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Original Article

Heightened attention to supplementation is needed to improve the vitamin D status of breastfeeding mothers and infants when sunshine exposure is restricted

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Abstract

Although exclusively breastfed infants are at increased risk of vitamin D (vit D) deficiency if vit D supplementation is lacking and sun exposure is limited, assessment of both risk factors in the first year of life is lacking. We evaluated the contribution of vit D intake and sunlight exposure to vit D status in 120 healthy, breastfeeding mother–infant dyads, who were followed up for 1 year. Vitamin D intake and skin sunlight exposure were evaluated using questionnaires. Serum 25-hydroxyvitamin D, parathyroid hormone (PTH) and alkaline phosphatase levels were determined post-natally in mothers at 4 weeks and in infants at 4, 26 and 52 weeks. Vitamin D supplementation was low (<20%) and sunlight exposure was common (93%) in study infants. At 4 weeks, 17% of mothers were vit D deficient (<50 nmol L^{-1}) and 49% were insufficient (50–<75 nmol L^{-1}), while 18% of infants were severely vit D deficient (<25 nmol L^{-1}) and 77% were deficient (<50 nmol L^{-1}). At 26 weeks, winter/spring birth season and shorter duration of months of exclusive breastfeeding were protective of vit D deficiency in infants. Vitamin D deficiency in infants decreased to 12% at 52 weeks with sunlight exposure. Serum PTH levels were significantly higher in severely vit D deficient than sufficient infants. Vitamin D deficiency was widespread in early post-partum breastfeeding mothers and infants, and declined to one in eight infants at 52 weeks due mostly to sunshine exposure. When sunlight exposure is limited or restricted, intensified vit D supplementation of breastfeeding mothers and infants is needed to improve vit D status.

Keywords: vitamin D deficiency, breastfeeding, sunlight exposure, infants, mothers.

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Introduction

Severe vitamin D (vit D) deficiency presenting as rickets is a public health problem worldwide (Holick 2006; Thacher *et al*. 2006), and there are increasing reports of its occurrence especially among breastfed infants in minority groups in the United States and elsewhere (Weisberg *et al*. 2004; Dawodu *et al*. 2005; Thacher *et al*. 2006; Strand *et al*. 2007). A recent study found that one-third of infants with vit D deficiency, who had radiographs performed, had evidence of bone under-mineralization (Gordon *et al*. 2008). In addition, vit D insufficiency and low vit D intake in childhood has been linked to increased risk of acute lower respiratory tract infections (Wayse *et al*. 2004; McNally *et al*. 2009) and autoimmune diseases such as type 1 diabetes (Hypponen *et al*. 2001). Vitamin D insufficiency in adults is reported to be associated

with increased risk of autoimmune diseases, certain types of cancer, and cardiovascular and infectious diseases (Holick 2007).

Breastfeeding without infant vit D supplementation, a lack of sunlight exposure and maternal vit D deficiency are reported as important risk factors for vit D deficiency rickets (Weisberg *et al*. 2004; Dawodu *et al*. 2005; Holick 2006; Thacher *et al*. 2006; Strand *et al*. 2007). The American Academy of Pediatrics (AAP) recommends that children younger than 6 months of age avoid direct sunlight because of concern about skin cancers in later life and, thus, should receive vit D supplementation to prevent vit D deficiency (AAP 1999; Wagner & Greer 2008). However, compliance with the recommendation for vit D supplementation appears low. Previous excellent studies (Greer *et al*. 1982; Ho *et al*. 1985; Specker & Tsang 1987) performed over 2 decades ago showed relationships among sun exposure, vit D supplementation and maternal vit D on infant vit D status. However, despite increasing reports of vit D deficiency in breastfeeding infants, recent studies that attempted to quantify the impact of sun exposure, vit D supplementation and maternal vit D status in relation to vit D status in breastfeeding infants during infancy (Andiran *et al*. 2002; Dawodu *et al*. 2003; Challa *et al*. 2005) are scarce. Such information is of public health importance because it could help identify and guide appropriate intervention strategies to avoid vit D deficiency in breastfeeding mothers and infants.

We conducted this study to investigate the prevalence and relationship of vit D deficiency in breastfeeding mother–infant pairs in Cincinnati, OH, and the effect of maternal vit D status, infant vit D supplementation and sun exposure on infant vit D status during the first year of life.

Methods

Subjects

One hundred and twenty breastfeeding mother–infant pairs enrolled in this longitudinal observational study were participants in an International Human Milk Research Collaborative to examine the association between mothers' nutritional status, human milk factors and child health outcomes during the first 2 years of life. The protocol of the parent study was approved by Cincinnati Children's Hospital Institutional Review Board and the mothers gave written informed consent. The sample size of the parent study was determined to assess the association between bioactive factors in human milk, and growth and the prevalence of respiratory infections and diarrhoea during the first 2 years of life. Subjects recruited were healthy, singleton term infants (≥37 weeks) whose mothers were aged 18–49 years and planned to provide at least 75% of feedings from breast milk for at least 3 months. Infants who were born prematurely (<37 weeks) or mothers with health problems that could interfere with breastfeeding were excluded. Healthy mothers were recruited from mothers who delivered at The Christ Hospital in Cincinnati, OH, between March 2007 and July 2008. This report concerns the prevalence and risk factors of vit D deficiency in mother–infant pairs at 4 weeks post-partum (baseline) and in the infants at longitudinal follow-up during the first year of life.

Study design

At 2 weeks post-partum, a registered nurse visited the family home for enrolment. Post-partum visits at 4, 13, 26 and 52 weeks of life took place at the Cincinnati

Key messages

- Vitamin D (vit D) deficiency is widespread and detectable soon after birth in breastfeeding mothers and their infants in Cincinnati.
- Maternal compliance with recommended infant vit D supplementation is poor.
- Infant exposure to sunshine exceeds AAP recommendations. At 26 and 52 weeks of age, season of birth and weekly hours of sun exposure are major determinants of vit D status.
- We suggest intensified vit D supplementation strategy that ensures vit D sufficiency during pregnancy and in breastfeeding mothers and infants, especially when sunlight exposure is limited or restricted.

Table 1. Calculation of percent body surface area (% BSA) exposed to sunlight*

Usual outdoor daylight attire in past week:			% BSA assigned for:	
Body part	Type of covering	Response	Mother	Infant
Head	Hat, cap or head scarf	Yes	0	0
		No	3	12
Neck	Clothing or scarf	Yes	0	0
		No	2	2
Face	Scarf	Yes	0	0
		No	4	7
Arms	Length of sleeves	Long	0	0
		Short	6	6
		Sleeveless	14	14
Hands	Gloves	Yes	0	0
		No	5	5
Legs	Length of pants or dress	Long	0	0
		Short (below knee)	7	5
		Short (knee length)	14	10
		Short (mid-thigh)	23	16
Feet	Amount of coverage	Covered	0	0
		Barefoot or sandals	7	7

*Total % BSA exposed to sunlight calculated as the sum of % BSA associated with subject's responses for each body part.

Center for Clinical Research to complete baseline demographic data and follow-up questionnaires. Blood samples were collected by venipuncture from the mother at 4 weeks post-partum and at weeks 4, 26 and 52 from the infants. A 24-h dietary recall questionnaire, including dietary supplements, was administered three times between weeks 4 and 13 for the mother. Nutrition Data Systems for Research (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA) was used to assess the micronutrient intake from foods reported in the dietary recall interview, while maternal vit D supplementation was assessed from the list of vitamin supplements intake reported during the interview. The total maternal vit D intake included both dietary and supplemented sources of vit D. Mothers also indicated the intake of any vitamins or medications provided to the infant within the past 24 h during weekly phone interviews beginning at 2 weeks post-partum. Maternal report of giving the infant a multivitamin, vit D supplement or cod liver oil was included as evidence of infant vit D supplementation.

Sun exposure behaviour questionnaires (Table 1) were administered by a registered nurse to mother and her infant at weeks 4, 26 and 52. The questionnaire is a modification of previous questionnaires used in assessing skin exposure to sunlight in adults and infants (Specker *et al*. 1985b; Dawodu *et al*. 1998; Barger-Lux & Heaney 2002). Table 1 also provides the method for calculating the total percent of body surface area (% BSA) exposed while outdoors for mothers and infants. A sun exposure index was estimated by multiplying the % BSA exposure by hours of sunlight exposure per week. Sun exposure index has been shown to correlate with vit D status in adults and children (Specker *et al*. 1985b; Dawodu *et al*. 1998; Barger-Lux & Heaney 2002). Collection of data on sunscreen use began after the study was underway and was thus incomplete.

Laboratory tests

Serum concentrations of 25-hydroxyvitamin D [25(OH)D] were determined by radioimmunoassay (DiaSorin, Stillwater, MN, USA) to assess the vit D status of mothers and infants. The intra- and inter-assay coefficient of variation for 25(OH)D measurement were 4% and 11%, respectively. In the mothers, vit D deficiency was defined as serum 25(OH)D < 50 nmol L^{-1} (Holick 2007; Holick *et al*. 2011; Institute

of Medicine 2011), while 25(OH)D between 50 and 75 nmol L^{-1} was considered vit D insufficient (Bischoff-Ferrari *et al*. 2006; Holick 2007). Vitamin D deficiency was also defined as serum 25(OH)D < 50 nmol L^{-1} in the infants (Wagner & Greer 2008; Holick *et al*. 2011). For this study, serum 25(OH)D levels <25 nmol L^{-1}, consistent with higher risk of rickets (Dawodu *et al*. 2005; Institute of Medicine 2011), was considered as severe vit D deficiency in the infants. Serum intact parathyroid hormone (PTH) was measured by immunoradiometric assay (Dia-Sorin). The normal adult range for serum PTH in our laboratory is 13–54 pg mL^{-1}. The range for infants using this assay is not established and the adult range was adopted as in other studies (Zeghoud *et al*. 1997; Liang *et al*. 2010). In addition, bone serum alkaline phosphatase (ALP) was measured by enzyme immunoassay (Quidel MicroVue BAP, Santa Clara, CA, USA) because significantly elevated levels have been reported in breastfed infants with severe vit D deficiency (Ziegler *et al*. 2006). The normal range using the assay is 6–34 IU L^{-1} for adult and 8–142 IU L^{-1} for children (0–2 years).

Statistical analysis

Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). For the baseline analysis, we estimated the prevalence of the different categories of vit D status and assessed the contribution of the selected key risk factors to vit D deficiency in mothers and in infants using univariate and multivariate analysis of variance (ANOVA). Comparisons of mean 25(OH)D concentrations for different categories of vit D status were made using ANOVA with Tukey–Kramer adjustments for multiple comparisons. Differences in percentages of categories of vit D status were compared using χ^2 or Fisher's exact test. Pearson correlations were used for correlation analyses.

Seasons of birth were categorised as winter/spring (October–March) and summer/fall (April–September). At each time point, we conducted univariate ANOVA or linear regression analysis and selected characteristics with a univariate $P < 0.10$ to consider for repeated measures multivariate analysis of vit D status. Analysis of covariance (ANCOVA) with backward selection was employed to determine which subset of characteristics was most predictive of vit D status. The longitudinal analysis of factors affecting infant vit D status was assessed using repeated measures ANCOVA, adjusting for key covariates and multiple measurements per infant over time. Infants' longitudinal changes in vit D status across time were tested using the paired McNemar test of symmetry.

Results

Ninety-nine mothers were non-Hispanic white, 17 African American and 4 identified as other ethnic groups. The mean (standard deviation) age was 31.5 (5.2). Eighty-two mothers had completed at least 4 years of college education. A significantly higher proportion (75%) of non-Hispanic white mothers had completed at least 4 years of college education compared with 35% of African American mothers ($P \leq 0.001$).

Vitamin D status of mothers and infants

Of the 120 mother–infant pairs enrolled in the study, serum 25(OH)D levels were available in 119 mothers and in 87 (73%) of the infants at 4 weeks post-partum. There were no significant differences in the demographics, sun exposure behaviours and maternal vit D status between mother–infant pairs with and without 4-week serum 25(OH)D levels (results not shown). We examined predictors of vit D status in the mothers (Table 2) and infants (Table 3) using categories of serum 25(OH)D concentrations. At 4 weeks post-partum, a total of 79 (66%) mothers were either vit D insufficient (50–75 nmol L^{-1}, $n = 59$) or deficient (<50 nmol L^{-1}, $n = 20$). In the mothers, black race and winter/spring season were significant factors associated with higher percentage of vit D deficiency in univariate analysis (Table 2). African American mothers were nine times [odds ratio, OR (95% confidence interval, CI): 9.3 (3.0, 29.1)] much more likely to be vit D deficient than mothers of other races.

Of the 87 infants, a total of 67 (77%) were vit D deficient [serum 25(OH)D < 50 nmol L^{-1}], of which 51 had 25 (OH)D levels of 25–50 nmol L^{-1} and 16 were severely deficient [serum 25(OH)D < 25 nmol L^{-1}] at

Table 2. Predictors of maternal vitamin D status at 4 weeks post-partum

Variable	Maternal serum 25(OH)D concentration status						
	n	Serum 25(OH)D (Mean ± SD)	*P*-value for ANOVA	Number (%)			
				<50 nmol L^{-1}	50–<75 nmol L^{-1}	≥75 nmol L^{-1}	*P*-value for difference*
All subjects vit D status	119	70.2 ± 23.2		40.4 ± 9.4	63.6 ± 7.0	94.9 ± 19.2	
Ethnicity							
White	98	73.2 ± 21.5	<0.001	11 (11%)	49 (50%)	38 (39%)	<0.001
African American	17	49.1 ± 18.1		9 (53%)	7 (41%)	1 (6%)	
Other	4	85.9 ± 37.9		0 (0%)	3 (75%)	1 (25%)	
Maternal education status							
Completed 4 year college	82	71.8 ± 22.7	0.28	11 (13%)	42 (51%)	29 (35%)	0.34
Did not complete 4 year college	37	66.8 ± 24.3		9 (24%)	17 (46%)	11 (30%)	
Maternal BMI class							
Not obese (BMI <30)	77	72.7 ± 22.9	0.12	9 (12%)	39 (51%)	29 (38%)	0.10
Obese (BMI ≥ 30)	42	65.8 ± 23.5		11 (26%)	20 (48%)	11 (26%)	
Maternal vit D supplementation							
Any supplementation	93	72.7 ± 21.1	0.08	11 (12%)	47 (51%)	35 (38%)	0.20
No supplementation	14	61.2 ± 28.6		4 (29%)	7 (50%)	3 (21%)	
Season							
Winter/spring	41	60.9 ± 15.0	0.001	11 (27%)	23 (56%)	7 (17%)	0.009
Summer/fall	78	75.1 ± 25.3		9 (12%)	36 (46%)	33 (42%)	
Sun exposure time							
Lowest quartile (<1.83 h)	29	69.8 ± 17.4	0.44	2 (7%)	18 (62%)	9 (31%)	0.38
Highest quartile (>8.54 h)	30	65.9 ± 21.2		6 (20%)	16 (53%)	8 (27%)	
Sun exposure (BSA)							
Lowest quartile (<19%)	29	65.7 ± 20.6	0.13	6 (21%)	15 (52%)	8 (28%)	0.35
Highest quartile (>49%)	26	75.3 ± 25.1		3 (12%)	11 (42%)	12 (46%)	
Sun index							
Lowest quartile (<42.5)	29	68.1 ± 17.8	0.47	3 (10%)	18 (62%)	8 (28%)	0.67
Highest quartile (>338.4)	30	64.7 ± 18.7		6 (20%)	16 (53%)	8 (27%)	
Sunscreen usage							
Any sunscreen usage	18	72.9 ± 25.4	0.60	3 (17%)	7 (39%)	8 (44%)	0.73
No sunscreen usage	28	69.3 ± 20.7		5 (18%)	14 (50%)	9 (32%)	

25(OH)D, 25-hydroxyvitamin D; ANOVA, analysis of variance; BSA, body surface area; SD, standard deviation; vit D, vitamin D. *For vit D level, mean comparisons made with ANOVA. For tests of proportions, differences were tested using chi-square or Fisher's exact test, as appropriate.

Table 3. Predictors of infant vitamin D status at 4 weeks of age

Variables	Infants' serum 25(OH)D concentration status						
	n	Serum 25(OH)D (Mean ± SD)	*P*-value for ANOVA	Number (%)			
				<25 nmol L^{-1}	25–<50 nmol L^{-1}	≥50 nmol L^{-1}	*P*-value for difference*
All subjects vit D status	87	41.0 ± 16.3		18.7 ± 3.5	39.2 ± 7.0	63.5 ± 10.1	
Ethnicity							
White	69	43.5 ± 15.6	0.008	8 (12%)	43 (62%)	18 (26%)	0.005
African American	9	26.9 ± 14.4		6 (67%)	2 (22%)	1 (11%)	
Other	9	35.7 ± 16.1		2 (22%)	6 (67%)	1 (11%)	
Maternal education status							
Completed 4 year college	58	42.4 ± 17.1	0.25	9 (16%)	33 (57%)	16 (28%)	0.29
Did not complete 4 year college	29	38.1 ± 14.5		7 (24%)	18 (62%)	4 (14%)	
Maternal vit D supplementation							
Any supplementation	71	40.0 ± 15.7	0.12	13 (18%)	43 (61%)	15 (21%)	0.54
No supplementation	11	48.2 ± 17.4		1 (9%)	6 (55%)	4 (36%)	
Infant vit D supplementation							
Supplemented prior to 4 weeks of age	3	51.5 ± 34.0	0.26	1 (33%)	0 (0%)	2 (67%)	0.07
Not supplemented prior to 4 weeks of age	84	40.6 ± 15.6		15 (18%)	51 (61%)	18 (21%)	
Season							
Winter/Spring	31	34.0 ± 14.7	0.002	10 (32%)	17 (55%)	4 (13%)	0.03
Summer/Fall	56	44.9 ± 15.9		6 (11%)	34 (61%)	16 (29%)	
Sun exposure time							
Lowest quartile (<0.67 h)	22	34.1 ± 12.6	0.003	6 (27%)	14 (64%)	2 (9%)	0.03
Highest quartile (>5.83 h)	19	48.3 ± 16.3		1 (5%)	10 (53%)	8 (42%)	
Sun exposure (BSA)							
Lowest quartile (<12%)	22	36.4 ± 16.1	0.04	7 (32%)	11 (50%)	4 (18%)	0.03
Highest quartile (>43%)	17	46.7 ± 12.8		0 (0%)	13 (77%)	4 (24%)	
Sun index							
Lowest quartile (<10.75)	22	32.4 ± 12.8	<0.001	8 (36%)	12 (55%)	2 (9%)	0.01
Highest quartile (>161.5)	21	49.0 ± 14.7		1 (5%)	12 (57%)	8 (38%)	
Sunscreen usage							
Any sunscreen usage	1	60.0 ± N/A	0.13	0 (0%)	0 (0%)	1 (100%)	0.21
No sunscreen usage	32	35.6 ± 15.6		9 (28%)	17 (53%)	6 (19%)	
Maternal vit D status							
Deficient (<50 nmol L^{-1})	15	35.5 ± 18.7	0.08	6 (40%)	5 (33%)	4 (27%)	0.04
Insufficient (50–<75 nmol L^{-1})	45	39.6 ± 15.8		8 (18%)	30 (67%)	7 (16%)	
Sufficient (≥75 nmol L^{-1})	26	46.7 ± 15.0		2 (8%)	15 (58%)	9 (35%)	

25(OH)D, 25-hydroxyvitamin D; ANOVA, analysis of variance; BSA, body surface area; SD, standard deviation; vit D, vitamin D. *For vit D level, mean comparisons made with ANOVA. For tests of proportions, differences were tested using chi-square or Fisher's exact test, as appropriate.

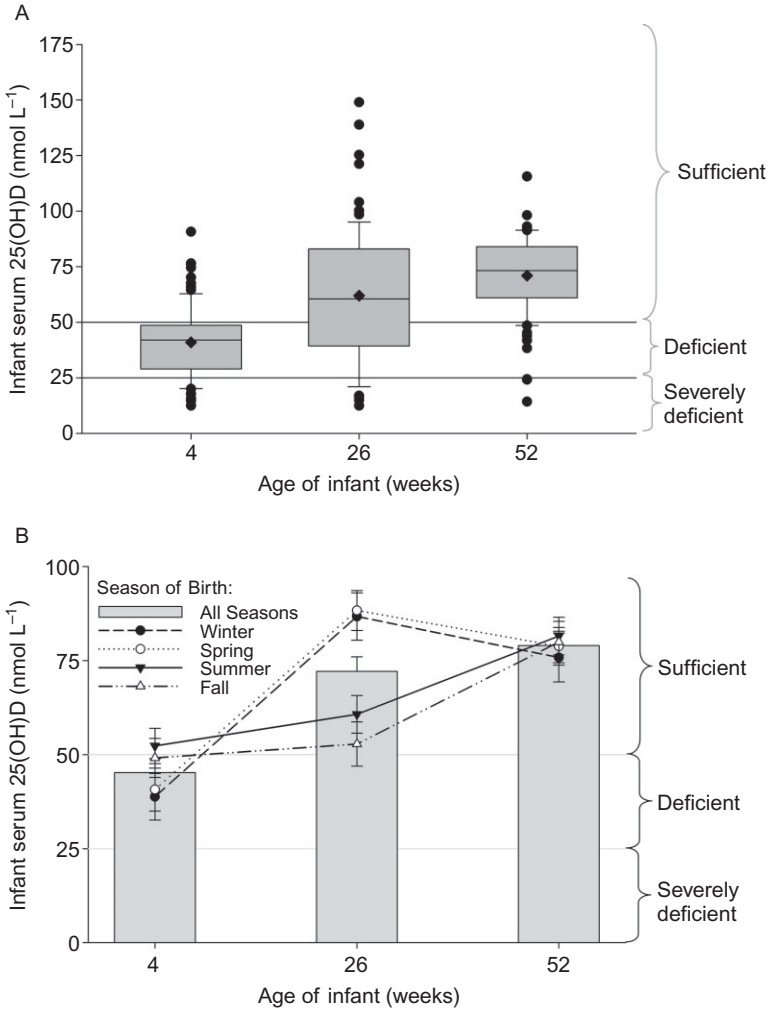

Fig. 1. (a) Infant serum 25-hydroxyvitamin D [25(OH)D] (nmol L^{-1}) measurements at 4, 26 and 52 weeks of age. Box indicates median and interquartile range; whiskers delineate 90th and 10th percentiles, with individual values outside those percentiles denoted by closed circles. Diamonds within the boxes designate the unadjusted mean levels. Horizontal lines show thresholds for vitamin D deficiency and severe deficiency. (b) Adjusted mean ± standard error of infants' serum 25(OH)D (nmol L^{-1}) by age for each birth season. Means are adjusted for race, maternal education level, maternal vitamin D sufficiency, maternal vit D supplementation, maternal % body surface area exposed to sunlight, exclusive breastfeeding, infant vitamin D supplementation and formula use. Lines represent the trajectory of serum 25(OH)D (nmol L^{-1}) over time for infants born during each season. Horizontal lines show thresholds for vitamin D deficiency and severe deficiency.

4 weeks of age. In univariate analysis, black race ($P = 0.005$), maternal vit D status ($P = 0.04$) winter/spring birth season ($P = 0.03$), lowest quartile sun exposure time ($P = 0.03$), % BSA exposure ($P = 0.03$) and sun index ($P < 0.001$) were associated with higher prevalence of vit D deficiency and severe vit D deficiency (Table 3). African American infants were 13 times [OR (95% CI): 13.6 (2.9, 63.2)] much more likely to be severely vit D deficient than other infants.

Unadjusted mean serum 25(OH)D levels increased from 41.0 nmol L^{-1} at 4 weeks ($n = 87$) to 62.1 nmol L^{-1} at 26 weeks ($n = 77$) and to 71.0 nmol L^{-1} at 52 weeks ($n = 80$) (all pairwise $P < 0.02$ after adjustment for multiple comparisons; Fig. 1a). In repeated measures longitudinal analysis, mean serum 25(OH)D levels [±standard error (SE)] similarly increased from 45.0 (±3.8) nmol L^{-1} at 4 weeks to 72.6 (±4.0) nmol L^{-1} at 26 weeks and to 80.3 (±4.2) nmol L^{-1} at 52 weeks (all pairwise $P < 0.02$), adjusting for the infant characteristics of race, exclusive breastfeeding, formula use, infant vit D supplementation, season of measurement and an interaction between season and infant age, and the maternal characteristics of education level, vit D sufficiency and supplementation, and % BSA exposed to sunlight.

The changes in infant's adjusted serum 25(OH)D levels reflected both the season of birth and the age (Fig. 1b). Infants' 25(OH)D levels did not differ by season of birth at either 4 or 52 weeks of age (all pairwise $P > 0.7$). However, infant's 25(OH)D levels

Table 4. Sunlight exposure for mothers and infants by season of measurement

			n	Winter	Spring	Summer	Fall	*P*-value
Mother	4 weeks	% BSA	120	10.0 (7.0, 14.0)^{A}	34.0 (27.0, 50.0)^{BC}	43.0 (34.0, 50.0)^{B}	27.0 (14.0, 39.0)^{C}	<0.001
		Hours	120	1.7 (0.7, 6.0)^{A}	7.5 (3.4, 12.5)^{B}	4.3 (2.1, 8.0)^{AB}	4.0 (2.1, 8.0)^{AB}	0.02
		Sun index	120	18.8 (6.0, 54.0)^{A}	340 (84.0, 544)^{B}	198 (90.6, 384)^{B}	108 (29.2, 188)^{B}	<0.001
Infant	4 weeks	% BSA	118	3.5 (0.0, 9.0)^{A}	21.0 (12.0, 33.0)^{B}	42.0 (32.0, 48.0)^{C}	26.0 (9.0, 42.0)^{B}	<0.001
		Hours	120	1.3 (0.3, 3.8)	3.8 (1.8, 7.0)	2.3 (0.7, 4.3)	3.8 (0.8, 6.0)	0.07
		Sun index	118	1.2 (0.0, 12.0)^{A}	54.0 (27.0, 236)^{B}	63.8 (27.2, 220)^{B}	50.0 (15.2, 175)^{B}	<0.001
	26 weeks	% BSA	91	9.0 (5.0, 14.0)^{A}	14.0 (12.0, 24.0)^{AC}	37.0 (30.0, 55.0)^{B}	26.0 (14.0, 32.0)^{C}	<0.001
		Hours	93	1.0 (0.3, 2.2)^{A}	2.0 (1.0, 4.5)^{A}	14.0 (7.5, 16.5)^{B}	2.5 (1.0, 5.8)^{A}	<0.001
		Sun index	91	5.2 (0.0, 19.5)^{A}	40.0 (8.3, 94.5)^{AC}	473 (278, 880)^{B}	44.0 (24.0, 165)^{C}	<0.001
	52 weeks	% BSA	87	9.0 (7.0, 12.0)^{A}	26.0 (14.0, 32.0)^{B}	49.0 (43.0, 55.0)^{C}	35.5 (26.0, 44.0)^{B}	<0.001
		Hours	92	1.8 (1.2, 7.0)^{A}	5.0 (1.7, 11.2)^{A}	10.5 (5.4, 14.0)^{B}	6.0 (3.4, 8.9)^{AB}	0.003
		Sun index	87	24.0 (0.0, 74.0)^{A}	104 (33.8, 271)^{B}	528.5 (224, 753)^{C}	272 (76.3, 457)^{B}	<0.001

BSA, body surface area. Median (interquartile range) is reported. Median differences were tested using the Kruskal–Wallis test, and pairwise *P*-values were calculated using the Tukey–Kramer adjustment for multiple comparisons. Medians with different superscript in a given row are significantly different. Sun index is defined as the product of % BSA and weekly hours of sun exposure.

were higher for those who reached 26 weeks during summer or fall (i.e. born in winter/spring) than those who reached 26 weeks during winter or spring (i.e. born in summer/fall; all pairwise $P < 0.02$).

Conversely, the prevalence of vit D deficiency declined from 77% at 4 weeks to 31% at 26 weeks and 12.5% at 52 weeks, while the prevalence of severe vit D deficiency declined from 18% at 4 weeks to 13% at 26 weeks and 4% at 52 weeks. The decline in deficiency was stronger between 4 weeks and both 26 and 52 (McNemar test $P \leq 0.001$) than between 26 and 52 weeks (McNemar test $P = 0.054$).

Infant milk source changed during the follow-up and impacted vit D status. Fifteen out of 120 (12.5%) infants were fed formula at 4 weeks, while 31 out of 96 (32%) used formula at 26 weeks. At 26 weeks, any formula usage was associated with higher serum 25(OH)D levels (adjusted mean ± SE: 83 ± 5.3 nmol L^{-1} vs. 63 ± 4.3 nmol L^{-1}, $P < 0.001$).

Vitamin D intake

Ninety-three of the 107 (87%) mothers with data on dietary intake reported taking vit D supplements. The median (interquartile range) vit D intake from diet and supplement combined was 540 IU day^{-1} (420, 640). There were no ethnic differences in daily vit D intake. Maternal vit D intake was not associated with vit D status in this cohort ($P = 0.59$). Mothers' report of vit D supplementation compared with no supplementation showed only a trend to higher serum 25(OH)D ($P = 0.08$) and did not impact her category of vit D status at 4 weeks post-partum (Table 2).

Only 32 (27%) of the infants ever received vit D supplementation. Supplementation was initiated before 4 weeks of age in only 6 (5%) and in 19% by 6 months of age. In view of the small number of infants supplemented with vit D at 4 weeks of age, we could not make a valid assessment of the effect of supplementation on infant vit D status. Maternal vit D supplementation status in the first month post-partum did not affect the infant vit D status (Table 3).

Sun exposure behaviours

Sun exposure behaviours in mothers and infants varied by season and age of infant (Table 4). At 4 weeks post-partum, mothers reported significantly more hours of direct sunlight exposure $week^{-1}$ in spring than winter. Median % BSA exposed outdoors and sun index were lower in winter than in other seasons (Table 4). Maternal sun exposure time, % BSA exposed and sun index did not affect her category of vit D status (Table 2). In this study, there was no interaction between race and maternal sun index and serum 25(OH)D concentrations.

A total of 93%, 92% and 96% of the infants were reportedly exposed to direct sunlight at 4, 26 and 52 weeks, respectively. The median duration of reported direct sunlight exposure week^{-1} by 4-week-old infants did not vary significantly by season, but the median % BSA was higher in summer than other seasons ($P < 0.001$). The sun index was lower in winter than other seasons (Table 4). There were positive correlations between mothers and infants in the duration of sun exposure ($r = 0.47$, $P < 0.001$) and % BSA exposed while outdoors ($r = 0.60$, $P < 0.001$). There were no ethnic differences in the sun exposure behaviours. As noted before, duration of sun exposure, % BSA exposed and sun index were significant determinants of infants' vitamin D status in univariate analysis (Table 3).

Of 47 mothers with information at 4 weeks post-partum on sunscreen use, 18 (38%) reported using sunscreen and of those 95% used a sun protection factor (SPF) >8. In contrast, of these 47 mothers, only one reported using sunscreen on her infant at 4 weeks of age, while 31 (34%) of 92 mothers reported using sunscreen on their infants at 52 weeks of age. In this subset, sunscreen usage did not significantly affect maternal or infant vit D levels (Tables 2,3).

Independent predictors of vitamin D sufficiency

Based on the significant factors on univariate analysis, multivariable models were constructed at each time point to determine independent predictors of maternal and infant vit D status. At 4 weeks post-partum, vit D sufficient mothers [serum 25(OH)D $\geq$ 75 nmol L^{-1}] were significantly more likely to be white [OR (95% CI): 8.3 (1.8, 38.8), $P = 0.007$] and to be measured during the summer/fall seasons [4.5 (1.7, 11.8), $P = 0.002$] compared with vit D insufficient or deficient mothers.

In infants, higher 25(OH)D levels at 4 weeks of age were independently associated with non-Hispanic white race ($\beta \pm$ SE: 14.3 $\pm$ 3.9 nmol L^{-1}, $P = 0.001$) and summer/fall season of birth ($\beta \pm$ SE: 12.6 $\pm$ 3.3 nmol L^{-1}, $P = 0.001$). At 26 weeks of age, independent predictors of infant vit D sufficiency were being born in winter/spring seasons [OR (95% CI): 10.7 (2.6, 43.9), $P < 0.001$] and shorter duration of exclusive breastfeeding [OR (95% CI): 0.6 (0.4, 0.9) per month of exclusive breastfeeding, $P = 0.003$]. At 52 weeks of age, only 4-week 25(OH)D levels were significantly associated with vit D sufficiency. However, excluding 4-week 25 (OH)D levels as a potential covariate, current weekly hours of sun exposure [OR (95% CI): 1.25 (1.03, 1.51) per hour of sun exposure, $P = 0.01$] was the only other independent predictor of vit D sufficiency in infants at 52 weeks.

Serum PTH and ALP concentrations and vitamin D status

There were negative correlations between serum 25(OH)D levels and PTH values in mothers ($r = -0.4$, $P < 0.001$) at 4 weeks (Fig. 2) post-partum, with the highest PTH levels in vit D deficient mothers (25.6 $\pm$ 8.2), followed by insufficient mothers (20.1 $\pm$ 8.2), and lowest in vit D sufficient mothers (15.2 $\pm$ 6.0) (all $P < 0.02$). There was no significant correlation between serum ALP and maternal 25(OH)D ($r = -0.17$, $P = 0.06$) and the ALP levels in mothers with different categories of vit D status were not different ($P = 0.3$).

Serum 25(OH)D levels also correlated with PTH values in infants at 26 ($r = -0.3$, $P = 0.01$) and 52 weeks ($r = -0.5$, $P < 0.001$), but not at 4 weeks ($P = 0.56$) (Fig. 2). Serum 25(OH)D concentrations showed negative weak correlations with serum ALP values ($r = -0.16$, $P = 0.02$). Neither PTH nor ALP levels demonstrated a non-linear relationship with 25(OH)D levels (data not shown). The relationships between categories of infant vit D status and serum PTH and ALP levels during the first year follow-up are summarised in Table 5. Serum PTH levels were significantly higher only in severely vit D deficient compared with sufficient infants at 26 weeks. The mean ALP levels did not differ significantly by categories of vit D status in infants.

Discussions

In this prospective cohort study, vit D deficiency and insufficiency is widespread in breastfeeding mothers and their infants at 4 weeks in Cincinnati. Based on the available evidence, the AAP, IOM and the Endo-

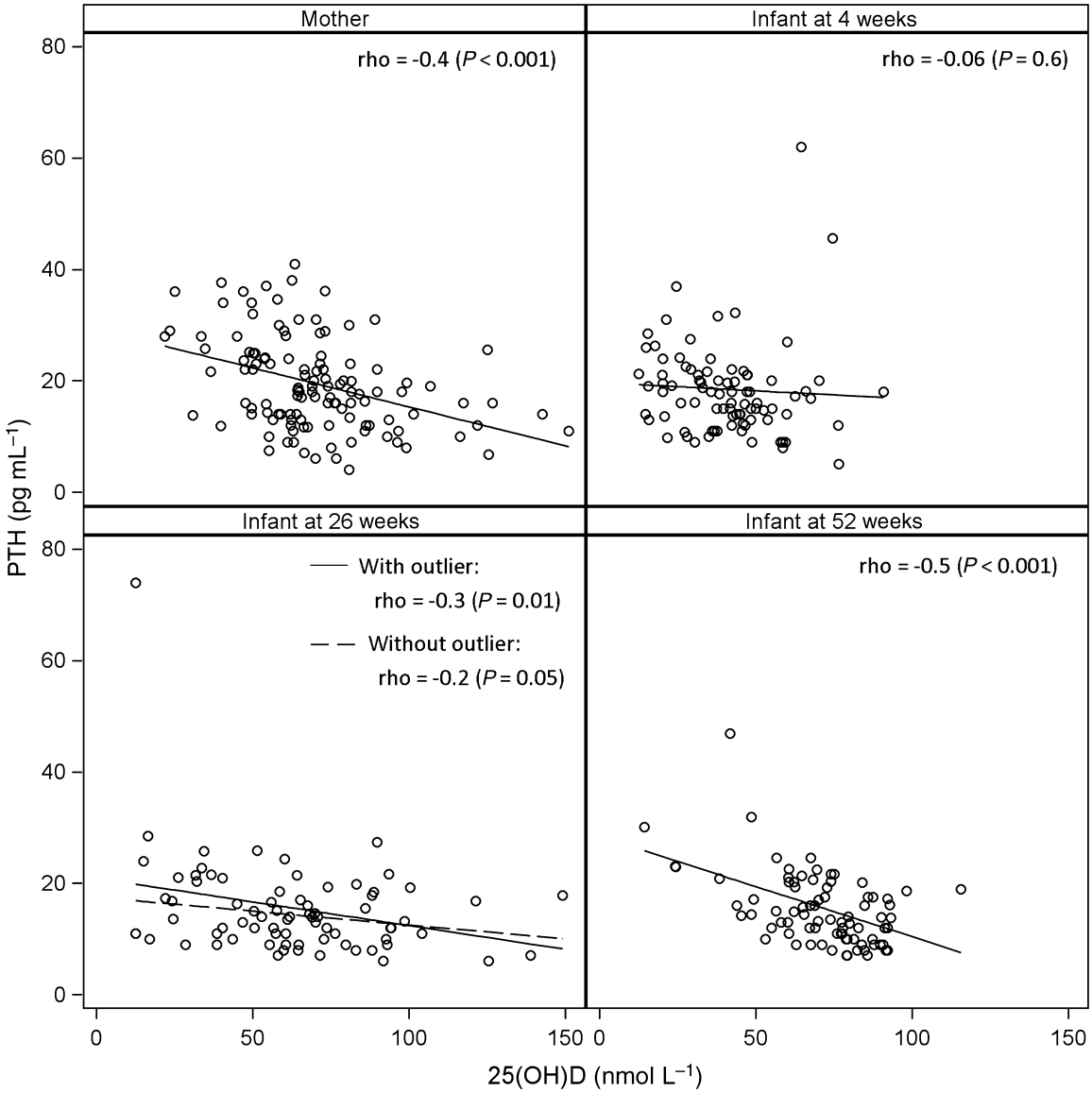

Fig. 2. Serum 25-hydroxyvitamin D [25(OH)D] levels and intact parathyroid hormone levels for mothers at 4 weeks post-partum and for infants at 4, 26 and 52 weeks of age. Pearson's correlation coefficients and *P*-values are reported. Solid lines are regression lines for the data. Dashed line for infants at 26 weeks excludes one outlying value from the regression calculation. PTH, parathyroid hormone.

crine Society Guidelines (Institute of Medicine 2011; Wagner & Greer 2008; Holick *et al*. 2011) recommend serum 25(OH)D concentrations ≥50 nmol L^{-1} in infants and children. Three out of four infants in this study had serum 25(OH)D levels <50 nmol L^{-1} by the first month of life. A recent US study also found that 24 (73%) of 33 fully breastfeeding infants had serum 25(OH)D levels <50 nmol L^{-1} at 1 month of age (Wagner *et al*. 2010). In another study from India, 90% of breastfed infants <6 months old were reported to have serum 25(OH)D levels <50 nmol L^{-1} (Seth *et al*. 2009). Therefore, vit D deficiency [as defined by serum 25(OH)D <50 nmol L^{-1}] occurs early in breastfed infants and appears to be a public health problem. This should be of concern in view of the promotion of exclusive breastfeeding and the additional recogni-

Table 5. Longitudinal assessment of infant serum PTH and ALP by vitamin D status

Measurement	Age (weeks)	*n*	Vitamin D status						Omnibus *P*-value
			Severely deficient (<25 nmol L^{-1})		Deficient (25–<50 nmol L^{-1})		Sufficient (≥50 nmol L^{-1})		
			n	Value	*n*	Value	*n*	Value	
PTH (pg mL^{-1})	4	87	16	21.3 ± 1.9	51	17.1 ± 1.1	20	18.5 ± 1.7	0.04
	26	76	9	22.9 ± 2.5^{A}	14	16.7 ± 2.0^{AB}	53	13.6 ± 1.0^{B}	
	52	80	3	25.3 ± 4.3	7	23.0 ± 2.8	70	14.1 ± 0.9	
ALP (IU L^{-1})	4	66	12	223 ± 44	38	279 ± 24	16	199 ± 38	0.78
	26	72	9	115 ± 50	12	180 ± 42	51	140 ± 21	
	52	76	3	166 ± 87	7	167 ± 57	66	184 ± 18	

ALP, alkaline phosphatase; PTH, parathyroid hormone. Least square means ± standard errors are reported. Mean differences were tested using repeated measures analysis of variance, and pairwise *P*-values were calculated using the Tukey–Kramer adjustment for multiple comparisons. Means in a row with different superscript letters are significantly different.

tion of increased risk of non-skeletal complications of vit D deficiency in children (Hypponen *et al*. 2001; Wayse *et al*. 2004; McNally *et al*. 2009).

We also showed that 18% and 12% of breastfed infants at the age of 4 and 26 weeks, respectively, had severe vit D deficiency, which is consistent with higher risk of rickets (Dawodu *et al*. 2005; Institute of Medicine 2011). Infants born to African American mothers were 13 times much more likely than other infants to be severely vit D deficient soon after birth. In a recent study from Iowa, 5 (6%) of 77 light skin infants and 3 (43%) of 7 black skin-pigmented infants, respectively, had severe vit D deficiency at 9 months of age (Ziegler *et al*. 2006). These findings are in agreement with the clinical experience of high prevalence of rickets among breastfed black infants in the United States (Weisberg *et al*. 2004). In India (Seth *et al*. 2009), Greece (Challa *et al*. 2005) and the United Arab Emirates (Dawodu *et al*. 2003), 43%, 21–30% and 82%, respectively, of exclusively breastfed infants <6 months old were reported to be severely vit D deficient. Taken together, these results indicate that vit D deficiency among exclusively breastfed infants appears to be a global health problem and the cases presenting as rickets vastly underestimate the magnitude of vit D deficiency.

The negative correlation between 25(OH)D and PTH levels is supportive of suboptimal vit D status. However, significantly higher PTH was only demonstrated when serum 25(OH)D levels were <25 nmol L^{-1} in infants. Serum ALP was not associated with vit D deficiency in the infants at 4 weeks of age and during follow-up at 26 and 52 weeks. In contrast, a recent study found an association between elevated ALP and severe vit D deficiency [serum 25(OH)D < 12.5 nmol L^{-1}] during the first 9 months of life (Ziegler *et al*. 2006). In this study, we could not evaluate the relationship between ALP and severe vit D deficiency as defined by serum 25(OH)D < 12.5 nmol L^{-1} because there were only four patients with serum 25(OH)D of 12.5 nmol L^{-1} and none with values <12.5 nmol L^{-1}. The results would suggest that significantly elevated serum PTH and ALP levels in infancy may only be associated with very low vit D status. This premise needs to be investigated in future large studies.

Vitamin D deficiency in infants at 4 weeks of age was associated with maternal vit D deficiency. The finding agrees with the results of other studies (Specker *et al*. 1985b; Dawodu *et al*. 2003; Challa *et al*. 2005; Seth *et al*. 2009) and indicate a possible contribution of maternal vit D deficiency to vit D deficiency in breastfed infants. Theoretically, vit D deficient lactating mothers would be expected to have been vit D deficient during pregnancy, although the current study did not evaluate vit D status of mothers before delivery. Maternal vit D deficiency during pregnancy is a significant risk factor for vit D deficiency in infants at birth (Lee *et al*. 2007; Merewood *et al*. 2010). Therefore, the early post-natal high rate of vit D deficiency

likely results, in part, from poor previous vit D transplacental transfer (Hollis & Wagner 2004; Lee *et al.* 2007; Merewood *et al.* 2010) and low human milk vit D content (Specker *et al.* 1985a; Wagner *et al.* 2006).

Currently, the AAP recommends vit D supplementation soon after birth to prevent vit D deficiency in breastfed infants. It is, therefore, disappointing that only 5% of infants were reported to have received a vit D supplement by 4 weeks of age and only 19% in the first 6 months, despite the fact that the majority (68%) were still fully breastfed at 6 months. This low supplementation rate is consistent with the findings of 5–19% vit D supplementation rates among fully breastfed infants in other US studies (Davenport *et al.* 2004; Perrine *et al.* 2010; Taylor *et al.* 2010). Why then are breastfed infants not receiving vit D supplementation as recommended by recent publications from the AAP (Gartner & Greer 2003; Wagner & Greer 2008)? Previous studies that attempted to answer this question concluded that a majority of paediatric health care providers do not recommend vit D supplementation for breastfed infants (Davenport *et al.* 2004; Shaikh & Alpert 2004; Taylor *et al.* 2010). The reasons include the assumption that breast milk contains sufficient vit D (Davenport *et al.* 2004; Shaikh & Alpert 2004; Sherman & Svec 2009), infants receive adequate sunlight exposure (Shaikh & Alpert 2004; Sherman & Svec 2009), recommendation of supplementation might discourage mothers from breastfeeding (Taylor *et al.* 2010) and that rickets is rare (Shaikh & Alpert 2004). On the contrary, rickets is not rare, especially among unsupplemented breastfed black infants in the United States (Weisberg *et al.* 2004). Although assessment of vit D content of human milk was not conducted, most of the breastfeeding mothers in this study would be expected to produce milk with insufficient vit D for their nursing infants because of the high prevalence of maternal vit D insufficiency (Wagner *et al.* 2006). It is hoped that the results of this and other recent studies (Ziegler *et al.* 2006; Wagner *et al.* 2010) will alert paediatric health care providers to the magnitude of vit D deficiency and insufficiency in breastfeeding mothers and their infants, and increase the awareness of the need to counsel for vit D supplementation of breastfeeding mother and her infant. Adequate supplementation should be emphasised, not only to prevent rickets but because of the reported association between vit D deficiency and increased risk of other childhood complications such as respiratory infections, wheezing and type 1 diabetes (Hypponen *et al.* 2001; Wayse *et al.* 2004; Holick 2007; McNally *et al.* 2009).

Contrary to the AAP recommendations to restrict sun exposure (AAP 1999), more than 90% of breastfed infants <6 months old in this study were reported by mothers to be exposed to direct sunlight and there was a positive correlation between skin sun exposure and vit D status of the infants. An older study of breastfed infants from Cincinnati (Specker *et al.* 1985b) also found a positive correlation between serum 25(OH)D and sun exposure score (minutes spent outside weighted by BSA exposed while outside). The authors concluded that vit D synthesis from skin exposure to sunlight contributes to the vit D status in largely unsupplemented exclusively breastfed infants. In this study of breastfeeding infants with very low rate of vit D supplementation, the changes in the serum 25(OH)D levels at follow-up reflected the seasonal changes in sunlight exposure. Higher serum 25(OH)D levels at 26 weeks in infants born in winter/spring and who reached 26 weeks during summer/fall is consistent with a higher cumulative endogenous synthesis of vit D due to higher sunlight skin exposures in summer/fall than winter/spring seasons. Weekly hours of sun exposure positively correlated with vit D status of infants during the first year. In addition to sun exposure, the higher vit D status during the follow-up possibly reflected an increase dietary intake of vit D because infants who received breast milk and fortified formula had higher serum 25(OH)D levels than those on breast milk only.

It is of interest that the median sun exposure of 3.8 h week^{-1} at 4 weeks of age in this study is similar to the average duration of sun exposure time of 4 h week^{-1} in another study over two decades ago from Cincinnati (Greer *et al.* 1982). These findings may indicate a lack of change in sun exposure behaviours of breastfed infants and that sun exposure still contributes to vit D status of breastfed infants during the first year of life despite the recommendation to limit sun exposure in infants. As mothers still expose infants to direct sunlight, compliance with the AAP

recommendation to avoid direct sun exposure (AAP 1999) could theoretically reduce endogenous vit D3 synthesis and contribute to higher prevalence of vit D deficiency in infants without corrective vit D supplementation. Therefore, it is important to quantify the contribution of sun exposure when evaluating vit D requirements in breastfed infants in future studies. Another important future research study is to investigate if there is a minimal-risk UVB skin exposure relative to skin cancer that promotes adequate vit D endogenous synthesis (Institute of Medicine 2011).

A significant finding in this study is the high prevalence of maternal vit D deficiency despite reported median daily intake of 540 IU of vit D, which is above the recommended daily intake of 200–400 IU vit D at the time of the study (Hollis & Wagner 2004; Holick 2007). This finding is in agreement with a recent study from Boston, MA, which showed that 35% of women who took daily prenatal vitamin containing 400 IU of vitamin D were vit D deficient at delivery (Merewood *et al*. 2010). In another study from Boston of mostly black mothers, 50% had serum 25(OH)D < 30 nmol L^{-1} at delivery, despite the fact that the majority were taking daily 400–600 IU vit D (Lee *et al*. 2007). At 4 weeks post-partum, 17% of the mothers in this cohort were vit D deficient and 66% were insufficient. This is not surprising because this amount of vit D supplementation is known to be inadequate to prevent vit D deficiency in pregnant and lactating mothers (Hollis & Wagner 2004; Wagner *et al*. 2006; Lee *et al*. 2007; Merewood *et al*. 2010). In addition, the majority (95%) of mothers with data on the use of sunscreen reportedly used sunscreen with >8 SPF, which blocks endogenous synthesis of vit D3 by >95% (Matsuoka *et al*. 1987) and could have masked the effect of sun exposure on maternal vit D status. It is possible that inadequate endogenous vit D synthesis and a lack of corrective vit D intake likely contributed to the high prevalence of vit D insufficiency in the mothers, although relationship between sunscreen use and 25(OH)D could not be confirmed in a small sample of mothers with information on sun screen use in this study. Inadequate sun exposure and vit D intake were proposed in breastfeeding mothers in other high risk populations (Dawodu *et al*. 2003; Bhalala *et al*. 2007; Seth *et al*. 2009). In a setting of breastfeeding, vit D deficiency is better viewed as a mother–infant health problem. We suggest that global preventive strategies should ensure mother–infant vit D sufficiency and should start by ensuring vit D sufficiency during pregnancy.

The limitations of this study include a moderate sample size and population may not be representative of all communities. However, we examined these as potential bias and found that the composition of ethnic groups in the study was similar to the distribution in Hamilton County where the study subjects were enrolled. In addition, 28% of the infants did not have serum 25(OH)D measured at 4 weeks of age because blood samples were not available. However, infants with serum 25(OH)D levels were representative of the study population because there were no significant differences in ethnicity, sun exposure behaviours, vit D supplementation and maternal vit D status between mother–infant pairs with and without 4-week infant serum 25(OH)D values (results not shown). The assessment of sun exposure behaviour and infant vit D supplementation was based on reports by the mothers and are subject to recall bias; however, prospective collection of this information using 24-h recalls may minimise this bias. In addition, assessment of sun exposure did not include data on time of day and the proportion of the body area being assessed that was actually exposed to direct sunlight. These factors affect endogenous vit D synthesis (Holick 2007). Therefore, caution is necessary in determining the magnitude of direct skin sun exposure and the rate of vit D supplementation use. Nevertheless, the study provides new information on sun exposure behaviours, and vit D supplementation of breastfeeding mothers and infants, type of infant milk feeding and the contribution of these factors to vit D status of breastfed infants during the first year of life.

Conclusions

Vitamin D deficiency is widespread early post-partum in breastfeeding mothers and their infants in Cincinnati, and severe vit D deficiency is common in the infants, especially African Americans. Winter/spring seasons, low skin sun exposure and longer duration of exclusive breastfeeding are independent determi-

nants of vit D deficiency in breastfeeding infants, with low rate of reported vit D supplementation during the first year of life. We suggest that there is a need to intensify corrective vit D supplementation starting during pregnancy to prevent vit D deficiency in breastfeeding mothers and infants when sunlight exposure is purposely avoided or restricted.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Contributions

AD, JGW and ALM participated in the study design, data analysis and the initial draft of the manuscript. JGW PMH and ALM provided statistical guidance in the analysis. AD, JGW, ALM, PMH, LZ, BSD and JEH assisted in the interpretation of results. All the co-authors contributed to the preparation and review of the content of the manuscript.

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