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ORIGINAL ARTICLE



Oral vitamin D supplementation is associated with full enteral feeding in very low birth weight infants

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ABSTRACT

Background and aims: As vitamin D is mostly transferred to the fetus during the third trimester, preterm infants are born with lower vitamin D stores. However, most clinical guidelines do not suggest clearly when to initiate vitamin D in very low birth weight (VLBW) infants. This retrospective study aimed to assess whether the initiation of oral vitamin D supplementation was associated with full enteral feeding (FEF) in VLBW infants.

Methods: A total of 383 VLBW infants (gestational age, 24–32 weeks; birth weight, 570–1500g) admitted to our neonatal intensive care unit between October 2018 and December 2022 were included in this study. To assess the independent association between oral vitamin D and FEF, univariate or multivariate Cox analyses were performed, adjusting for 16 major confounders (birth weight, initiation of enteral feeding, sepsis, the enteral feed volume when oral vitamin D started, etc.). Time-varying coefficients method is used to accommodate the non-constant hazard ratio implied by the proportional hazards assumption violation.

Results: Multivariate Cox regression for the time to reach full enteral feeding (T-FEF) and time to add human milk fortifier (T-HMF) were analyzed respectively. Delayed oral vitamin D supplementation (after postnatal day 13) was independently and negatively associated with the cumulative probability of achieving FEF ($B = -5.088$, RR 0.006 (0.001–0.057), $p < .00001$). Delayed oral vitamin D supplementation was independently and negatively associated with the cumulative probability of adding human milk fortifier (HMF) ($B = -3.115$, RR 0.044 (0.006–0.334), $p = .002$). The hazard effect of the delayed supplementation diminished over time, with the $RR = \text{EXP}(-5.088 + 1.447 \times \text{Ln}(T\text{-FEF}))$ or $RR = \text{EXP}(-3.115 + 0.729 \times \text{Ln}(T\text{-HMF}))$, respectively.

Conclusions: Our research suggests that earlier initiation of oral vitamin D is associated with improved FEF in VLBW infants.

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KEYWORDS

Initiation of oral vitamin D supplementation; full enteral feeding; very low birth weight infants; multivariate Cox regression; human milk fortifier

Introduction

Vitamin D is a fat soluble vitamin involved in multiple physiological processes, including bone development, immune system modulation, intestinal mucosal defense, microbial flora development, etc. [1–3]. As vitamin D is mostly transferred to the fetus during the third trimester, preterm infants are born with lower vitamin D stores. A high prevalence of moderately severe vitamin D deficiency with mean serum levels of 25 hydroxy vitamin D (25(OH)D) at 9.5–25 ng/mL during 1–3 days

after birth in preterm infants has been reported [4]. Additionally, 53–80% of very low birth weight (VLBW) infants admitted to a neonatal intensive care unit (NICU) in New York had either deficient (<20 ng/mL) or insufficient (20–29 ng/mL) vitamin D status at birth and only achieved vitamin D sufficiency by 3–4 weeks while receiving 102–400 IU/day vitamin D [5,6]. A report from Central Europe showed that serum 25(OH)D was <20 ng/mL in 71.3% of mothers, in 91.5% of cord blood samples, and even in almost 60% of preterm newborns

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after 8 weeks of oral supplementation of 800–1000 IU/day vitamin D [7].

Tremendous evidence points to the central role of vitamin D in maintaining mucosal barrier function and intestinal immunity, which is a research highlight in the gastrointestinal field nowadays [1–3,8]. Therefore, vitamin D might be involved in gastrointestinal disorders in infants [9]. Maternal vitamin D oral supplementation during gestation was found to improve intestinal health and microbial composition in suckling piglets [10]. Several studies have highlighted that low vitamin D concentrations in pregnant women may increase neonate susceptibility to celiac disease [11,12]. Additionally, a recent meta-analysis suggested that the risk of clinical relapses for inflammatory bowel disease might be reduced with vitamin D supplementation compared to placebo or no treatment [13,14].

Beginning and achieving full enteral nutrition is a key step in the care of preterm infants, particularly VLBW infants with immature gastrointestinal function [15], and is challenging in critically ill preterm infants [16]. Owing to the high prevalence of vitamin D deficiency and its possible involvement in intestinal disorders in VLBW infants, we hypothesized that earlier oral supplementation with vitamin D may improve feeding in VLBW infants. However, most clinical guidelines do not clearly suggest when to initiate vitamin D administration in VLBW infants, and there is no standard rule among various NICUs worldwide. A checklist example of a neonatal enteral nutrition feeding protocol from the American Society for Parenteral and Enteral Nutrition even suggested to add vitamin D after reaching full enteral feedings (FEFs) [17], which may delay the oral supplementation of vitamin D in VLBW infants. In order to support earlier oral supplementation of vitamin D in preterm infants, we explored whether earlier initiation of oral vitamin D was associated with FEF in VLBW infants.

Materials and methods

Study design

In this retrospective cohort study, VLBW infants (gestational age at 24–32 weeks, and birth weight at 570–1500 g) admitted to the NICU of Baoan Women's and Children's Hospital in Shenzhen City, Guangdong Province, Southern China, in 48 h after birth, between October 2018 and December 2022, were included and categorized into the group with early oral supplementation (within 13 days) or delayed oral supplementation (after day 13) of vitamin D. The cutoff value for grouping was set at day 13 to obtain the most

balanced data between groups regarding gestational age ($p = .083$) compared with all other integer cutoff values ($p < .05$) (see [Supplementary Tables S1 and S2](#)). Clinical and demographic data were collected from the patients' medical records until discharge. The exclusion criteria were as follows: malformations, genetic defects, severe asphyxia (Apgar score < 5 and cord blood gas pH < 7.0), admission to the NICU at ≥ 48 h of postnatal age, hospital stay < 21 days, and death or treatment abandon. Finally, 281 VLBW and 102 extremely low birth weight (ELBW; birth weight < 1000 g) infants were included in the study ([Figure 1](#)).

Feeding protocol

Our hospital's NICU adopts standardized feeding procedures as described in the previously published paper [18]. Enteral feeding was initiated mostly in the first 48 h of life at 10–20 mL/kg/day divided into eight meals with their own mother's milk or donor milk. Human milk fortifier (HMF) was added when enteral feeding reached 80–100 mL/kg. Aspirate residuals from an orogastric tube and abdominal aspect were checked before each meal. In the absence of signs of feeding intolerance for 24 h, enteral feeding was increased daily by 10–20 mL/kg. Enteral nutrition was discontinued in the case of erythematic abdominal wall, absence of bowel sounds, blood in the stools, or bile or blood in aspirates associated with a radiologic marker of necrotizing enterocolitis (NEC) $\geq$ Bell stage II. Parenteral nutrition (PN) was maintained through a central line in all infants to ensure adequate intake of fluids, electrolytes, and nutrients until FEF was achieved. Iron was supplemented orally according to recommendations.

Vitamin D supplementation

Due to the adult dosage form of fat-soluble vitamin injection used in our hospital, only 20–30 IU/day vitamin D3 was supplemented in PN route from 2 to 5 days after birth until FEF reached. Therefore, vitamin D3 drops (400 IU/drop, oil-based solution) were mainly supplemented at 800–1000 IU/day via nasogastric tube or directly into the oral cavity between feeds (≥ 30 min after prior feeding). Doses were documented using barcode scanning with dual-nurse verification. No redosing was performed if vomiting occurred within 15 min post-administration. The initiation was decided by clinical assessment: the medium (quartile) initiation time of oral vitamin D3 were at day 9.40 (8.37, 14.37) and day 11.42 (9.37, 16.35) in VLBW and ELBW infants, respectively. The timing of vitamin D initiation in

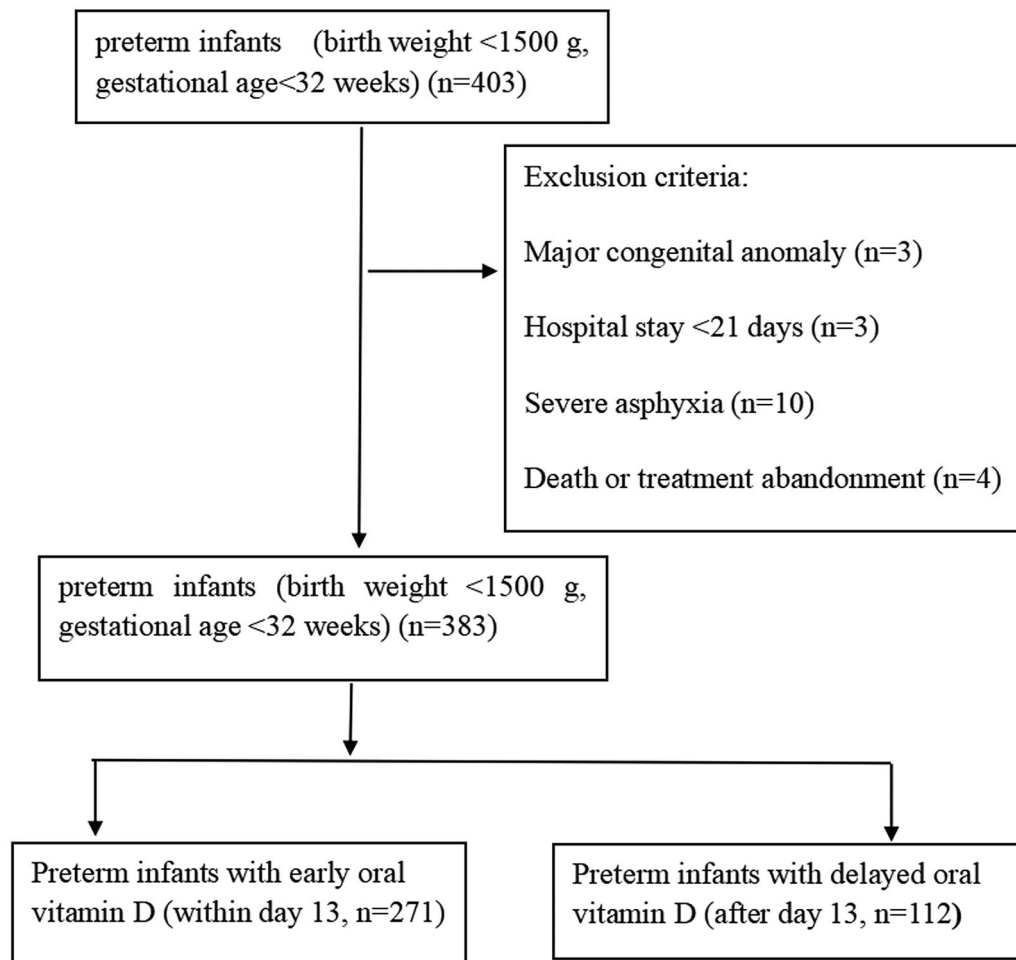


Figure 1. Flowchart of the retrospective cohort study population. VLBW infants admitted to the NICU of our hospital in Southern China in 48h after birth, between October 2018 and December 2022, were included and categorized into the group with early oral supplementation (within 13 days, $n = 271$) or delayed oral supplementation (after day 13, $n = 112$) of vitamin D. The cutoff value for grouping was set at day 13 to obtain the most balanced data between groups regarding gestational age ($p = .083$) compared with all other integer cutoff values ($p < .05$). Clinical and demographic data were collected from the patients' medical records until discharge. A total of 383 infants were enrolled: 281 VLBW (1000–1499 g) and 102 ELBW (<1000 g).

preterm infants is not standardized in current guidelines. Though timing varied, all infants ultimately received vitamin D (≥ 800 IU/day) by 4 weeks postnatal age.

Outcome measures

The main outcome was the time to reach full enteral feeding (T-FEF, when enteral feeding reached ≥ 150 mL/kg/day); the secondary outcome was the time to add HMF (T-HMF, when enteral feeding reached 80–100 mL/kg).

Data collection

Data regarding gestational age, birthweight, mode of delivery, multiple birth, gender, Apgar score, presence of patent ductus arteriosus (PDA), small for gestational age (SGA), time-to-discharge, initiation of enteral nutrition,

T-HMF, T-FEF, and following data took place before the T-FEF: initiation and dose of oral vitamin D, the enteral feed volume when oral vitamin D started (mL/kg/d), presence of mechanical ventilation, occurrence of sepsis (clinically diagnosed or culture-proven), and gastrointestinal complications as secondary outcomes describe above, were collected and double-checked by information engineers and researchers not in charge of the clinical management of the participants. Discharge was decided when the infant's body-weight reached at ≥ 2 kg stably and can afford milk feeding at ≥ 30 mL/meal.

Statistical analysis

In the survival analysis, less than 5% of cases (T-FEF (7/383) and T-HMF (19/383), respectively) were right-censored due to the absence of the endpoint event by the study cutoff. The Kolmogorov–Smirnov

test was used to determine whether variables were normally distributed. For continuous variables of non-normal distribution, groups were compared with independent-sample Mann–Whitney's test. For continuous variables of normal distribution, groups were compared with the independent-sample *t*-test. The Chi-square test and Fisher's exact test were used for categorical variables [18]. To assess the independent association of initiation of oral vitamin D with the main and secondary outcomes, univariate and multivariate Cox regression analyses were performed, adjusting for the indicated major confounders.

We assessed the proportional hazards (PHs) assumption using the log-minus-log survival plots, indicating a violation of the PH assumption. We introduced an interaction term between the group with delayed oral supplementation of vitamin D after day 13 (delayed oral vitamin-D grouping) and the logarithm of T-FEF (group $\times$ $\ln(\text{T-FEF})$) into the Cox model. This approach allows the effect of the delayed initiation to vary as a function of time, thereby accommodating the non-constant hazard ratio implied by the PH assumption violation. Similarly, we also introduced an interaction term between the enteral feed volume and the logarithm of T-FEF (the enteral feed volume $\times$ $\ln(\text{T-FEF})$) into the Cox model.

Statistical analyses were performed using IBM SPSS Statistics version 29.0 (Armonk, NY). *p* Values less than .05 (two-sided) were considered statistically significant.

Ethics approval and consent to participate: This study was approved by the Institutional Review Board of Shenzhen Baoan Women's and Children's Hospital (LLSCHY-2023-04-12-01), which waived the need for informed consent for this retrospective study. All methods were carried out in accordance with relevant guidelines and regulations.

Results

Main demographic and clinical characteristics of the study population

Among the 383 VLBW infants, 112 were supplemented with vitamin D after 13 days of life (delayed oral vitamin-D group, see Table 1). The cutoff value for grouping was set at day 13 to obtain the most balanced data between groups regarding gestational age ($p = .083$) compared with all other integer cutoff values ($p < .05$). Birth weight, SGA, cesarean section, and initiation of enteral feeding might be associated with delayed oral supplementation of vitamin D (Table 1). The T-FEF and the T-HMF were likely increased in the delayed oral vitamin-D group (both $p < .001$).

Table 1. Main demographic and clinical characteristics of the study population.

Characteristics	Early oral vitamin-D (<i>n</i> = 271)	Delayed oral vitamin-D (<i>n</i> = 112)	<i>p</i>
Gestational age (weeks, (P25, P75)) ^c	29.4 (28.0, 30.7)	29.0 (27.0, 30.7)	.083
Birth weight (g, <i>M</i> (P25, P75)) ^c	1220 (1000, 1390)	1155 (900, 1300)	.004
Admission time (day, <i>M</i> (P25, P75)) ^c	0.58 (0.43, 0.78)	0.563 (0.35, 0.84)	.628
Male (<i>n</i> (%)) ^b	140 (51.7%)	60 (53.6%)	.733
Cesarean delivery (<i>n</i> (%)) ^b	182 (67.2%)	65 (58.0%)	.090
Multiple birth (<i>n</i> (%)) ^b	95 (35.1%)	34 (30.4%)	.376
Small for gestational age (<i>n</i> (%)) ^b	28 (10.3%)	19 (17.0%)	.072
Patent ductus arteriosus (<i>n</i> (%)) ^b	185 (68.3%)	80 (71.4%)	.542
Invasive mechanical ventilation (<i>n</i> (%)) ^b	131 (48.3%)	62 (55.4%)	.211
Apgar score at 1 min (<i>M</i> (P25, P75)) ^c	9 (8, 10)	9 (8, 10)	.411
Hospital stay (day, <i>M</i> (P25, P75)) ^c	55.6 (48.4, 71.7)	64.6 (53.8, 79.6)	.000
Initiation of enteral feeding (days, <i>M</i> (P25, P75)) ^c	1.6 (1.4, 2.6)	2.4 (1.4, 3.4)	.088
Time ^a to add HMF (days, <i>M</i> (P25, P75)) ^c	16.4 (12.4, 21.4)	22.4 (16.4, 30.4)	.000
Time ^a to reach full enteral feeding (days, <i>M</i> (P25, P75)) ^c	24.0 (18.1, 32.9)	30.6 (22.5, 37.8)	.000

Abbreviation: HMF, human milk fortifier.

^aLess than 5% case data is censored in T-HMF (19/383) and T-FEF (7/383), respectively.

^bThe Chi-square test and Fisher's exact test were used for categorical variables.

^cFor continuous variables of non-normal distribution, groups were compared with independent-sample Mann–Whitney's test.

Factors associated with unbalanced grouping or enteral feeding

The 16 factors, whose data only before the T-FEF were retrieved and sorted by the value of test probability in Table 2, might be associated with unbalanced grouping or enteral feeding in this study. The following 10 factors might be associated with unbalanced grouping at a cutoff value of 13 days (all factors with $p < .1$ were chosen): mean dose of vitamin D, birth weight, intravenous glucocorticoids, intestinal surgery, hemo-dynamically significant patent ductus arteriosus (hsPDA) treated with ibuprofen, SGA, gestational age, initiation of enteral feeding, cesarean delivery, and feeding intolerance. Initiation of oral vitamin D and mean dose of vitamin D are the determinant factors in this study hypothesis. The use of intravenous glucocorticoids demonstrated severity status, and hsPDA treated with ibuprofen might reflect the degree of gastrointestinal disturbance after birth.

Table 2. Factors associated with unbalanced grouping or enteral feeding before the time to reach full enteral feeding.

Unbalanced or associated factors before the T-FEF	Early oral vitamin-D (n = 271)	Delayed oral vitamin-D (n = 112)	p	Univariate Cox regression	
				RR (CI) for T-FEF ^a	RR (CI) for T-HMF ^a
Initiation of oral vitamin D (days, M (P25, P75)) ^c	9.4 (7.4, 10.4)	15.4 (14.4, 18.4)	.000	0.949 (0.929–0.970)**	0.942 (0.921–0.963)**
Mean dose of vitamin D (IU/day, M (P25, P75)) ^c	930 (900, 1300)	900 (800, 992)	.000	1.000 (0.999–1.000)#	1.000 (1.000–1.000)
The enteral feed volume when oral vitamin D started (mL/kg/d, M (P25, P75)) ^c	50.4 (24.6, 83.3)	69.7 (40.6, 114.3)	.005	1.012 (1.009–1.014)**	1.012 (1.009–1.014)**
Birth weight (g, M (P25, P75)) ^c	1220 (1000, 1390)	1155 (900, 1300)	.004	1.001 (1.001–1.002)**	1.001 (1.002–1.003)**
Intravenous glucocorticoids (n (%)) ^b	20 (7.4%)	17 (15.2%)	.019	0.583 (0.411–0.828)**	0.611 (0.430–0.866)#
Intestinal surgery (n (%)) ^b	2 (0.7%)	5 (4.5%)	.040	0.164 (0.066–0.406)**	0.215 (0.088–0.526)**
hsPDA treated with ibuprofen (n (%)) ^b	52 (19.2%)	31 (27.7%)	.067	0.933 (0.730–1.194)	0.763 (0.596–0.977)#
Small for gestational age (n (%)) ^b	28 (10.3%)	19 (17.0%)	.072	0.888 (0.652–1.210)	0.843 (0.611–1.164)
Gestational age (weeks, (P25, P75)) ^c	29.4 (28.0, 30.7)	29.0 (27.0, 30.7)	.083	1.164 (1.104–1.227)**	1.193 (1.133–1.256)**
Initiation of enteral feeding (days, M (P25, P75)) ^c	1.6 (1.4, 2.6)	2.4 (1.4, 3.4)	.088	0.853 (0.785–0.925)**	0.832 (0.760–0.910)**
Cesarean delivery (n (%)) ^b	182 (67.2%)	65 (58.0%)	.090	1.046 (0.846–1.293)	1.153 (0.930–1.429)
Feeding intolerance (n (%)) ^b	33 (12.2%)	21 (18.8%)	.093	0.637 (0.474, 0.856)**	0.586 (0.429, 0.801)**
Sepsis (n (%)) ^b	67 (24.7%)	36 (32.1%)	.136	0.627 (0.497, 0.791)**	0.643 (0.509, 0.813)**
NEC ≥ Bell stage II (n (%)) ^b	4 (1.5%)	5 (4.5%)	.166	0.296 (0.146, 0.599)**	0.566 (0.280, 1.145)
Invasive mechanical ventilation (n (%)) ^b	131 (48.3%)	62 (55.4%)	.211	0.653 (0.531, 0.803)**	0.607 (0.492, 0.747)**
Gastrointestinal bleeding (n (%)) ^b	25 (9.2%)	15 (13.4%)	.225	0.545 (0.388, 0.767)**	0.562 (0.397, 0.795)**

Abbreviations: RR, relative risk in multivariate Cox regression analyses; CI, confidential interval; hsPDA, hemo-dynamically significant patent ductus arteriosus; NEC, necrotizing enterocolitis; T-FEF, time to reach full enteral feeding; T-HMF, time to add HMF.

^aLess than 5% case data is censored in T-HMF (19/383) and T-FEF (7/383), respectively.

^bThe Chi-square test and Fisher's exact test were used for categorical variables.

^cFor continuous variables of non-normal distribution, groups were compared with independent-sample Mann-Whitney's test.

**p < .001.

#p < .05.

Univariate Cox regression analyses revealed that gastrointestinal complications, sepsis, invasive mechanical ventilation, and some unbalanced factors were associated with T-FEF or T-HMF ($p < .001$), except for NEC, which was not associated with T-HMF (Table 2). Therefore, all 16 factors listed in Table 2 were chosen as determinant or confounding factors in the subsequent multivariate Cox regression analyses.

Earlier initiation of oral vitamin D was independently associated with full enteral feeding

Multivariate Cox regression analyses for T-FEF and T-HMF, adjusted for 16 factors listed in Table 2 were performed, respectively. We rigorously assessed the PH assumption using the log-minus-log survival plots, indicating a violation of the PH assumption. Therefore, we used time-varying coefficients method to accommodate the non-constant hazard ratio implied by the PH assumption violation.

The delayed supplementation was associated with a reduced achievement of FEF ($B = -5.088$, RR 0.006 (0.001–0.057), $p < .00001$). There is significant interaction of delayed oral vitamin-D supplementation with $\ln(T-FEF)$ ($B = 1.447$, RR 4.250 (2.230–4.857), $p = .00001$). The hazard effect of the delayed supplementation diminished over time, with the $RR = \text{EXP}(-5.088 + 1.447 \times \ln(T-FEF))$ (Table 3 and Figure 2(A)). The delayed supplementation was associated with a reduced addition of HMF ($B = -3.115$, RR 0.044 (0.006–0.334), $p = .002$). There is

significant interaction of delayed oral vitamin-D supplementation with $\ln(T-HMF)$ ($B = 0.729$, RR 2.072 (1.146–3.747), $p = .016$). The hazard effect of the delayed supplementation diminished over time, with the $RR = \text{EXP}(-3.115 + 0.729 \times \ln(T-HMF))$ (Table 3 and Figure 2(B)).

Discussion

This retrospective cohort study included 383 VLBW infants admitted to a level III NICU between October 2018 and December 2022 in Shenzhen City, Southern China. To assess the independent association between initiation of oral vitamin D and enteral feeding, multivariate Cox regression analyses were performed, adjusted for 16 major confounders (birth weight, initiation of enteral feeding, etc.). This study suggests that earlier initiation of oral vitamin D is independently associated with FEF.

A daily challenge for neonatologists is how to optimally initiate, advance, and reach full enteral nutrition to meet the nutrient, energy, and fluid needs of VLBW infants while minimizing risk [15,19]. It is essential to establish suitable FEF for each infant as soon as possible to optimize extrauterine growth. However, the benefits of enteral nutrition in premature infants are offset by gastrointestinal complications in premature infants. To diminish the dilemma in the “daily challenge”, this study suggested initiation of oral vitamin D much earlier than postnatal day 13, which might significantly smooth the feeding process in VLBW infants.

Table 3. Factors independently associated with the T-FEF and T-HMF (multivariate Cox regression analyses).

Factors	T-FEF ^a			T-HMF ^a		
	<i>B</i>	RR (CI)	<i>p</i> ^b	<i>B</i>	RR (CI)	<i>p</i> ^b
Delayed oral vitamin-D	-5.088	0.006 (0.001–0.057)	.00001	-3.115	0.044 (0.006–0.334)	.002
Delayed oral vitamin-D × ln(T-FEF or T-HMF)	1.447	4.250 (2.023–8.087)	.00001	0.729	2.072 (1.146–3.747)	.016
The enteral feed volume when oral vitamin D started	0.304	1.355 (1.312–1.399)	.00000	0.075	1.078 (1.063–1.094)	.00000
The enteral feed volume × ln(T-FEF or T-HMF)	-0.019	0.914 (0.905–0.923)	.00000	-0.019	0.982 (0.977–0.986)	.00000
Mean dose of vitamin D (IU/day)	0.001	1.000 (1.000–1.001)	.00000	0.001	1.001 (1.001–1.001)	.00000
Birth weight (g)	0.000	1.000 (0.999–1.001)	.862	0.002	1.002 (1.001–1.002)	.0001
Intravenous glucocorticoids	-0.114	0.892 (0.593–1.342)	.585	0.244	1.276 (0.842–1.934)	.521
Intestinal surgery	-1.940	0.144 (0.051, 0.402)	.0002	-1.287	0.276 (0.100, 0.763)	.013
hsPDA treated with ibuprofen	0.006	1.006 (0.755–1.339)	.969	-0.020	0.981 (0.742–1.295)	.890
Small for gestational age	-0.113	0.893 (0.590–1.352)	.593	-0.095	0.910 (0.597–1.386)	.659
Gestational age (weeks)	0.088	1.092 (0.980–1.216)	.110	-0.007	0.993 (0.891–1.107)	.902
Initiation of enteral feeding (days)	-0.067	0.935 (0.853–1.025)	.153	-0.107	0.898 (0.810–0.997)	.043
Cesarean delivery	-0.034	0.967 (0.757–1.234)	.785	0.091	1.096 (0.847–1.417)	.486
Feeding intolerance	-0.371	0.690 (0.497–0.958)	.027	-0.259	0.772 (0.551–1.080)	.131
Sepsis	-0.242	0.785 (0.611–1.008)	.058	-0.100	0.905 (0.698–1.174)	.451
NEC ≥ Bell stage II	-0.476	0.621 (0.288–1.339)	.224	0.523	1.702 (0.738–3.921)	.212
Invasive mechanical ventilation	0.129	1.137 (0.895–1.445)	.292	-0.095	0.910 (0.714–1.159)	.443
Gastrointestinal bleeding	-0.439	0.645 (0.439, 0.947)	.025	-0.277	0.758 (0.524, 1.098)	.143

Abbreviations: hsPDA, hemo-dynamically significant patent ductus arteriosus; NEC, necrotizing enterocolitis; T-FEF, time to reach full enteral feeding; T-HMF, time to add HMF; RR, relative risk; CI, confidential interval.

^aLess than 5% case data is censored in T-HMF (19/383) and T-FEF (7/383), respectively.

^bThe values $p < 0.05$ are highlighted in bold.

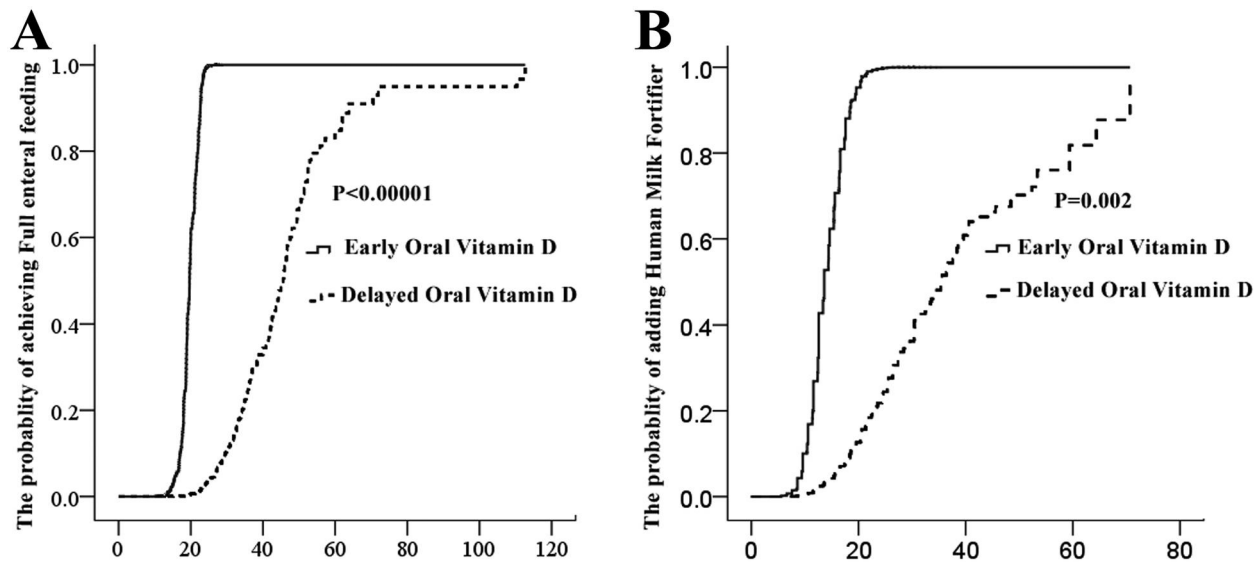


Figure 2. The cumulative probability of achieving full enteral feeding (FEF) or adding human milk fortifier (HMF). Multivariate Cox regression analyses for T-FEF and T-HMF, adjusting for 16 factors listed in Table 2 were analyzed respectively. (A) The delayed supplementation was associated with a reduced achievement of FEF ($B = -5.088$, RR 0.006 (0.001–0.057), $p < .00001$). There is significant interaction of delayed oral vitamin-D supplementation with $\ln(T-FEF)$ ($B = 1.447$, RR 4.250 (2.230–8.087), $p = .00001$). The hazard effect of the delayed supplementation diminished over time, with the $RR = \exp(-5.088 + 1.447 \times \ln(T-FEF))$. (B) The delayed supplementation was associated with a reduced addition of HMF ($B = -3.115$, RR 0.044 (0.006–0.334), $p = .002$). There is significant interaction of delayed oral vitamin-D supplementation with $\ln(T-HMF)$ ($B = 0.729$, RR 2.072 (1.146–3.747), $p = .016$). The hazard effect of the delayed supplementation diminished over time, with the $RR = \exp(-3.115 + 0.729 \times \ln(T-HMF))$.

Earlier initiation of oral vitamin D intake was independently associated with improved enteral feeding

At birth, most VLBW preterm infants have a biochemical vitamin D deficiency. The American Academy of

Pediatrics has recommended a dose of 200–400 IU/d of vitamin D for preterm infants, and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition recommends 800–1000 IU/d. However, most guidelines do not clearly suggest when to initiate

vitamin D administration in VLBW infants, and there is no standard rule among NICUs in different countries worldwide.

The multivariate Cox regression analyses in this study showed that delayed initiation of vitamin D was independently and negatively associated with the cumulative probability of achieving of FEF and addition of HMF, though the hazard effect of the delayed supplementation diminished over time. Our research suggests that earlier initiation of oral vitamin D is associated with FEF in VLBW infants.

Possible protective role of vitamin D in the premature gastrointestinal tract

As the largest immune organ, the ontogeny of the preterm gastrointestinal tract (GIT) includes the development of complex pro- and anti-inflammatory pathways that protect it from pathogens and toxins. Increasing evidence points to the central role of vitamin D in maintaining mucosal barrier function and intestinal immunity [1–3]. A recent study found that both maternal/neonatal vitamin D levels were significant predictors of NEC in 145 preterm infants [20]. Furthermore, vitamin D ameliorates neonatal NEC by suppressing TLR4 in a mice model [21].

The vitamin D/vitamin D receptor (VDR) signaling pathway regulates the expression of various components of intestinal mucosal tight junctions (TJs) and adherens junctions (AJs) [1–3]. Furthermore, vitamin D/VDR signaling affects intestinal Paneth cells and intraepithelial lymphocytes (IELs) viability, secretion of antimicrobial peptides (AMPs), and suppression of inflammatory responses. Vitamin D also exerts its modulatory effects on gastrointestinal health by regulating the composition and metabolic activity of the gut microbiome [1–3]. Vitamin D deficiency has been associated with the disruption of gut barrier integrity, translocation of bacteria into the bloodstream, and systemic inflammation [22]. Oral supplementation of vitamin D is beneficial to alleviate and reduce relapse in Crohn's disease and ulcerative colitis [23–25].

The primary and processing imbalances between the groups in this cohort study were extensively and intensively considered. (1) Only data of confounders that took place before the T-FEF in each infant were retrieved for analyses; (2) the cutoff value for grouping was set at day 13 to obtain the most balanced data between groups regarding gestational age ($p = .083$ between groups) compared with all other integer cutoff values ($p < .05$); (3) all included factors with $p < .1$ between groups and indicated medically significant factors (such as gastrointestinal complications, sepsis, hSPDA treated with ibuprofen, use of intravenous

glucocorticoids, etc.) were included as confounders in multivariate Cox regression analyses; (4) even balanced factors ($p > .1$ between groups), which were significantly associated with T-FEF or T-HMF in univariate Cox analyses, were included as confounders.

Although baseline 25(OH)D measurements were not obtained, longitudinal monitoring of vitamin D status was conducted in most cases at two clinically relevant intervals (Supplementary Table S2) in most preterm infants: (1) at 3–6 weeks postnatally, the group with early oral supplementation within 13 days (early oral vitamin-D group) ($n = 236$) demonstrated significantly higher serum concentrations (median (IQR): 17.8 (15.7–20.5) ng/mL) compared to the delayed oral vitamin-D group ($n = 88$; 16.3 (13.5–19.3) ng/mL; $p = .002$), indicating earlier and higher supplementation of vitamin D (Table 3) elevated the serum level of 25(OH)D, which might be associated with improved enteral feeding and addition of HMF. (2) During pre-discharge evaluation, vitamin D levels showed comparable values between groups, with median concentrations of 20.1 (16.4–24.6) ng/mL in the early oral vitamin-D group ($n = 260$) versus 19.1 (15.9–23.0) ng/mL in the delayed oral vitamin-D group ($n = 102$; $p = .195$).

Theoretically, both early supplementation and modestly elevated 25(OH)D levels may promote intestinal feeding. Emerging evidence indicates that vitamin D supplementation in deficient individuals preferentially enhances plasma levels of bioactive 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) over its inactive metabolite 24,25-dihydroxyvitamin D ($24,25(\text{OH})_2\text{D}$) [26]. In our cohort, the majority of preterm infants exhibited vitamin D deficiency at birth. Early oral supplementation could promptly improve intestinal barrier function, local immunity, and microbiota, thereby facilitating enteral feeding. Moreover, the modest higher 25(OH)D levels observed in the early supplementation group may further enhance intestinal protection. This is because vitamin D activity in the gut involves not only endocrine regulation but also autocrine and paracrine mechanisms, which enable the local activation of 25(OH)D to bioactive $1,25(\text{OH})_2\text{D}$ within intestinal tissues [26,27]. Elevated 25(OH)D levels in plasma and intestinal tissues likely occur concurrently, and the synergistic effects of these secretory systems may strengthen local intestinal protection.

As to persistent Vitamin D deficiency in this study, current thresholds (<20 ng/mL) for vitamin D deficiency (established in children) may not be appropriate for preterm VLBW infants, particularly in populations with distinct genetic contexts [28]. Our data reinforce the need for population-specific reference ranges, as the functional requirements for vitamin D in preterm gut development might differ from those governing skeletal health in children [29]. The persistent vitamin D

deficiency observed in our cohort aligns with findings from Munshi et al. [5] but contrasts with the rapid improvement reported by Fort et al. [30]. We propose that this discrepancy arises from critical differences in supplementation timing. Munshi et al. [5]: Oral vitamin D supplementation initiated late (median 19.7 days postnatally in ELBW infants) only after achieving enteral feeding ≥ 140 mL/kg/day resulted in a paradoxical "dose-irrelevant" elevation of serum 25(OH)D (deficiency rates: 25% at 28 days and 22% at 8 weeks despite escalating doses to 800–1200 IU/day). Fort et al. [30]: Supplementation early commenced (within the first postnatal week) regardless of feeding volume demonstrated a clear dose-dependent effect: deficiency rates at 28 days decreased to 16% (200 IU/day) and 0% (800 IU/day). These studies indicated that earlier initiation of vitamin D might improve absorption of vitamin D and enteral feeding in a dose irrelevant manner.

Our study has the limitation of being a retrospective cohort with inevitably unbalanced grouping and universal oral supplementation of vitamin D, but without a standard regimen with fixed ranks of initiation time and dosage. We did not detect 25(OH)D levels on the first day of life or in cord blood, which could exhibit the baseline of vitamin D deficiency in the VLBW infants. Additionally, other unknown residual confounding risks (e.g. institutional practices, clinician preferences) may also influence our conclusion. A large sample, double-blind, randomized clinical trial comparing vitamin D supplementation initiated at different enteral feeding stages after birth is required to validate the hypothesis and results of this study.

Conclusions

This retrospective cohort study included 383 VLBW infants in a level III NICU center in Southern China, suggesting that earlier initiation of oral vitamin D supplementation might promote FEF. The primary and processing unbalance between groups in this cohort study was extensively and intensively considered. Our research suggests that earlier initiation of oral vitamin D is associated with FEF in VLBW infants. A large sample, double-blind, randomized clinical trial comparing vitamin D supplementation initiated at different enteral feeding stages after birth is required to validate the hypothesis and results of this study.

Author contributions

CRedit: **Yie Huang**: Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft; **Ping Zhou**: Data curation, Methodology,

Resources, Supervision, Writing – review & editing; **Ruiping Wu**: Data curation, Investigation, Software, Validation, Visualization; **Xiaomei Fan**: Funding acquisition, Resources, Supervision; **Ping Zheng**: Conceptualization, Resources, Writing – review & editing; **Xintian Shen**: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Data availability statement

Data described in the manuscript, code book, and analytic code will be made available upon request.

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