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Cord Blood Vitamin D Deficiency is Associated With Respiratory Syncytial Virus Bronchiolitis

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KEY WORDS

bronchiolitis, nutrition, pregnancy, infant, immunity

ABBREVIATIONS

RSV—respiratory syncytial virus
LRTI—lower respiratory tract infection
25-OHD—25-hydroxyvitamin D

Drs Belderbos, Houben, and Bont conceived and designed the research; Drs Belderbos and Bont conducted research; Drs Lentjes and Wilbrink were responsible for the biochemical assays; Mrs Bloemen contributed to data collection; Dr Rovers assisted in statistical analyses; and Dr Kimpen contributed to writing of the manuscript.

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WHAT'S KNOWN ON THIS SUBJECT: Respiratory syncytial virus is the most important pathogen that causes lower respiratory tract infections in infants. Cross-sectional studies in developing countries have show that vitamin D deficiency is associated with increased risk and severity of infant viral respiratory tract infections.

WHAT THIS STUDY ADDS: Results of this prospective birth cohort study demonstrated that vitamin D deficiency is highly prevalent among healthy newborns in Western countries, and that neonates who are vitamin D deficient at birth have an increased risk of developing respiratory syncytial virus respiratory tract infections during infancy.

abstract

BACKGROUND: Respiratory syncytial virus (RSV) is the most important pathogen causing severe lower respiratory tract infection (LRTI) in infants. Epidemiologic and basic studies suggest that vitamin D may protect against RSV LRTI.

OBJECTIVE: To determine the association between plasma vitamin D concentrations at birth and the subsequent risk of RSV LRTI.

DESIGN: A prospective birth cohort study was performed in healthy term neonates. Concentrations of 25-hydroxyvitamin D (25-OHD) in cord blood plasma were related to RSV LRTI in the first year of life, defined as parent-reported LRTI symptoms in a daily log and simultaneous presence of RSV RNA in a nose-throat specimen.

RESULTS: The study population included 156 neonates. Eighteen (12%) developed RSV LRTI. The mean plasma 25-OHD concentration was 82 nmol/L. Overall, 27% of neonates had 25-OHD concentrations <50 nmol/L, 27% had 50-74 nmol/L and only 46% had 25-OHD 75 nmol/L. Cord blood 25-OHD concentrations were strongly associated with maternal vitamin D3 supplementation during pregnancy. Concentrations of 25-OHD were lower in neonates who subsequently developed RSV LRTI compared with those who did not (65 nmol/L versus 84 nmol/L, $P = .009$). Neonates born with 25-OHD concentrations <50 nmol/L had a sixfold (95% confidence interval: 1.6-24.9; $P = .01$) increased risk of RSV LRTI in the first year of life compared with those with 25-OHD concentrations ≥ 75 nmol/L.

CONCLUSIONS: Vitamin D deficiency in healthy neonates is associated with increased risk of RSV LRTI in the first year of life. Intensified routine vitamin D supplementation during pregnancy may be a useful strategy to prevent RSV LRTI during infancy. *Pediatrics* 2011;127:e1513–e1520

Respiratory syncytial virus (RSV) is the most important respiratory pathogen in young children, in whom it causes significant morbidity.[1,2] Although >90% of all infants encounter RSV before the age of 2 years, only 10% develop a severe lower respiratory tract infection (LRTI). Several risk factors for RSV LRTI have been described, but the majority of infections occur in infants without any known risk factors.[1,2] Insight into the factors that predispose infants to RSV LRTI may result in new strategies to prevent infection.

Vitamin D is an essential nutrient, with functions that extend beyond its classical role in bone metabolism. Vitamin D regulates >1000 human genes, with receptors present in most cells throughout the body.[3] In Westernized countries, 40% of pregnant women and 50% of newborns and infants have vitamin D insufficiency.[4–8] Concentrations of vitamin D in the fetus and newborn are dependent on and correlated with maternal serum 25-hydroxyvitamin D (25-OHD) concentrations.[9–12] Accordingly, maternal vitamin D insufficiency has been related to many diseases in the offspring, including type 1 diabetes,[13] multiple sclerosis,[14] schizophrenia,[15] infant wheeze,[16] and acute respiratory infections.[12,17]

Basal and epidemiologic evidence suggests that vitamin D may protect against severe RSV LRTI. In vitro, vitamin D decreases the inflammatory response of airway epithelial cells to RSV infection, without jeopardizing viral clearance.[18] In humans, RSV occurs in a seasonal pattern with peaks in winter, when serum concentrations of vitamin D are lowest.[19] Genetic polymorphisms in the vitamin D receptor are associated with hospitalization for acute LRTIs, predominantly RSV bronchiolitis, in infancy.[20] Furthermore, several studies have demonstrated that plasma concentrations 25-OHD are lower in infants hospitalized for acute LRTI compared with healthy controls.[10–12,21,22] However, these studies were cross-sectional and included a limited number of subjects.

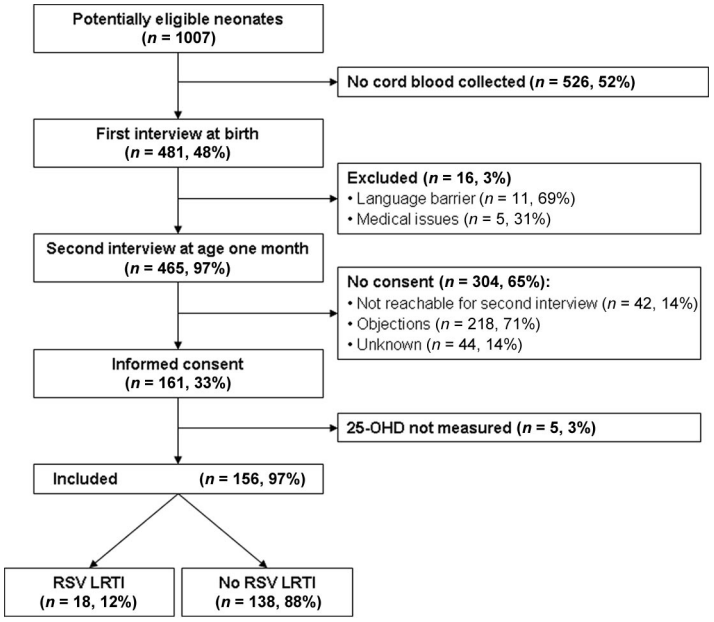

FIGURE 1
Flow chart of study population.

In this healthy birth cohort study, we aimed to determine the association between cord blood vitamin D status and the subsequent risk of RSV LRTI in the first year of life.

METHODS

Study Design and Recruitment Criteria

This study was part of a prospective birth cohort study on early life determinants of RSV LRTI performed in 2 medical centers in Utrecht, Netherlands. Study design and recruitment criteria have been published previously.[23] Eligible study participants were healthy newborns, born after uncomplicated gestation of ≥37 weeks. To avoid extensive counseling of parents just after delivery, a 2-step approach was used (Fig 1). The first consent interview took place soon after delivery. During this interview, parents were informed that cord blood had been collected and were provided with oral and written information on the study. A second interview was scheduled when the infant was aged 1 to 3 weeks, during which we provided additional information and obtained informed consent. The study was approved by the ethics review board of the University Medical Center Utrecht and the Diakonessen Hospital Utrecht, and parents of all participants provided written informed consent for study participation.

Clinical Characteristics

Data on baseline characteristics and use of vitamin D supplements were collected from hospital charts and standardized parental questionnaires.[24] Maternal ethnicity was defined as caucasian or other on the basis of country of birth. Season of birth was designated as: winter (December, January, February), spring (March, April, May), summer (June, July, August), or fall (September, October, November). To assess the relation between cord blood 25-OHD levels and sun exposure, monthly hours of sunshine in the Neth-

erlands during the study period were obtained from the archives of the Dutch Royal Meteorological Institute.[10]

Plasma Vitamin D Measurement

Cord blood was collected immediately after delivery and anticoagulated by use of sodium heparin. Plasma was prepared by centrifugation (10 minutes at 500 × *g*), and stored at −80°C. Plasma 25-OHD concentrations (nmol/L, to convert to ng/mL divide by 2.496) were measured with the Modular E170 analyzer (Roche, Basel, Switzerland). Interassay variability for pooled serum analyses was 19% at 33 nmol/L 25-OHD, 12% at 62 nmol/L, and 10% at 99 nmol/L. Plasma concentrations of 25-OHD were analyzed both as a continuous variable and divided into quartiles (<25 nmol/L, 25–49 nmol/L, 50–74 nmol/L, and ≥75 nmol/L).[7] Because of the low number of neonates in the <25-nmol/L 25-OHD group ($n = 7$), for outcome analyses the lower quartiles (<25 and 25–49 nmol/L) were pooled.

Primary and Secondary Outcomes

The primary outcome was defined as parent-reported RSV LRTI, which was defined as (1) LRTI symptoms, and (2) simultaneous presence of RSV RNA in a nose-throat specimen. Parents were instructed to record the presence and severity of respiratory symptoms during the first year of life in a daily log.[23] LRTI symptoms were defined by 2 independent researchers who used strict criteria: moderate or severe cough or wheeze of any severity lasting for at least 2 days. At the second day of every respiratory episode, parents obtained a nose-throat swab specimen. Samples were sent to the researchers in viral transport medium and frozen at 80°C.[25] The presence of RSV RNA was determined by real-time polymerase chain reaction as described previously.[23]

The secondary outcome, "physician-attended RSV LRTI" was defined as (1) respiratory illness for which the general practitioner or pediatrician was visited, and (2) simultaneous presence of RSV RNA in a nose-throat swab.

Statistical Analysis

Cord blood plasma 25-OHD concentrations were normally distributed, and means were compared by using the Student's *t* test. χ^2 analysis was used to test associations between categorical variables. Seasonality of 25-OHD was tested by fitting the data to a sine function with a period of 12 months in a nonlinear regression model. Statistical significance of seasonal distribution was determined by comparing the resulting sinusoidal model with the best fitting linear model, using the F test. Logistic regression analysis was performed to determine the effect of cord blood 25-OHD concentrations on the risk of subsequent RSV LRTI adjusted for potential confounders. Because of the limited number of cases, only a restricted number of potential confounders could be analyzed. The variables birth month, birth weight, and maternal ethnicity showed the highest association with both cord blood 25-OHD concentration and risk of RSV disease in single-variable analyses, and were therefore included in regression models. To adjust for birth month, 2 approaches were used. In the first approach, we used "deseasonalization" of 25-OHD concentrations (Supplemental Fig 4).[26] In this approach, the predicted 25-OHD concentrations based on birth month for each subject, derived from the sinusoidal model, were subtracted from the actual observed value. Subsequently, the overall mean was added and the resulting deseasonalized 25-OHD concentrations were analyzed using logitistic regression analysis, also adjusting for maternal ethnicity and birth weight. In the second approach, we used regression analysis in which we adjusted for birth ± 10 weeks from the start of the RSV season (yes versus no), next to maternal ethnicity and birth weight. Analyses were performed in SPSS 15.0 (SPSS Inc, Chicago, IL).

RESULTS

Population Characteristics

From November 2006 to December 2009, 1007 neonates were eligible for study participation (Fig 1). Of these, cord blood was collected from 481 neonates (48%), and for 161 (33%) of these infants the parents agreed to their participation in follow-up. Because of technical reasons, plasma 25-OHD concentrations were not measured in 5 (3%) of participating neonates, which resulted in a final cohort of 156 neonates. Baseline characteristics did not differ between participating subjects and nonparticipants (Supplemental Table 2). Of the participating neonates, 18 (12%) developed RSV LRTI in their first year of life, of whom 10 neonates had a physician-attended RSV LRTI. Neonates who subsequently developed RSV LRTI had a higher birth weight (3903 vs 3523 g, $P = .001$), and trended toward higher gestational age (40.4 vs 39.9 weeks; $P = .06$) compared with those who did not, as observed previously (Supplemental Table 3).[27] There were no differences in birth season, number of siblings, maternal ethnicity, mode of feeding, and use of vitamin D supplements between neonates who did and those who did not develop RSV LRTI.

High Prevalence of Vitamin D Deficiency in Healthy Newborns

The mean cord blood plasma 25-OHD concentration among healthy newborns was 82 nmol/L (SE: 3.5 nmol/L). Overall, 4% of neonates had 25-OHD levels of <25 nmol/L and 23% had levels of <50 nmol/L; 27% had 25-OHD levels of 50 to 74 nmol/L, and only 46% had levels of 75 nmol/L or higher.

TABLE 1 Characteristics of the Study Population According to Cord Blood Vitamin D Status

	Cord Blood 25-OHD Concentration				*P*
	<25 nmol/L (*n* = 7)	25–49 nmol/L (*n* = 29)	50–74 nmol/L (*n* = 48)	≥75 nmol/L (*n* = 72)	
Birth weight, g (SE)	3237 (167)	3529 (90)	3604 (74)	3574 (50)	.246
Gestational age, wk (SE)	39.8 (0.41)	40.1 (0.21)	40.2 (0.14)	39.8 (0.13)	.345
Any siblings, *n* (%)	0 (0)	12 (41)	18 (38)	31 (43)	.17
Male gender, *n* (%)	4 (57)	13 (45)	24 (50)	30 (42)	.75
Birth season, *n* (%)					.012
Winter	3 (43)	11 (38)	16 (33)	13 (18)	
Spring	1 (14)	6 (21)	7 (15)	18 (25)	
Summer	1 (14)	3 (10)	7 (15)	28 (39)	
Fall	2 (29)	9 (31)	18 (38)	13 (18)	
Maternal ethnicity, *n* (%)					
Caucasian	2 (29)	15 (52)	33 (69)	63 (88)	<.001
Other	5 (71)	14 (48)	15 (31)	9 (13)	
Ever breastfed, *n* (%)	6 (100)	19 (79)	33 (87)	51 (81)	.56
Vitamin D supplement use during pregnancy, *n* (%)	6 (100)	13 (68)	23 (62)	24 (39)	.005
Neonatal vitamin D supplement use, *n* (%)	4 (67)	12 (63)	30 (81)	46 (75)	.50

Use of Vitamin D Supplements During Pregnancy Increases Cord Blood 25-OHD Concentrations

Of participating women, 46% reported that they used supplements that contained vitamin D during pregnancy. The majority of these supplements (97%) were multivitamin preparations that contained a daily dose of 400 IU (10 μg) vitamin D3. Of these women, 74% used supplements during the first trimester, 86% during the second trimester, and 81% during the third trimester. In total, 54% of participating women used vitamin D supplements throughout pregnancy. Maternal use of vitamin D supplementation during pregnancy was associated with increased concentrations of 25-OHD in cord blood (73 vs 96 nmol/L; $P = .003$). After birth, 75% of all neonates received vitamin D supplements (daily recommended dose 400 IU vitamin D3) during the first month of life. Characteristics of participants according to vitamin D status at birth are shown in Table 1.

25-OHD Concentrations Show a Seasonal Pattern

To explore the seasonal variation in the concentrations of 25-OHD, cord blood 25-OHD concentrations and birth month were fitted to a sinusoidal model. Cord blood concentrations of 25-OHD showed a seasonal distribution with a baseline level of 84 nmol/L and an amplitude of 15 nmol/L ($P = .001$, Fig 2A). Maximum fitted concentrations of cord blood 25-OHD were observed in newborns born in July, and concentrations reached their nadir in January. Of neonates born in winter, 33% had cord blood 25-OHD concentrations <50 nmol/L and 70% had concentrations <75 nmol/L, compared with 10% and 28% of neonates born in summer (Fig 2B; χ^2 $P = .012$). Seasonality of 25-OHD concentrations was present for all birth years in our cohort (data not shown). We also related cord blood 25-OHD levels to monthly hours of sunshine during the study period, according to data from the Royal Netherlands Meteorological Institute,[10] and found a strong correlation between cord blood 25-OHD levels and monthly sun hours (Fig 2C; $R = 0.196$; $P = .01$).

Cord Blood Vitamin D Concentrations Are Associated With RSV LRTI in the First Year of Life

Plasma concentrations of 25-OHD at birth were related to the risk of RSV LRTI in the first year of life (Fig 3). Newborns who subsequently developed RSV LRTI had 1.3-fold lower cord blood concentrations of 25-OHD compared with those who did not (65 ± 7 vs 84 ±

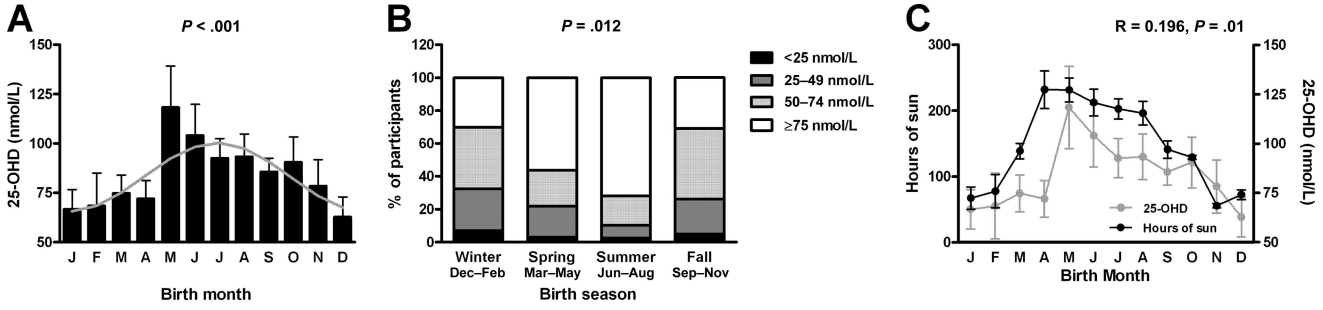

FIGURE 2
High prevalence of vitamin D deficiency in healthy newborns. Concentrations of 25-OHD in cord blood plasma ($n = 156$) were measured with the Modular E170 analyzer, and related to birth month (A) or birth season (B). Seasonality of cord blood 25-OHD was assessed by fitting the data to the best-fitting linear and sinusoidal model. C, monthly hours of sun during the study period were obtained from the archives of the Dutch Royal Meteorological Institute[10] and related to cord blood 25-OHD by using Pearson correlation. Bars represent mean + SE of the mean (A and C) or percent of subjects (B).

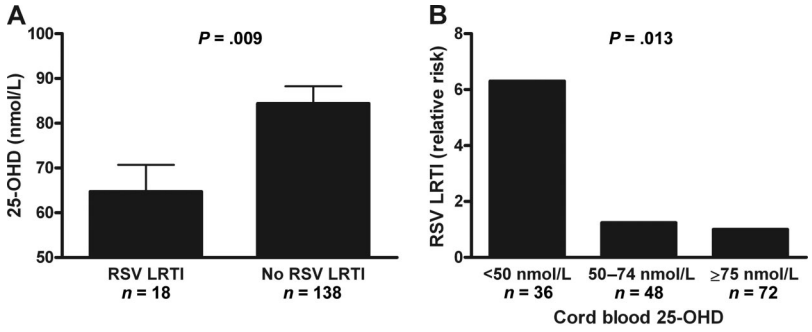

FIGURE 3

Association between cord blood vitamin D concentrations and RSV LRTI in the first year of life. A, cord blood concentrations of 25-OHD in neonates who subsequently developed RSV LRTI ($n = 18$) and those who did not ($n = 138$). B, risk of RSV LRTI per quartile of 25-OHD levels. Because of the limited number of cases, the lower quartiles (<25 nmol/L, $n = 7$; and 25–49 nmol/L, $n = 29$) were pooled. Bars represent mean + SE of the mean (A) or risk of RSV LRTI relative to neonates with 25-OHD concentrations ≥ 75 nmol/L (B).

11 nmol/L; $P = .009$) (Fig 3A). Logistic regression analysis, with correction for birth month, birth weight, and maternal ethnicity as potential confounders, demonstrated that cord blood 25-OHD concentrations were independently associated with subsequent risk of RSV LRTI (B = 0.978 [95% CI: 0.959–0.997]; $P = .024$). Sensitivity analyses, corrected for birth ± 10 weeks of the start of the RSV season, maternal ethnicity, and infant birth weight, also revealed a significant negative association between cord blood 25-OHD and risk of RSV LRTI (B = 0.976 [95% CI: 0.957–0.996]; $P = .018$). With the use of the secondary outcome physician-attended RSV LRTI, a similar trend was observed (63 vs 83 nmol/L; B = 0.983 [95% CI: 0.962–1.004]; $P = .117$).

We also analyzed the risk of RSV LRTI in neonates who were born with cord blood 25-OHD levels of <50 nmol/L, 50 to 75 nmol/L, and ≥75 nmol/L (Fig 3B). Compared with neonates with cord blood 25-OHD levels of ≥75 nmol/L, the adjusted relative risk of RSV LRTI was 6.2 (95% CI: 1.6–24.9; $P = .01$) in neonates with 25-OHD levels of <50 nmol/L.

DISCUSSION

In this prospective birth cohort study, we demonstrated that 54% of healthy newborns in the Netherlands are born with insufficient 25-OHD concentrations required for maximum health,[28,29] and that low plasma concentrations of 25-OHD at birth are associated with increased risk of RSV LRTI in the first year of life.

RSV is the most important respiratory pathogen in infancy, yet the mechanisms responsible for severe RSV disease are incompletely understood. Although antibody therapy is recommended for infants at high risk of severe infection, the majority of infections occur in infants without any known risk factors,[30] for whom no preventive strategies are currently available. Micronutrient supplementation to pregnant women and their newborns could be an easy and affordable strategy to prevent RSV LRTI.

The prevalence of vitamin D deficiency in our cohort was comparable to reported prevalences in other Westernized countries.[6,28,29,31] Cord blood vitamin D concentrations demonstrated a seasonal pattern, with maximum concentrations in newborns born in July and lowest concentrations in newborns born in December. This vitamin D concentration peak, which was relatively early compared with peaks observed in previous cohort studies,[11,32] may have occurred because pregnant mothers experienced extraordinarily high sun exposure in spring months during the study period (Fig 1C). In addition, the partial association between hours of sunshine and cord blood 25-OHD levels indicates that other factors, including time spent outdoors, use of sun protection,[33,34] and nutritional intake of vitamin D by the mother might contribute to cord blood vitamin D status.

To our knowledge, this is the first longitudinal study to demonstrate a relationship of plasma 25-OHD concentrations at birth to the subsequent risk of RSV LRTI. Previous cross-sectional studies have related low plasma concentrations of 25-OHD to increased severity of respiratory tract infections.[12,17,22] In Turkey and rural Bangladesh, plasma 25-OHD concentrations during infection were observed to be lower in children hospitalized with acute LRTI compared with age-matched healthy controls,[12,22] and subclinical vitamin D deficiency was associated with predisposition to acute respiratory tract infection in Indian children.[17] In addition, a recent cohort study in 284 Finnish children hospitalized for acute wheezing demonstrated a significant association between plasma vitamin D levels and risk of viral coinfection, specifically coinfections with RSV, rhinovirus, or both.[8] In contrast, studies in Canada failed to show a difference in plasma 25-OHD between children with and without respiratory tract infection.[21,35]

Several explanations may account for the protective effect of vitamin D at birth against subsequent RSV LRTI, which we observed in our study. Severe RSV infection is thought to arise from an interplay between the host immune response, airway anatomy, and RSV viral load. All these factors may be affected by vitamin D.

Vitamin D has immune modulatory properties that may influence the development of the fetal and neonatal immune system. Low vitamin D intake

during pregnancy is associated with increased incidence of diseases related to immune dysfunction in the offspring, including type 1 diabetes, asthma, and allergic rhinitis.[16,36,37] In vitro, vitamin D has many immune modulatory functions, which include induction of tolerogenic dendritic cells,[38] development of $CD4^+CD25^+Foxp3^+$ regulatory T cells,[39] activation of T-cell signaling,[40] and elaboration of tolerizing and antiinflammatory cytokines, including interleukin 10.[39,41,42] Moreover, results of a recent study demonstrates that maternal vitamin D intake during pregnancy increases expression of tolerogenic genes in cord blood,[43] which suggests that the immune modulatory function of vitamin D may already be occurring prenatally.

In addition, vitamin D may modulate early lung development. In animal models, vitamin D has been shown to promote lung development and surfactant production.[44–46] In humans, $1,25(OH)_2D$, the biologically active form of vitamin D, promotes surfactant production,[47] and downstream effectors of vitamin D have been detected in fetal lungs as early as 14 weeks' gestational age.[48] Vitamin D might thus accelerate fetal lung development, thereby potentially protecting against RSV disease.

Vitamin D also has many antimicrobial properties that may result in decreased viral load during infection.[42] Neonates who are born with vitamin D deficiency may also have lower serum concentrations during the neonatal period and infancy, which may confound the demonstration of any association between cord blood vitamin D and RSV LRTI during infancy. We did not measure vitamin D concentrations during RSV infection. However, 75% of neonates and infants in our cohort received vitamin D supplements after birth (400 IU per day), and there was no association between postnatal vitamin D supplement use and cord blood vitamin D levels (data not shown) or risk of RSV LRTI (Supplemental Table 3).

Our results suggest that strategies aimed at improving maternal vitamin D status during pregnancy might decrease the risk of RSV disease in the offspring. In agreement with recommendations of the American Association for Pediatrics and the World Health Organization, the Dutch Health Council recommends daily supplementation of 400 IU (10 μg) vitamin D to all pregnant women and breastfed newborns.[49] However, the optimal dose of vitamin D supplementation is still under debate. Especially during pregnancy, doses up to 4000 IU per day may be needed to obtain optimal maternal and neonatal health.[29,42,50–52] In addition, adherence to the current guidelines is generally poor.[53] In our cohort, only 46% of women reported use of vitamin D–containing supplements during pregnancy. Although vitamin D supplementation during pregnancy resulted in increased cord blood 25-OHD concentrations, we did not find a significant association with risk of RSV LRTI. However, the current study was insufficiently powered to answer this question. The association between cord blood 25-OHD concentrations and subsequent RSV LRTI suggests that larger clinical trials should be conducted to investigate the effect of vitamin D supplementation during pregnancy on the susceptibility to RSV LRTI in the offspring.

Potential limitations of this study warrant discussion. The sample size was relatively low and the number of cases in our cohort was relatively small. Limited statistical power particularly affected analyses that used cord blood 25-OHD quartiles, which resulted in wide confidence intervals. Nevertheless, despite the low number of cases, we were able to demonstrate significant differences in RSV risk. In addition, lack of parental compliance may have caused misclassification of infants who did experience RSV LRTI, but whose parents forgot to take a nose-throat swab or fill out the diary. However, because of the low incidence of RSV LRTI, we do not think that this limitation had a significant effect on our conclusions. Another limitation was that detailed information on sun exposure and dietary habits was not available for analysis. As a surrogate marker of sun exposure, birth month was included into our analysis. Similar results were found, which indicated that cord blood 25-OHD is an independent predictor of RSV LRTI.

In conclusion, vitamin D deficiency was highly prevalent in Dutch newborns, and cord blood 25-OHD concentrations were associated with susceptibility to subsequent RSV LRTI. Increased awareness of vitamin D status of pregnant women and intensified routine vitamin D supplementation may help prevent RSV LRTI during infancy. Randomized trials are required to address this question.

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