childhood in terms of future cardiovascular health is not clear.

The purpose of this study was to (1) describe the relative concentrations of total and bioavailable 25OHD in cord blood and early childhood samples in a racial/ethnically diverse population and (2) evaluate the association of each form of vitamin D in cord blood with early-childhood cardiovascular risk factors. We hypothesized that there would be racial/ethnic differences in vitamin D levels at each time point, and that low cord blood levels would be associated with cardiovascular risk factors in childhood, independent of vitamin D levels in childhood.

Methods

Participants

This study included a subset of participants from Healthy Start, which is an ongoing, longitudinal prebirth cohort of 1410 ethnically diverse pregnant women and their offspring in Colorado. From 2009 to 2014, women were recruited from the obstetric clinic at the University of Colorado Anschutz Medical Campus. Women were eligible if they were pregnant with a single fetus, were aged ≥16 years, and had no history of chronic disease (diabetes mellitus, cancer, psychiatric conditions, or steroid-dependent asthma) or obstetric complications (previous delivery <25 weeks, previous stillbirth). Participants completed research visits in early pregnancy (median 17 weeks' gestation), mid-pregnancy (median 27 weeks' gestation), at delivery (median 1 day after birth), and in early infancy (median 5 months), late infancy (median 22 months), and early childhood (median 5 years as of October 1, 2018). The study protocol was approved by the Colorado Multiple Institutional Review Board. Maternal participants provided written informed consent, and offspring participants aged ≥7 years provided written assent. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Blood Sample Collection

Cord blood samples were obtained at delivery, stored on ice for up to 20 minutes, and processed by centrifugation. Serum aliquots were stored at 4°C for up to 24 hours before being transported (on ice) to an −80°C freezer for long-term storage. Childhood blood samples were obtained at the childhood visit, allowed to clot for 15 to 30 minutes, and processed by centrifugation. Serum aliquots were immediately stored in an −80°C freezer. Cord blood and childhood serum samples were stored for up to 6.4 (mean, 4.8±0.6) and 3.0 years (mean, 1.9±0.6), respectively, before analysis of vitamin D. Funds were available to measure vitamin D in a subset of cord blood samples (n=660) and childhood samples (n=275), which were selected based on stored sample volume.

Vitamin D Measurements

According to the free-hormone hypothesis, biological activity of a hormone depends on the concentration of the hormone that is free, or unbound to a protein, in the blood.[32] Approximately 90% of 25OHD is bound tightly to VDBP, whereas the remaining 10% is bound loosely to albumin or completely unbound (free) in the circulation.[33] Estimation of bioavailable 25OHD requires calculating total 25OHD and the relative amounts bound to VDBP, bound to albumin, and unbound. Given the low binding affinity of 25OHD to albumin, both free and albumin-bound 25OHD are considered to be bioavailable.

Assays were performed by the University of Colorado Clinical and Translational Sciences Institute Core Laboratory. Total 25OHD was measured with the iSYS 25OHD assay (ImmunoDiagnostic Systems, Tyne & Wear, UK). This assay is US Food and Drug Administration approved, certified in the Vitamin D Standardization Program,[34,35] and has been validated against liquid chromatography/tandem mass spectrometry methods.[36,37] It has 100% cross-reactivity with

$25OHD_2$ and $25OHD_3$.[38] VDBP was measured with the Human Vitamin D BP Quantikine ELISA (R&D Systems, Minneapolis, MN). Albumin was measured on the Beckman/Coulter AU480 chemistry analyzer using Beckman Coulter reagents (Beckman Coulter, Brea, CA). Published equations were used to estimate free and bioavailable vitamin D.[27,39] Free 25OHD was calculated as total 25OHD/((albumin binding coefficient×[albumin])+(VDBP binding coefficient×[VDBP]), using genotype-nonspecific binding affinity coefficients for VDBP (0.7×10^9 M^{-1}) and albumin (6×10^5 M^{-1}).[27] Albumin-bound 25OHD was calculated as [free 25OHD]×albumin binding coefficient×[albumin]. Free and albumin bound 25OHD are summed to calculate bioavailable 25OHD. Total and bioavailable 25OHD are reported in nmol/L, and free 25OHD is reported in pmol/L.

Cardiovascular Risk Factor Assessments

Blood pressure, arterial stiffness, body size, and body composition were measured in offspring at the childhood visit. Systolic and diastolic blood pressure (mm Hg) were measured in a seated position, after 5 minutes of rest, with an automated blood pressure monitor (Dinamap V100, GE Carescape; GE Healthcare, Waukesha, WI). Three readings were taken, with the average used for analysis. Offspring carotid-femoral pulse wave velocity was measured while supine by tonometry (SphgymoCor CPVH; AtCor Medical Pty Ltd, Sydney, New South Wales, Australia). Spot ECG was used to record heart rhythms, and the distance between the suprasternal notch and the carotid and femoral artery measurement sites was measured. Tonometry was used to obtain waveforms at each site, which are gated by the R-wave on the ECG. Carotid-femoral pulse wave velocity was calculated by the distance between the carotid and femoral sites (in meters) divided by the difference in time that the foot of the R-wave is recorded at each site (in seconds). Greater velocity (m/s) indicates greater arterial stiffness. Height was measured to the nearest 0.1 cm with a stadiometer and weight to the nearest 0.1 kg using an electronic scale, while the child was dressed in light indoor clothing and no shoes. Age- and sex-specific body mass index (BMI) z-scores were calculated using the Centers for Disease Control and Prevention standards[40,41] and used as an assessment of body size. Offspring body composition was measured by whole-body air displacement plethysmography (BOD POD with Pediatric Option; COSMED, Rome, Italy). This device uses a 2-compartment model to estimate fat mass (adipose tissue; g and percent of total mass) and fat-free mass (water, bone, etc; g and percent of total mass). Trained personnel took 2 measurements on each child, with a third measurement taken if the percent fat mass differed by >2%. The average of the 2 closest readings was used for analysis.

Covariates

Maternal age at delivery was calculated from the self-reported date of maternal birth and date of delivery. Maternal race/ethnicity, education, household income, and number of previous term pregnancies (gravidity) were obtained from self-report at enrollment. Prepregnant weight, gestational weight gain, and gestational diabetes mellitus were obtained from medical records. Smoking in pregnancy (including frequency and number of cigarettes) was assessed by self-report at the prenatal and delivery research visits. Physical activity in pregnancy was assessed at the prenatal and delivery research visits with the Pregnancy Physical Activity Questionnaire.[42] Maternal average daily kilocalories was estimated from up to 8 days of 24-hour recall data processed with the National Cancer Institute's measurement error model,[43,44] as described previously.[45] Offspring gestational age at birth was estimated by prenatal ultrasound measurements and/or self-reported first day of last menstrual period. Offspring sex was obtained from maternal report at the delivery visit. Offspring age was calculated from the date of birth recorded at the delivery visit and offspring age at the childhood study visit.

Statistical Analyses

We calculated unadjusted means (SDs) of total, bioavailable, and free 25OHD among all eligible participants and by racial/ethnic groups (Hispanic [all races], non-Hispanic white, non-Hispanic black, and other). Because the majority of vitamin D is synthesized by sun exposure and influenced by skin pigmentation (ie, melanin), we used maternal race/ethnicity for cord blood analyses, and child race/ethnicity for childhood blood analyses. We used a general linear univariate model to determine whether there were racial/ethnic differences in vitamin D levels, using separate models for each time point. Cord blood analyses were adjusted for sex, gestational age at birth, and maternal race/ethnicity. Childhood analyses were adjusted for sex, child age, and child race/ethnicity. When the main effect of race was significant, we used Tukey-adjusted *P* values to determine significant differences between each pair of racial/ethnic groups.

We also examined the association between cord blood vitamin D levels with childhood cardiovascular risk factors. We used separate general linear univariate models for each type of vitamin D (total, bioavailable, and free) and each cardiovascular outcome. Given our interest in racial/ethnic differences, we tested for an interaction between cord blood vitamin D levels and race/ethnicity. When nonsignificant, this interaction was removed from the model, and only main effects were interpreted. Models were sequentially adjusted for potential confounders identified from published literature.[22,24–26] Model 1 was adjusted for child sex and childhood

values of age, the relevant 25OHD (eg, when total 25OHD in cord blood was the predictor, total 25OHD in childhood was included as a covariate), and (for blood pressure and pulse wave velocity outcomes only) childhood BMI z-score. Model 2 included the model 1 covariates plus maternal race/ethnicity, prepregnant BMI, gestational weight gain, and gestational diabetes mellitus. Model 3 included the model 2 covariates plus maternal prenatal smoking, physical activity, daily kilocalories, age at delivery, education, income, gravidity, and offspring gestational age at birth.

Healthy Start participants were included in the descriptive analysis if they had cord blood or childhood blood samples available for vitamin D analyses as of January 30, 2018. Participants were included in the regression analysis if they had vitamin D measured in both cord blood and childhood blood samples, had at least 1 cardiovascular risk factor measured at the childhood visit, and complete data on covariates. All analyses were conducted in SAS software (version v9.4; SAS Institute Inc, Cary, NC). A 2-sided $P<0.05$ was considered statistically significant.

Results

The final analyses included data from 715 of the 1410 Healthy Start participants (Figure 1), with 632 and 258 contributing data to the descriptive analysis of 25OHD levels in cord blood and childhood, respectively. Of these, 175 were included in the childhood cardiovascular outcomes analysis. Participant characteristics for the full Healthy Start cohort (n=1410) and the 2 analytical subsets (n=715 and 171) are reported in Table 1.

Unadjusted means and SDs of total, free, and bioavailable 25OHD are reported in Table 2, overall and by racial/ethnic groups at each time point. After adjustment for age and sex, we observed significant racial/ethnic differences for all vitamin D types and time points (Figure 2, all $P<0.05$). In cord blood samples, non-Hispanic whites had significantly higher levels of total 25OHD compared with all other groups (all Tukey, $P<0.0001$). Non-Hispanic blacks had significantly higher levels of bioavailable and free 25OHD compared with Hispanics (all Tukey, $P<0.02$), whereas non-Hispanic whites and other races had intermediate levels that were not significantly different from any other group. In childhood blood samples, non-Hispanic whites had significantly higher levels of total, bioavailable, and free 25OHD compared with Hispanics and non-Hispanic blacks (all Tukey, $P<0.001$). Participants of other races had significantly higher free 25OHD compared with non-Hispanic blacks (Tukey, $P=0.04$).

In terms of the association between cord blood vitamin D levels and childhood cardiovascular outcomes, we did not observe any significant interaction between vitamin D levels and race/ethnicity; thus, only main effects are reported. We observed significant inverse associations of cord blood levels of total 25OHD with childhood systolic ($P<0.01$ in all models) and diastolic blood pressure ($P=0.01$ in all models; Table 3). Each 25-nmol/L increase in total 25OHD at birth was

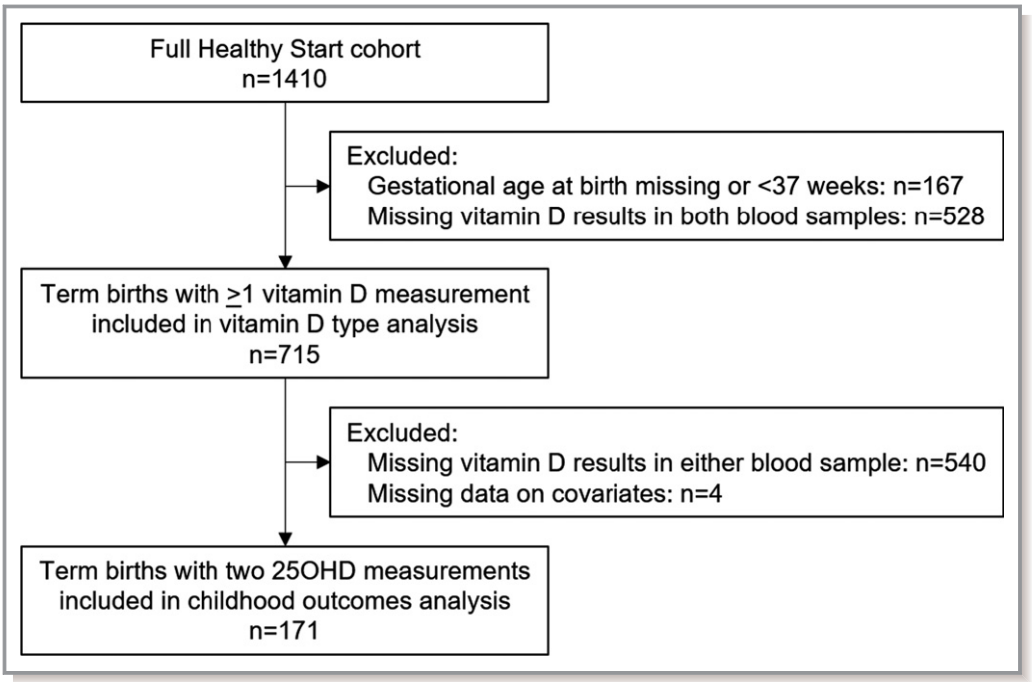

Figure 1. Participant flow diagram. 25OHD indicates 25-dihydroxyvitamin D.

Table 1. Participant Characteristics

	Full Healthy Start Cohort		Vitamin D Type Analysis		Childhood Outcome Analysis	
	n	Mean (SD) or n (%)	n	Mean (SD) or n (%)	n	Mean (SD) or n (%)
Maternal characteristics						
Age, y	1410	27.8 (6.2)	715	27.6 (6.2)	171	27.8 (6.1)
Race, n	1410		715		171	
Hispanic		351 (25%)		180 (25%)		40 (23%)
Non-Hispanic white		751 (53%)		387 (54%)		88 (51%)
Black		219 (16%)		109 (15%)		34 (20%)
Other		89 (6%)		39 (5%)		9 (5%)
Education, n	1410		715		171	
<High school degree		204 (14%)		109 (15%)		30 (18%)
High school degree		259 (18%)		131 (18%)		23 (13%)
Some college or 2-y degree		334 (24%)		173 (24%)		41 (24%)
4-y degree		309 (22%)		149 (21%)		40 (23%)
Graduate degree		304 (22%)		153 (21%)		37 (22%)
Household income, n	1410		715		171	
<$40 000		414 (29%)		217 (30%)		51 (30%)
$40 000 to $70 000		260 (18%)		138 (19%)		27 (16%)
>$70 000		460 (33%)		225 (31%)		63 (37%)
Missing/do not know		276 (20%)		135 (19%)		30 (18%)
Gravidity (live births), n	1410	1.4 (1.5)	715	1.3 (1.5)	171	1.2 (1.3)
Prepregnant BMI, kg/m^2	1406	25.7 (6.2)	715	26.2 (6.6)	171	27.2 (7.8)
Gestational weight gain, kg	1404	13.2 (6.9)	715	14.1 (6.6)	171	13.6 (6.4)
Gestational diabetes mellitus, n	1270	55 (4%)	688	30 (4%)	171	9 (5%)
Prenatal smoking, n	1410	124 (9%)	715	66 (9%)	171	13 (8%)
Daily oral vitamin D intake during pregnancy (IU)	1363	641 (497)	696	645 (476)	169	602 (421)
Daily energy intake in pregnancy, kcal	1363	2062 (387)	697	2058 (385)	171	2076 (381)
Maternal physical activity in late pregnancy (METS)	1311	166.8 (90.8)	704	166.4 (86.5)	171	168.4 (90.2)
Offspring characteristics						
Female, n	1342	646 (48%)	715	341 (48%)	171	79 (46%)
Race, n	1410			715		
Hispanic		394 (28%)		208 (29%)		49 (29%)
Non-Hispanic white		724 (51%)		370 (52%)		83 (49%)
Black		210 (15%)		102 (14%)		30 (18%)
Other		82 (6%)		35 (5%)		9 (5%)
Gestational age at birth, weeks	1331	39.2 (1.9)	715	39.6 (1.1)	171	39.5 (1.1)
Season of birth, n	1363		715		171	
Summer (June, July, August)		405 (30%)		222 (31%)		62 (36%)
Fall (September, October, November)		328 (24%)		167 (23%)		40 (23%)
Winter (December, January, February)		305 (22%)		148 (21%)		22 (13%)
Spring (March, April, May)		325 (24%)		178 (25%)		47 (27%)

Continued

Table 1. Continued

	Full Healthy Start Cohort		Vitamin D Type Analysis		Childhood Outcome Analysis	
	n	Mean (SD) or n (%)	n	Mean (SD) or n (%)	n	Mean (SD) or n (%)
Cord blood 25OHD						
Total, nmol/L	660	55.8 (21.2)	632	55.8 (21.1)	171	54.9 (21.4)
Bioavailable, nmol/L	625	9.0 (4.7)	599	9.0 (4.7)	161	9.0 (5.1)
Free, pmol/L	625	25.5 (13.4)	599	25.4 (12.8)	161	24.8 (13.0)
Childhood 25OHD						
Total, nmol/L	275	77.9 (21.5)	258	77.7 (21.5)	171	78.0 (21.4)
Bioavailable, nmol/L	275	10.0 (2.7)	258	10.0 (2.7)	171	10.0 (2.7)
Free, pmol/L	275	25.2 (6.5)	258	25.2 (6.5)	171	25.3 (6.5)
Age at childhood visit, y	516	4.7 (0.6)	351	4.7 (0.7)	171	4.7 (0.6)
Systolic blood pressure, mm Hg	512	97.7 (9.2)	348	97.7 (8.7)	170	98.1 (8.1)
Diastolic blood pressure, mm Hg	512	56.8 (6.0)	348	57.0 (6.1)	170	57.1 (6.3)
Pulse wave velocity, m/s	340	4.4 (0.9)	225	4.4 (0.8)	125	4.4 (0.9)
BMI z-score	511	0.16 (1.09)	349	0.21 (1.13)	171	0.23 (1.07)
Waist-to-height ratio	511	0.48 (0.04)	348	0.48 (0.04)	170	0.48 (0.04)
Total mass, kg	442	18.0 (3.2)	309	18.4 (3.3)	153	18.4 (3.6)
Fat-free mass, kg	442	14.1 (2.4)	309	14.3 (2.4)	153	14.4 (2.4)
Fat mass, kg	442	3.9 (1.6)	309	4.0 (1.7)	153	4.0 (1.7)
Adiposity, %	442	21.6 (6.2)	309	21.6 (6.3)	153	21.4 (5.7)

25OHD indicates 25-dihydroxyvitamin D; BMI, body mass index; METS, metabolic equivalents.

associated with a 2.5–mm Hg decrease in systolic blood pressure and 1.7–mm Hg decrease in diastolic blood pressure at a mean age of 4.7 years (SD, 0.6). There was no significant association between cord blood total 25OHD with any other cardiovascular risk factors. Cord blood levels of bioavailable and free 25OHD were not significantly associated with any cardiovascular risk factor in childhood (data not shown).

Discussion

In this diverse prebirth cohort, we observed lower systolic and diastolic blood pressure among children with higher total 25OHD levels at birth. This association was independent of 25OHD levels in childhood and did not differ by race/ethnicity. We did not observe any association between cord blood total 25OHD and childhood arterial stiffness or body size and composition, nor any relationship of bioavailable 25OHD with any cardiovascular risk factor. Our findings suggest that intrauterine exposure to vitamin D may contribute to early-life programming of offspring blood pressure, and that optimizing prenatal vitamin D may be a potential strategy for reducing the risk of elevated blood pressure in childhood.

Our blood pressure findings are consistent with previous studies: The ALSPAC (Avon Longitudinal Study of Parents and Children) reported lower systolic blood pressure at 9.9 years among children born to mothers with higher total 25OHD levels at 25 weeks' gestation,[22] and the Odense Child Cohort study reported lower systolic and diastolic blood pressure in 3-year-old female children who had higher total 25OHD in cord blood.[46] The ALSPAC results were also independent of childhood 25OHD levels, again demonstrating that low intrauterine vitamin D levels may have effects on offspring blood pressure that persist after the exposure period ends. In vitro studies provide a plausible biological pathway for this effect: 1α,25-dihydroxyvitamin D (1,25OHD), the active vitamin D hormone, has been shown to suppress renin gene expression[47,48] and regulate both vascular smooth muscle cell proliferation[11] and cardiomyocyte development.[49] Higher levels of active vitamin D also inhibit parathyroid hormone secretion and therefore may be able to help prevent the increases in blood pressure, vascular tone, and vascular stiffness that result from elevations in parathyroid hormone.[50] These studies suggest that low 25OHD levels during critical periods of fetal development may alter cardiovascular structure and function with lasting effects on blood pressure. We note that our effect estimates did not noticeably change upon further adjustment for potential covariates, which suggests that there is minimal confounding and adds credence to the above biological pathway. Randomized clinical trials are now needed to conclusively determine whether increasing fetal

Table 2. Vitamin D Levels Among All Participants and by Racial/Ethnic Groups

	All Participants		Hispanic		Non-Hispanic White		Non-Hispanic Black		Other	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Cord blood levels										
Total 25OHD, nmol/L	632	55.8 (21.1)	163	48.4 (19.4)	343	63.4 (19.7)	90	43.5 (18.7)	36	48.4 (19.5)
Bioavailable 25OHD, nmol/L	599	9.0 (4.7)	155	8.3 (4.9)	326	9.2 (4.1)	87	10.1 (6.0)	31	8.4 (4.4)
Free 25OHD, pmol/L	599	25.4 (12.8)	155	23.0 (13.4)	326	25.9 (11.4)	87	28.5 (15.6)	31	23.3 (12.5)
Childhood levels										
Total 25OHD, nmol/L	258	77.7 (21.5)	75	70.2 (16.6)	125	85.1 (22.8)	48	70.2 (17.2)	10	78.3 (25.8)
Bioavailable 25OHD, nmol/L	258	10.0 (2.7)	75	9.1 (2.2)	125	10.9 (2.8)	48	8.7 (1.9)	10	11.0 (3.5)
Free 25OHD, pmol/L	258	25.2 (6.5)	75	22.9 (4.9)	125	27.5 (6.8)	48	22.4 (5.3)	10	26.2 (8.1)

Data are unadjusted means (SDs), stratified by maternal race/ethnicity for cord blood samples and child race/ethnicity for childhood samples. 25OHD indicates 25-dihydroxyvitamin D.

vitamin D exposure can reduce offspring blood pressure in humans.

We note variation in the reported magnitude of the association between 25OHD and systolic blood pressure: we observed a 2.6–mm Hg decrease with every 25-nmol/L increase in cord blood 25OHD, whereas the Odense study reported a 0.7–mm Hg decrease with every 10-nmol/L increase in cord blood 25OHD,[46] and the ALSPAC study reported only a 0.48–mm Hg decrease with every 50-nmol/L increase in mid-gestational 25OHD.[22] These differences may be attributed to measurement timing for the exposure and outcome; indeed, 3 other studies found no association between maternal 25OHD in early-mid pregnancy with offspring blood pressure at 5 to 9 years of age.[24–26,51] It is possible that cord blood 25OHD levels, which reflect neonatal status at birth, are more relevant to future blood pressure than maternal 25OHD status earlier in gestation, but this can only be confirmed in future studies that include multiple

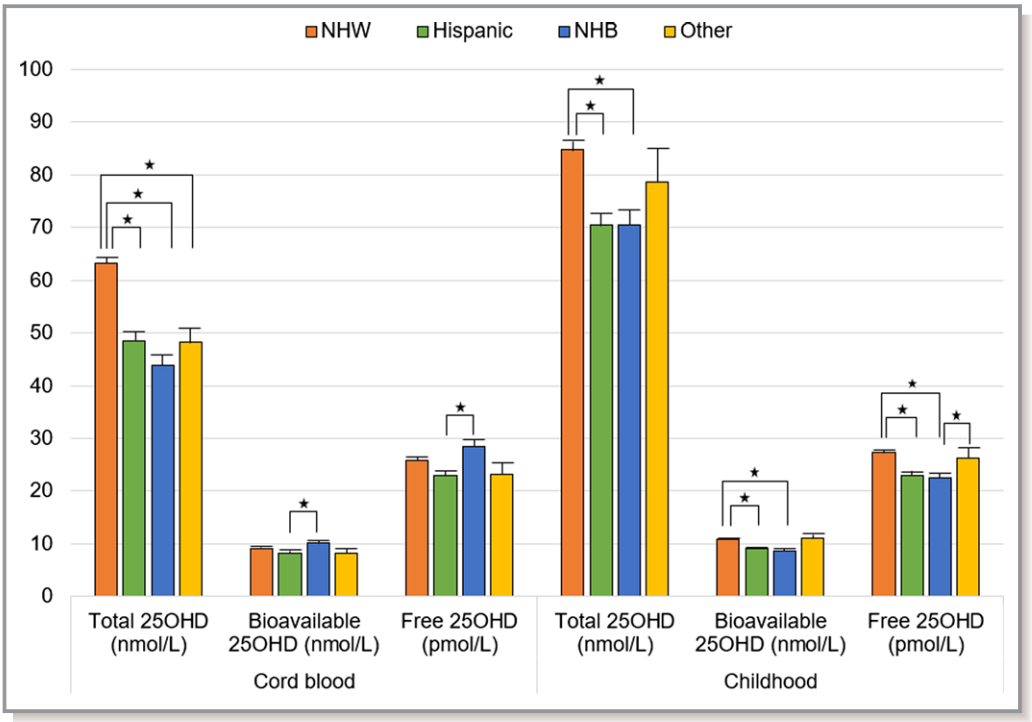

Figure 2. Age- and sex-adjusted levels of 25OHD, stratified by maternal race/ethnicity for cord blood measurements and child race/ethnicity for childhood measurements. 25OHD indicates 25-dihydroxyvitamin D; NHB, non-Hispanic black; NHW, non-Hispanic white. *Tukey, $P<0.05$ between denoted race/ethnicity groups.

Table 3. Association Between Cord Blood Total 25OHD (Per 25 nmol/L) and Cardiovascular Risk Factors at 4 to 6 Years

	n	Model 1		Model 2		Model 3	
		Estimate (SE)	*P* Value	Estimate (SE)	*P* Value	Estimate (SE)	*P* Value
Systolic blood pressure, mm Hg	170	−2.6 (0.7)	0.0003	−2.5 (0.7)	0.001	−2.5 (0.8)	0.002
Diastolic blood pressure, mm Hg	170	−1.5 (0.6)	0.01	−1.6 (0.6)	0.01	−1.7 (0.6)	0.01
Pulse wave velocity, m/s	125	−0.02 (0.10)	0.80	0.06 (0.10)	0.52	0.04 (0.11)	0.69
BMI z-score	171	0.05 (0.09)	0.63	0.13 (0.09)	0.14	0.13 (0.10)	0.21
Waist-to-height ratio	170	0.002 (0.004)	0.55	0.004 (0.004)	0.26	0.005 (0.004)	0.25
Total mass, g	153	148 (279)	0.60	343 (279)	0.22	351 (304)	0.25
Fat-free mass, g	153	32 (170)	0.85	139 (175)	0.43	102 (188)	0.59
Fat mass, g	153	116 (162)	0.47	205 (161)	0.21	249 (172)	0.15
Adiposity (%)	153	0.42 (0.55)	0.45	0.58 (0.56)	0.30	0.74 (0.59)	0.21

Model 1: adjusted for child sex and childhood (4–6 years) visit age, total 25OHD, and (for blood pressure and pulse wave velocity only) BMI z-score. Model 2: model 1 covariates+maternal race/ethnicity, prepregnant BMI, gestational weight gain, and gestational diabetes mellitus. Model 3: model 2 covariates+maternal prenatal smoking, prenatal physical activity, prenatal daily kilocalories, age at delivery, education, income, gravidity, and offspring gestational age at birth. 25OHD indicates 25-dihydroxyvitamin D; BMI, body mass index.

assessments throughout pregnancy to determine the critical window(s) of exposure. Although the 2.6–mm Hg difference we observed is modest and of questionable clinical relevance, it has the potential to be clinically relevant. For children aged 4 to 6 years, the difference in diagnostic thresholds for elevated systolic blood pressure (90th percentile) versus hypertension (95th percentile) is only 4 mm Hg.[52] Furthermore, our results suggest that increasing cord blood 25OHD from the minimum to the maximum observed in our sample (15 versus 120 nmol/L) would result in a reduction of systolic blood pressure of >10 mm Hg, which approaches the 14–mm Hg difference between the 90th and 50th percentiles at this age. This indicates that increases in neonatal 25OHD could be clinically meaningful for an individual child. At a population level, a 5–mm Hg decrease in systolic blood pressure among adults is projected to reduce cardiovascular and all-cause mortality by 7% to 14%, saving up to 28 000 lives per year.[53] Given that blood pressure tends to increase with age,[54] understanding and targeting factors that raise blood pressure levels early in life is important for preventing hypertension and related comorbidities later in life.

We did not observe any association between cord blood 25OHD levels and childhood arterial stiffness, body size, or body composition. Some studies in Europe and India have also reported no association between mid-gestational or cord blood 25OHD levels and arterial stiffness or adiposity at 5 to 9 years of age.[24–26,55] Other cross-sectional studies in pediatric populations have reported inconsistent results with regard to the association between 25OHD and arterial stiffness,[31,56–59] although clearer associations tend to be observed among participants with chronic health conditions (diabetes mellitus[57,58] or chronic kidney disease[56]). It is possible that healthy children do not have sufficient variability or subclinical impairment in arterial stiffness to detect an association with pulse wave velocity, especially in relatively small samples such as ours (n=125). In terms of adiposity, the Southampton Women's Study, Generation R, and Screening for Pregnancy Endpoints studies have all reported that higher maternal levels of 25OHD at 15 to 34 weeks' gestation were associated with significantly lower adiposity at 5 to 6 years of age.[23,51,60] Heterogeneity in study methods may account for the inconsistent results, as well as latitude-driven differences in background vitamin D levels. In vitro studies demonstrate that low levels of 25OHD promote adipogenesis[61–63] and limit lipolysis,[64] resulting in increased adiposity, and corroborating in vivo evidence would be helpful for understanding why past results have varied between populations. A meta-analysis could also be useful for synthesizing and systematic drawing conclusions from the accumulating observational evidence.

Despite previous reports that bioavailable 25OHD is a better predictor of health outcomes than total 25OHD,[28–30,39] we found no association between bioavailable 25OHD and any childhood cardiovascular risk factors. In a cross-sectional analysis of 47 adolescent females, low bioavailable 25OHD was significantly associated with greater endothelial dysfunction, although this relationship was attenuated after adjustment for potential confounders.[31] Other reports demonstrate the significance of bioavailable 25OHD in terms of bone mineral density,[28,39] osteoporotic fractures,[30] and end-stage renal disease.[29] However, at least 1 study has shown that total 25OHD is significantly correlated with 1,25OHD (the active vitamin D metabolite), whereas free 25OHD is not.[65] Other studies have reported that total 25OHD is more affected by disease states (liver disease,[66] obesity,[67] and multiple sclerosis[68]) and factors such as race/ethnicity[27,31,65] compared with free or bioavailable 25OHD, suggesting that the pathways underlying the associations between vitamin D

and health outcomes may not be specifically dependent upon vitamin D bioavailability. Alternatively, our null findings could be attributed to nonspecificity in VDBP affinity: We used a genotype-nonspecific binding affinity[27] because genetic information was not available. Polymorphisms in VDBP genes, which are often observed between racial/ethnic groups, have been noted to affect concentrations of bioavailable 25OHD.[27] Previous studies have also shown that measured free 25OHD has better precision than calculated 25OHD,[69–71] which could have limited our ability to detect associations. It is well recognized that vitamin D concentrations are affected by factors such as genetics, liver function, kidney disease, and pregnancy.[72] Because previous studies of prenatal vitamin D and offspring outcomes have not included free or bioavailable 25OHD,[73–77] additional studies in diverse populations are needed to understand the relative importance of each form of 25OHD in early life for offspring health.

We do provide novel evidence that there are racial/ethnic differences in total, bioavailable, and free 25OHD that varied between birth and 4- to 6-year measurements. Non-Hispanic whites had the highest levels of total 25OHD at birth and all 25OHD types in childhood. Non-Hispanic blacks had the highest levels of bioavailable and free 25OHD at birth, but the lowest levels of bioavailable and free 25OHD by 4 to 6 years of age. It is well known that vitamin D deficiency is more common among blacks than whites; however, previous reports indicate that blacks also have less VDBP, resulting in similar amounts of circulating 25OHD that is unbound or loosely bound to albumin (ie, similar bioavailable 25OHD).[27] Thus, we expected to see differences between whites and blacks in total 25OHD at both time points, as well as whites and the other racial/ethnic groups because of melanin-driven differences in vitamin D synthesis from sunshine exposure. But we did not expect to see differences in bioavailable 25OHD, particularly in divergent directions across the 2 time points. We confirmed that these results are not attributed to racial/ethnic misclassification by repeating the cord blood analysis with child race/ethnicity in place of maternal race/ethnicity; this replacement affected classification for only 3% of participants and did not change the results. We also confirmed, in an exploratory analysis, that these results were not attributed to differences in intake of vitamin D from food and/or dietary supplements. Rather, we hypothesize that these time-varying differences in 25OHD fractions are attributed to genotype-specific binding affinities (which was not measured in this study as noted above) or real differences in vitamin D exposure from dietary intake and sun exposure. Reports of bioavailable and free 25OHD in pediatric populations are sparse; thus, our work provides early contributions to our understanding of vitamin D availability in children. Importantly, the inverse association we observed between cord blood total 25OHD and childhood blood pressure was not modified by race/ethnicity, indicating its potential as a prevention strategy for all subpopulations.

Our study has strengths and limitations. We included a diverse sample with longitudinal assessments of total, bioavailable, and free 25OHD, which has not previously been reported. The use of cord blood samples allowed us to evaluate neonatal status at birth rather than using maternal status during pregnancy as a proxy. We measured body composition using the gold standard for offspring at birth and during childhood to obtain more-direct assessments of adiposity risk than weight or BMI alone. Our analysis was limited to the subset of participants with complete data, which was largely attributed to the cost and required sample volume for the vitamin D blood measurements. However, the analytical samples of 715 and 171 were similar to the total Healthy Start sample of 1410 in terms of maternal/child sociodemographics (age, sex, race/ethnicity, and education), prenatal exposures (maternal obesity, gestational diabetes mellitus, smoking, diet, and physical activity), offspring vitamin D levels (total, free, and bioavailable in cord blood and childhood blood), and offspring cardiovascular risk factors (blood pressure, pulse wave velocity, BMI, and body composition). This suggests that the subsets were representative of the larger sample and reduces concerns about selection bias. The sample size for some racial/ethnic groups was particularly small, which may have reduced power to detect interactions or obtain more-precise effect estimates. We did not include childhood physical activity or dietary intake as covariates in our analysis because of missing data for these variables, which would have reduced our analytical sample further. However, exploratory analyses of the smaller subsets with complete data for physical activity and diet resulted in similar findings, suggesting that our results are not confounded by these health behaviors. We did not adjust for multiple comparisons, and thus our blood pressure findings could be type 1 error, highlighting the need for replication in larger samples. However, we note that the significance of systolic blood pressure result would have persisted even if we had used a Bonferroni correction to adjust the threshold for significance (0.002 in the fully adjusted analysis compared with 0.05/9 outcomes=0.005). Furthermore, the finding is in agreement with previous studies.[22,46] Last, our use of genotype-nonspecific binding coeffecients for the calculation of bioavailable and free 25OHD may have resulted in measurement error.

In conclusion, we have shown that higher 25OHD levels at birth is associated with lower blood pressure at 4 to 6 years of age, independent of childhood 25OHD levels, race/ethnicity, and child BMI. Our study provides further evidence in support of the developmental origins of health and disease theory and highlights the importance of optimizing intrauterine nutritional exposures to improve for offspring health.

Continued follow-up of the Healthy Start cohort and confirmation of our findings in other studies, including clinical trials, will clarify the role of early-life vitamin D exposure on cardiovascular health as children enter adolescence and adulthood.

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Author Contributions

Sauder, Ringham, Glueck, and Dabelea designed the analysis. Stamatoiu and Leshchinskaya collected the data. Sauder and Stamatoiu conducted the analysis in consultation with Ringham and Glueck. Sauder wrote the first draft of the manuscript. All authors interpreted the data, revised the manuscript critically for intellectual content, and approved the final version of the manuscript. Sauder takes responsibility for data integrity and contents of the manuscript.

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Disclosures

None.

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