

Vitamin D Status as Related to Race and Feeding Type in Preterm Infants

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ABSTRACT

Background: Despite the higher prevalence of vitamin D deficiency in blacks, the vitamin D status of black preterm infants remains unknown. In addition, with the combination of parenteral and enteral nutritional support that preterm infants receive, the effect of vitamin D-deficient breast milk on vitamin D status is unknown.

Objective: To evaluate vitamin D status of preterm infants through the first month after delivery and compare status by race and feeding type.

Study Design: Thirty-six (36) preterm (≤ 32 weeks gestation) infants (19 black, 17 white) had assessment of feeding type, vitamin D intake, and serum 25-hydroxyvitamin D [25(OH)D] as a marker of vitamin D status at three time points in the first month after delivery.

Results: Black infants had a significantly lower mean 25(OH)D level on day 7–8 and day 14–15 evaluations than white infants [14.9 ± 6.6 versus 23.3 ± 9.3 ng/mL ($p = 0.021$) and 18.3 ± 7.3 versus 25.6 ± 10.3 ng/mL ($p = 0.048$), respectively], but the difference was no longer significant by day 28–30 evaluation [19.6 ± 7.7 versus 26.2 ± 11.6 ng/mL ($p = 0.26$)]. Vitamin D status was not significantly lower in infants receiving predominantly breast milk ($p = 0.6$). Vitamin D intake rose through the month as the amount and caloric density of enteral nutrition increased. Six infants had significant decrease in serum 25(OH)D values from day 14–15 to day 28–30 evaluation despite receiving > 400 IU/day vitamin D.

Conclusion: Differences in vitamin D status occurred between black and white infants and were significant through the first 2 weeks after delivery. Infants receiving predominantly breast milk did not have significantly worse vitamin D status than those receiving formula. The significant decline in serum 25(OH)D status observed in 28% of the infants was not related to breast milk intake.

INTRODUCTION

BLACK PRETERM INFANTS are at risk for low serum 25(OH)D status because of the high prevalence of vitamin D deficiency in black mothers.¹ A fetus receives all vitamin D from the mother, and an infant's serum 25(OH)D level at birth may be as low as half of the

mother's level.² The authors have previously studied cord blood 25(OH)D levels stratified by race and found black infants to have significantly lower cord blood 25(OH)D levels than white infants (10.5 ± 6.0 ng/mL versus 19.5 ± 9.6 ng/mL, respectively, $p < 0.0001$).³ Over 80% of the subjects in this study were full term. In evaluation of preterm infants over the past

30 years, studies of vitamin D status have not stratified by skin pigmentation or race, ignoring analysis of a population at high risk for deficiency.⁴⁻¹⁸

The risk for low 25(OH)D status in preterm infants also is a result of the difficulty in providing nutrition to these infants. Preterm infants have an immature gastrointestinal (GI) system that necessitates slow advancement of enteral feeds. Before achieving sufficient enteral feeds, a preterm infant relies on parenteral nutrition support, which is deficient in providing vitamin D when an infant weighs <1250 g.¹⁹ Once feedings are initiated, the preferred feeding for preterm infants is breast milk. However, breast milk often is low in vitamin D content unless the mother is receiving high-dose vitamin D (≥ 4000 IU/day) or has regular sunlight exposure.²⁰⁻²³ The single study comparing serum 25(OH)D status between preterm breast milk- and formula-fed infants provided at least 1000 IU/day vitamin D supplementation to all infants; therefore, it does not reflect the standard vitamin D supplementation (200 to 400 IU/day) provided to breast milk-fed infants in the United States.⁵

With the unstudied vitamin D status of a population at risk of deficiency because of race or nutritional inadequacies, the authors designed this observational study to evaluate serum 25(OH)D status in the first month after delivery in a cohort of preterm infants. The authors hypothesized that black infants would have significantly lower serum 25(OH)D early on and that infants receiving predominantly breast milk feedings would have significantly lower serum 25(OH)D than infants receiving predominantly formula feedings.

MATERIALS AND METHODS

Subjects

After receiving approval from the Institutional Review Board for Human Subjects at the Medical University of South Carolina, parents whose infants were admitted to the neonatal intensive care unit were approached for consent for their infant's participation if their infant met the following criteria: (a) ≤ 32 weeks

gestation; (b) first feeding within the first 7 days after delivery; and (c) absence of major congenital anomalies. The study was conducted during a 3-year period.

Study design

This is a prospective study of preterm infants ≤ 32 weeks' gestation who were followed for a 1-month study period as a part of an overall nutritional and gut maturity assessment. A component of the study was to measure the vitamin D status of this cohort over time as related to race and infant feeding status.

Sample size determination

The sample size was determined for the parent study of overall nutritional and gut maturity assessment with the primary outcome variable of gut permeability maturity. The study was not powered for the evaluation of serum 25(OH)D by race and feeding type. In assessment of vitamin D status in a previous study that was specifically powered to detect differences in serum 25(OH)D as a function of two vitamin D doses and race, the power calculation was 16 infants with 8 in each dose group and further stratification by race.²³

Study protocol

After receiving informed consent, infants who met criteria were followed for a 1-month study period. Data were collected using a standardized form developed by the investigators to ascertain information about prenatal history, delivery characteristics, health status, dose and volume of breast milk received, initiation and duration of parenteral nutrition, use of human milk fortifier, and episodes of feeding intolerance that resulted in an infant being designated NPO (nothing by mouth) for at least a 24-hour period. Birth head circumference was measured in this study because of reports in both preterm and term infants of association between head circumference and vitamin D status.^{12,24}

Each infant had blood samples collected on days 7-8, 14-15, and 28-30 after delivery. Infants who had been without enteral feedings for >72 hours before a study day exited the study, with the intention to collect data on

those premature infants who were without GI compromise. If an infant was without enteral feedings for <72 hours on a study day, then the infant missed that sample collection but continued participation in the study. Blood samples were sent to the General Clinical Research Center at MUSC for processing and were stored at -80°C until later analysis.

Assignment of feeding type

Feeding type was defined as predominantly (>80% feeding volume) breast milk, formula, or a combination of breast milk and formula. At each of the three time points, dose and volume of breast milk and formula over the interim period were recorded.

Measurement of vitamin D status

Circulating 25-hydroxy vitamin D [25(OH)D] was measured as an indicator of vitamin D status²⁵ at day 7–8, day 14–15, and day 28–30 after delivery. The vitamin D metabolite, 25(OH)D, was assessed using assays developed in the laboratory of Dr. Hollis and have been described in detail elsewhere.²⁶ A rapid, direct radioimmunoassay (RIA) for 25(OH)D was used to test for nutritional vitamin D status. The reagent for the ¹²⁵I-labeled RIA for 25(OH)D was purchased from the Diasorin Corp. (Stillwater, MN). The normal adult circulating levels of the serum 25(OH)D, in the authors' laboratory is 32 to 90 ng/mL. The lower limit of detection of this assay is 2 ng/mL.

Vitamin D intake calculation

No attempt was made to control vitamin D intake. Calculation of the daily vitamin D intake was an average of the vitamin D provided daily for the 7 days before the serum 25(OH)D measurements on day 7–8 and day 14–15. The daily vitamin D provided for the 14 days before the measurement on day 28–30 was averaged and recorded. Calculated vitamin D amounts were from formula, parenteral nutrition, and/or vitamin D supplements.

Breast milk

The vitamin D supplied in breast milk was not measured but estimated in this study. The

authors previously calculated the amount of vitamin D activity in breast milk by quantitation of vitamin D₂, vitamin D₃, 25(OH)D₂, and 25(OH)D₃ in the milk by competitive protein binding assay.²⁰ Quantitation of these measurements give a range of 20 to 70 IU/L vitamin D provided by breast milk when the mother is not receiving high-dose vitamin D or regular sunlight exposure, as previously reported.^{20–23} In the present study, an approximation of 50 IU/L was used for calculations. Table 1 shows the vitamin D activity estimated for breast milk.

Human milk fortifier

Once an infant achieved total feed volume of 120 to 150 cc/kg per day, human milk fortifier was added to all breast milk at a concentration to provide an additional 4 kcal/oz (3.6 g fortifier/100 mL milk). This concentration added 120 IU vitamin D to 100 mL milk (see Table 1).²⁷

Parenteral nutrition

When an infant weighed <2500 g, the amount of multivitamin solution added to parenteral nutrition was based on weight at a concentration of 160 IU/kg.¹⁹ An infant who weighed ≥ 2500 g received 400 IU/day vitamin D.

TABLE 1. VITAMIN D ACTIVITY SUPPLIED BY VARIOUS FEEDING TYPES

<i>Feeding type</i>	<i>20 kcal/oz*</i>	<i>24 kcal/oz*</i>
Breast milk	50 IU/L**	1201 IU/L** (breast milk fortifier added) [†]
Formula 1 [†]	1013 IU/L	1220 IU/L
Formula 2 [‡]	1600 IU/L	1905 IU/L

*For this study, the caloric density of breast milk was approximated to 20 kcal/oz without additives and 24 kcal/oz with breast milk fortifier concentrated to add 4 kcal/oz to breast milk.

**Vitamin D activity in breast milk ranges from 20 to 70 IU/L. An approximation of 50 IU/L was used for unsupplemented breast milk.

[†]Ross Products Handbook. Abbott Park, IL, Abbott Laboratories, 2004.

[‡]Mead Johnson Nutritional Products Handbook. Evansville, IN, Mead Johnson and Company, 2004.

Formula

The vitamin D content of the two formulas given at the institution during the study time period is shown in Table 1. For the purpose of this study, 20 kcal/oz is considered nonfortified formula and 24 kcal/oz is considered fortified formula. Formula feeds were fortified when an infant achieved total feed volume of 120 to 150 cc/kg per day.

Statistical analyses

Statistical analyses were performed using SAS software (SAS Institute, Cary, NC) and included two-tailed student's *t*-test, chi-square, ANOVA, and MANOVA. Significance was set at $p < 0.05$ *a priori*.

RESULTS

During the 3-year study period, 783 infants were born at <32 weeks gestation and were receiving enteral feeds before 7 days after delivery. Thirty-six (36) infants, 19 black and 17 white, were enrolled in the study as a convenience sample.

Clinical characteristics are given in Table 2. No significant differences existed between black and white infants in sociodemographic and clinical parameters.

Three infants (1 black and 2 white) did not have serum results at the first study visit on day 7–8. On day 14–15, 16 black and 12 white infants had serum laboratory measurements performed. On day 28–30, 10 black and 11 white infants had laboratory measurements. One infant did not have laboratory measurements on day 28–30 because of discontinuation of feeds when necrotizing enterocolitis developed after day 14–15. The other infants exited the study because of discharge from the hospital.

Table 3 presents the serum 25(OH)D levels measured at each of the three time points during the first month after delivery with comparison between the black and white infants. The black infants had a significantly lower mean 25(OH)D level at both of the first two evaluations: day 7–8 ($p = 0.021$) and day 14–15 ($p = 0.048$). By day 28–30, the difference was no longer significant ($p = 0.26$) (Fig. 1).

Eleven infants (6 black and 5 white), who received $>80\%$ of enteral nutrition as breast milk,

TABLE 2. SUBJECT DEMOGRAPHICS AND NUTRITIONAL SUPPORT CHARACTERISTICS

Characteristics	All infants (n = 36)	Black infants (n = 19)	White infants (n = 17)	p value
% male	31 (n = 11)	26 (n = 5)	35 (n = 6)	0.56
% receiving $>80\%$ BM feeds	31 (n = 11)	32 (n = 6)	29 (n = 5)	0.57
Birth weight (g)				
mean \pm S.D.	1275 \pm 300	1251 \pm 266	1302 \pm 340	0.57
median	1228	1230	1225	
Birth head circumference (cm)				
mean \pm S.D.	27.0 \pm 1.8	27.1 \pm 1.7	26.8 \pm 2.0	0.83
median	27.5	27.5	27.5	
Gestational age (wks)				
mean \pm S.D.	29.2 \pm 1.6	29.4 \pm 1.6	29.1 \pm 1.7	0.83
median	29.1	29.1	29.5	
Days receiving parenteral nutrition				
mean \pm S.D.	13.1 \pm 6.2	16.0 \pm 6.0	12.3 \pm 7.1	0.72
median	10.5	11	10	
Age at first enteral feed (days)				
mean \pm S.D.	3.8 \pm 1.5	3.6 \pm 1.3	4.0 \pm 1.7	0.41
median	4	3.5	4	
Age when full enteral feeds achieved (days)				
mean \pm S.D.	14.7 \pm 6.7	14.0 \pm 7.3	15.5 \pm 6.2	0.61
median	12	12	13	
Age when feeds fortified (days)				
mean \pm S.D.	16.0 \pm 6.2	15.3 \pm 6.9	16.6 \pm 5.6	0.49
median	14	13	14	

n = number of infants. *P* values compare black and white infants.

TABLE 3. 25(OH)D LEVELS AT THREE TIME POINTS IN THE FIRST MONTH AFTER DELIVERY

25(OH)D ng/mL	All infants	Black infants	White infants	p value
Day 7–8				
Mean \pm S.D.	18.6 \pm 8.8	14.9 \pm 6.6	23.3 \pm 9.3	0.021*
Median	16.2	13.7	24.3	
Range	7.6–37.8	7.6–33.2	9.1–37.8	
Number of subjects	33	18	15	
Day 14–15				
Mean \pm S.D.	21.5 \pm 9.3	18.3 \pm 7.3	25.6 \pm 10.3	0.048*
Median	19.4	16.0	24.9	
Range	8.5–40.2	9.5–40.2	8.5–39.9	
Number of subjects	28	16	12	
Day 28–30				
Mean \pm S.D.	23.6 \pm 10.5	19.6 \pm 7.7	26.2 \pm 11.6	0.258
Median	21.6	17.6	23.4	
Range	9.5–50.2	12.3–31.5	9.5–50.2	
Number of subjects	21	10	11	

P values compare black and white infants.

*Demonstrates significance. Test is ANOVA.

did not have statistically lower 25(OH)D levels than infants receiving <80% breast milk or all formula at 28 to 30 days after delivery (22.1 ± 11.5 ng/mL versus 23.7 ± 10.4 ng/mL). Vitamin D supplementation was not evaluated as a function of feeding type before the day 28–30 time point because of the presence of parenteral nutrition.

When evaluating the average daily amount of vitamin D intake in this cohort, the mean daily intake in the first week after delivery was 195 ± 53 IU. The mean daily intake increased over the month as infants received more enteral nutrition and fortification of feeds. Table 4

shows the variation for each race and feeding type over the month. No significant difference was found in vitamin D intake between black and white infants at any time point or between infants receiving predominantly breast milk (>80%) and those receiving either <80% of feed volume as breast milk (formula received instead of breast milk) or formula with no breast milk when compared at the end of the month.

Six infants (three black and three white) had significantly decreased serum 25(OH)D levels from day 14–15 to day 28–30 despite improved vitamin D intake over the month, including >400 IU/day vitamin D in the last 14 days of the month, as shown in Table 5. Their gestational ages ranged from 25 to 32 weeks, and their birth weights ranged from 1035 to 1746 g. None of the six patients received predominantly breast milk. Two of the six received no parenteral nutrition and four received parenteral nutrition for 8 to 19 days. Their average days for feeding initiation, full feed achievement, and feed fortification were similar to the other study patients. These infants account for 28% of those with serum 25(OH)D evaluation at the day 28–30 time point.

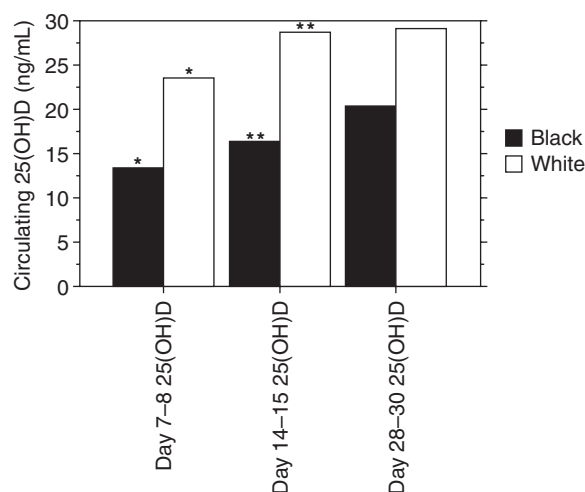


FIG. 1. Mean circulating 25(OH)D during first postnatal month as a function of race. * $p = 0.021$ and ** $p = 0.048$.

DISCUSSION

In this preterm infant population, the black infants had significantly lower 25(OH)D levels

TABLE 4. DAILY VITAMIN D INTAKE

<i>Vitamin D intake (IU/day)</i>	<i>Day 7–8</i>	<i>Day 14–15</i>	<i>Day 28–30</i>
All infants	195 ± 53 (<i>n</i> = 33)	223 ± 97 (<i>n</i> = 28)	507 ± 223 (<i>n</i> = 21)
Black	195 ± 50 (<i>n</i> = 18)	227 ± 81 (<i>n</i> = 16)	467 ± 222 (<i>n</i> = 10)
White	196 ± 58 (<i>n</i> = 15)	239 ± 113 (<i>n</i> = 12)	544 ± 223 (<i>n</i> = 11)
All infants receiving >80% of feed as BM	165 ± 49 (<i>n</i> = 11)	177 ± 49 (<i>n</i> = 8)	435 ± 279 (<i>n</i> = 6)

Mean ± standard deviation is given. *P* value >0.1 in comparison of vitamin D intake between black and white infants at all time points and comparison of vitamin D intake between infants receiving >80% of feed as BM and all other infants.

than the white infants at 7 to 8 days and 14 to 15 days after delivery, with this trend still evident at 28 to 30 days after delivery. The difference seen in the first 2 weeks was most likely caused by lower levels in the black mothers and the consequential effect on vitamin D status in the newborn infant. Although the disappearance of a significant difference between the races by 28 to 30 days after delivery may reflect the similar vitamin D intake received by the infants during the month, it also may reflect a Type 2 error given the small sample number at that time period. With mean vitamin D supplementation at approximately 200 IU/day in the first week after delivery and increasing to over 400 IU/day by the end of the first month, most infants of both races had improvement in vitamin D status.

The six infants who had significantly decreased serum 25(OH)D values, despite receiving >400 IU/day vitamin D by 3 weeks after delivery, generate consideration of the adequacy of 200 IU/day or even 400 IU/day as the recommended intake for all infants.^{6,28} As none of these infants received predominantly breast milk, the approximation of breast milk vitamin

D activity in this study is not responsible for this result. A linear relationship between vitamin D intake and serum 25(OH)D measurements has been shown in adults.²⁹ If a similar relationship exists in children, this decrease in serum 25(OH)D may demonstrate poor intestinal absorption of the vitamin D in formula by these preterm infants.

No significant difference was observed in vitamin D intake by feeding type. The infants receiving predominantly breast milk had lower vitamin D intake than those who received more formula or formula alone, but this difference was not significant. The lack of significant difference in vitamin D intakes between breast milk and formula-fed infants is most likely due to intake of breast milk fortifier by the majority of the breast fed infants by 16 days.

This study demonstrates that black preterm infants are at risk for lower serum 25(OH)D status than white infants. It also raises the question of whether the recommendations of 200 to 400 IU/day vitamin D are adequate to maintain stable vitamin D status in all preterm infants. These results are limited by lack of understanding sufficient serum 25(OH)D status in

TABLE 5. VITAMIN D STATUS AND INTAKE OF THE SIX INFANTS WITH INCREASING INTAKE AND DECREASING SERUM 25(OH)D

<i>Parameters</i>	<i>Day 7–8</i>	<i>Day 14–15</i>	<i>Day 28–30</i>
25(OH)D, ng/mL	21.4 ± 7.7* (10.6–29)	28.3 ± 9.4** (19–40)	17.0 ± 4.8** (12.3–24.4)
Vitamin D intake, IU/d	207 ± 26 (169–243)	224 ± 85 (102–245)	598 ± 164 (425–857)

Mean ± standard deviation and range shown. Vitamin D intake is the average daily intake for the 7 days before day 7–8 and day 14–15 and the 14 days before day 28–30 study days.

*Only four of the six infants had serum 25(OH)D values for day 7.

**In comparison with day 14–15 and day 28–30 serum 25(OH)D values, *p* is significant at 0.02.

infants. Historically, vitamin D deficiency in children is defined by the serum 25(OH)D level measured when rickets is clinically apparent. This level ranges from 5 to 11 ng/mL.³⁰ Although the study population did not demonstrate this magnitude of vitamin D deficiency, studies in adults have shown that a serum 25(OH)D level in the range of 15 to 32 ng/mL is required to promote optimal calcium absorption, bone mineral density, and normal parathyroid function, which are essential for maintaining normal bone health.^{31–33} Until vitamin D sufficiency is defined for the preterm infant population, the authors do not know if the drop in serum 25(OH)D seen in 28% of infants with evaluation at the end of the month, receiving the recommended vitamin D supplementation for infants, produced detrimental effects in calcium absorption and bone health.

In addition, knowledge that vitamin D plays a role in immune function is expanding exponentially.^{34,35} Vitamin D status has been associated with numerous long-latency immune-related chronic diseases such as diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and cancer by several epidemiologic and mechanistic studies.^{36–39} Uncovering these associations suggests that vitamin D insufficiency in early development increases long-term disease risk.⁴⁰ As assessment of the consequences of vitamin D status in infancy ensues, this study presents the vitamin D status achieved with current nutritional practices for a population at risk for vitamin D insufficiency because of race and feeding type.

CONCLUSION

In this cohort of very-low-birth-weight infants, black infants exhibited significantly lower serum 25(OH)D levels in the first 2 weeks after delivery when compared with white infants, with a trend that persisted at 28 to 30 days. Predominantly breast milk-fed infants did not demonstrate lower serum 25(OH)D levels than infants receiving less breast milk or formula alone. Six infants, representing 28% of the infants with serum 25(OH)D evaluation at the end of the month, had significantly lower vitamin D status at 4 weeks compared to 2 weeks

after delivery despite receiving an average of 425 to 857 IU/day. Improved understanding of vitamin D sufficiency in this population is needed to define the true requirements for vitamin D intake.

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