


Effect of Oral Vitamin D3 Supplementation in Exclusively Breastfed Newborns: Prospective, Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

Exclusively breastfed infants are at a high risk of vitamin D deficiency. Few studies have evaluated the effects of vitamin D supplementation. Hence, we conducted a prospective randomized controlled trial investigating the effects of oral vitamin D3 400 IU/d supplementation in exclusively breastfed newborns. Serum 25-hydroxy-vitamin D (25[OH]D) levels in pregnant women and their newborns were evaluated. Breastfed newborns were randomized to one of two regimens at age 10 days. One group received vitamin D3 supplementation at a dose of 400 IU/d (vD-400 group), whereas the placebo group received a liquid product without vitamin D3. Outcomes were assessed at 4 months of age. A total of 92 pregnant women and their infants were enrolled, and the data of 72 infants (37 in the vD-400 group and 35 in the placebo group) who completed the study at 4 months of age were assessed. The results showed severe vitamin D deficiency in 15.2% of mothers before delivery, while 54.3% had vitamin D deficiency. Moreover, 15.2% of newborns presented with severe vitamin D deficiency at birth, while 52.2% had vitamin D deficiency. Maternal vitamin D levels were significantly correlated with infant vitamin D levels at birth ($r = 0.816$, $p < 0.001$). At 4 months of age, weight, head circumference, serum 25(OH)D, phosphorus, and intact parathyroid hormone levels significantly differed between the vD-400 and placebo groups. However, the body length and bone mineral density of the two groups did not differ significantly. Regardless of vitamin D supplementation, participants with severe vitamin D deficiency had significantly higher intact parathyroid hormone levels and lower bone mineral content. In conclusion, among exclusively breastfed infants, oral supplementation with vitamin D3 at a dose of 400 IU/d from age 10 days increased 25(OH)D concentrations at 4 months of age, but it did not affect bone mineralization. © 2022 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: PTH/VIT D/EGF23; NUTRITION; CLINICAL TRIALS; DXA

Introduction

Vitamin D, a fat-soluble steroid hormone, promotes calcium (Ca) and phosphorus (P) absorption in the intestinal tract

and enhances bone mineral metabolism. Vitamin D plays an important role in human health, but vitamin D deficiency is common in both children and adults. Without an adequate calcium-phosphorus product (the value for serum calcium is multiplied

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by serum phosphorus value), the mineralization of the collagen matrix diminishes, leading to the classic signs of rickets in children and osteomalacia in adults.⁽¹⁻⁵⁾ The benefits of breastfeeding are unquestionable because breast milk contains almost all the necessary nutrients for the proper growth of an infant. Despite the numerous advantages of breast milk, emerging reports have shown that prolonged or exclusive breastfeeding is associated with certain nutritional diseases, such as vitamin D deficiency.⁽⁶⁻⁷⁾ Infants often do not have sufficient sunlight exposure to support adequate vitamin D production in the skin. Therefore, exclusively breastfed infants are especially at risk of vitamin D deficiency because the amount of vitamin D is limited in breast milk.^(1,3,4,8) Moreover, a low maternal vitamin D level during pregnancy further increases the risk of vitamin D deficiency in infants.⁽⁹⁾ Nutritional rickets is not rare in the United States and Taiwan, and 70% to 90% of children with nutritional rickets are exclusively breastfed without vitamin D supplementation.^(3,10) To ensure adequate vitamin D status, in 2008, the American Academy of Pediatrics (AAP) recommended that all infants should have a minimum daily vitamin D intake at a dose of 400 IU immediately after birth.⁽¹¹⁾ The Taiwan Pediatric Association revised its guidelines for breastfed infants in 2016 and suggested that exclusively or partially breastfed infants should be provided with 400 IU of daily oral vitamin D supplementation.⁽¹²⁾ Nevertheless, studies from the United States and Taiwan have shown that only 20.8% and 9.7% of breastfed infants, respectively, fulfilled the guideline recommendations.^(13,14) However, benefits of vitamin D supplementation remain largely unclear in Taiwanese infants. No study has evaluated the nutritional status and bone mineralization effect of daily oral supplementation of vitamin D at a dose of 400 IU in breastfed infants in Taiwan. Therefore, we conducted a prospective randomized double-blind controlled trial to investigate the effects of oral vitamin D3 supplementation in exclusively breastfed newborns. This study aimed to evaluate the vitamin D status of pregnant women and their newborns and the effects of daily oral vitamin D3 supplementation at a dose of 400 IU in breastfed infants.

Materials and Methods

Study design and study participants

From February 2018 to March 2019, we conducted a prospective, randomized, controlled trial including healthy mothers and their healthy term infants delivered at Hsinchu MacKay Memorial Hospital. The inclusion and exclusion criteria for the pregnant women and their newborn infants are shown in Fig. 1. A total of 92 mothers and 92 infants fulfilled the recruitment criteria and were enrolled in this study. The Institutional Review Board of MacKay Memorial Hospital approved the study (17MMHIS088e), and the parents of the newborns provided their informed consent.

Randomization and allocation

Ninety-two newborns who met the inclusion criteria were randomized in a 1:1 ratio to supplementation regimen and placebo control group at age 10 days. For the randomization, computer-generated allocation sequence and block randomization were performed by a research staff who was not involved in this study. The participants were blinded to the allocation.

Intervention: vitamin D supplementation

The intervention group of infants received vitamin D3 drops with each drop containing 400 IU of vitamin D3 (vD-400 group; $n = 46$) in 90% to 95% medium-chain-triglyceride (MCT) oil (Liquid P&B; ULong Pharmaceutical, Taipei, Taiwan), which is a natural vitamin D3 extracted from lanolin. The placebo group ($n = 46$) only received MCT oil manufactured by the same plant. The two supplements were identical in appearance, color, and taste.

Study follow-up

All infants were followed up from age 10 days by a study nurse who regularly contacted the mothers to ensure adherence to the treatment and exclusive breastfeeding of the infants. The supplementation continued for 4 months. At the end of the study, 80.4% (37 of 46) in the vD-400 group and 76.1% (35 of 46) in the placebo group completed the trial regimens and were enrolled for data assessment (Fig. 1).

Anthropometric measurements and biochemical analysis

The anthropometric measurements of the newborns, including length, weight, and head circumference, were performed at birth and 4 months of age. We assessed the 25(OH)D, Ca, P, alkaline phosphatase (ALP), and intact parathyroid hormone (iPTH) concentrations of the newborns 1 day after birth and at 4 months of age. We also determined the bone mineral density (BMD) and bone mineral content (BMC) of the infants at 4 months of age. For the biochemical analyses, serum samples were stored at 2°C to 8°C before assay. The 25(OH)D concentration was measured using chemiluminescence (DiasorinXL, LIAISON, Saluggia, Italy), whereas the iPTH level was measured using chemiluminescence (Abbott ARCHITECT, Biokit; reference 15 to 68.3 pg/mL; Abbott Park, IL, USA). Vitamin D deficiency, insufficiency, and sufficiency were defined as a serum 25(OH)D level of 10 to 19.9, 20 to 29.9, and 30 to 100 ng/mL, respectively, according to the Endocrine Society guidelines.⁽²⁾ Severe vitamin D deficiency was defined as a serum 25(OH)D level of <10 ng/mL.⁽¹⁵⁻¹⁸⁾ The levels of 25(OH)D of mothers were determined before delivery.

Analysis of bone mineralization

The BMC (g) and BMD (g/cm²) of the infants' lumbar spine (L₁ to L₄) were assessed using dual-energy X-ray absorptiometry (DXA; Horizon DXA System, Hologic Horizon W, APEX software, Hologic Inc., Marlborough, MA, USA) according to the manufacturer's instructions.

Statistical analysis

We analyzed the between-group differences using Fisher's exact test for categorical variables and *t* test for continuous variables. The paired *t* test was used to compare the mean differences of continuous variables before and after the interventions within each group. The bivariate Pearson correlation coefficients between the infant 25(OH)D levels and other parameters were assessed. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

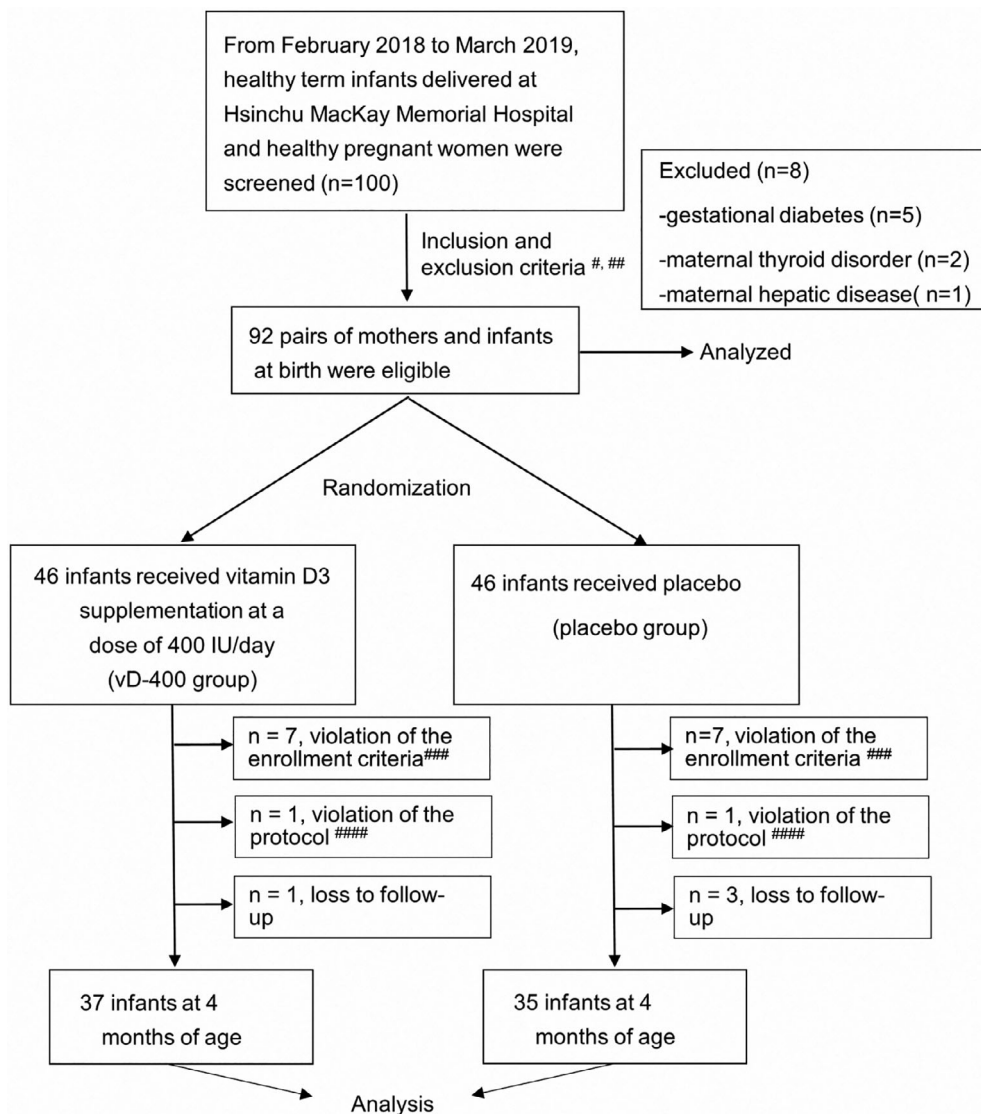


Fig. 1. Flowchart of the selection of maternal–infant pairs. #The inclusion criteria for pregnant women were as follows: age ≥ 18 years old, average daily outdoor sunlight exposure < 30 minutes, singleton pregnancy, preconception body mass index $< 25 \text{ kg/m}^2$, vitamin D supplementation at a dose $< 1000 \text{ IU/d}$ during pregnancy, and without systemic and autoimmune diseases. The exclusion criteria for pregnant women were as follows: gestational diabetes or diabetes, preeclampsia, oligohydramnios or polyhydramnios, history of repeated abortion, rheumatoid arthritis, thyroid or parathyroid disorders, hepatic and renal diseases, history of using aspirin and anticonvulsants or immunosuppressive drugs. ##The inclusion criteria for infants were as follows: birth weight $\geq 2500 \text{ g}$ and gestational age ≥ 37 weeks. The exclusion criteria for infants were as follows: preterm neonate, birth body weight $< 2500 \text{ g}$, congenital malformations, inborn errors of metabolism, endocrine disorders, liver or renal dysfunction, history of using anticonvulsant drugs, and serum 25(OH)D level $< 10 \text{ ng/mL}$ and Ca levels $< 8 \text{ mg/dL}$ after birth. ###Neonates with a 25(OH)D level $< 10 \text{ ng/mL}$ at birth were excluded, and they received vitamin D3 supplementation at a dose of 2000 IU/d . ####Did not breastfeed exclusively.

Results

A total of 92 newborns and their mothers were enrolled in the study (Fig. 1), and 46 newborns were randomly assigned to the intervention group (vD-400 group) to receive vitamin D3 supplementation and another 46 to the placebo group. Table 1 shows the demographic characteristics of the mothers and infants at birth. Forty of the studied infants were male, and their mean length, weight, and head circumference at birth were $50.1 \pm 1.3 \text{ cm}$, $3.2 \pm 0.3 \text{ kg}$, and $33.7 \pm 1.3 \text{ cm}$, respectively.

The mean 25(OH)D level of the mothers before delivery was $16.9 \pm 7.0 \text{ ng/mL}$. Among the 92 enrolled mothers, 15.2%, 54.3%, 22.8%, and 7.6% had severe vitamin D deficiency, vitamin D deficiency, vitamin D insufficiency, and vitamin D sufficiency, respectively. The mean 25(OH)D level of newborns at birth was $17.2 \pm 7.3 \text{ ng/mL}$. Among the 92 enrolled neonates, 15.2%, 52.2%, 27.2%, and 5.4% had severe vitamin D deficiency, vitamin D deficiency, vitamin D insufficiency, and vitamin D sufficiency, respectively. The percentages of vitamin D deficiency or insufficiency in the mothers and infants are similar. This is not

Table 1. Demographic Characteristics of Mothers and Infants at Birth

	Mothers ^a	Neonates
	N = 92	N = 92
Preconception BMI (kg/m ²)	21.0 ± 2.4 ^b	
Vitamin D supplementation (IU/d)	218 ± 175	
Sex, male (%)		40 (43.5)
25(OH)D (ng/mL)	16.9 ± 7.0	17.2 ± 7.3
VD severe deficiency, n (%)	14 (15.2)	14 (15.2)
VD deficiency, n (%)	50 (54.3)	48 (52.2)
VD insufficiency, n (%)	21 (22.8)	25 (27.2)
VD sufficiency, n (%)	7 (7.6)	5 (5.4)
Body length (cm)		50.1 ± 1.3
Body weight (kg)		3.2 ± 0.3
Head circumference (cm)		33.7 ± 1.3
Ca (mg/dL)		9.5 ± 0.5
P (mg/dL)		5.2 ± 0.6
ALP (IU/L)		163.3 ± 46.0
iPTH (pg/mL)		12.4 ± 23.7

BMI = body mass index; VD = vitamin D; Ca = calcium; P = phosphorus; ALP = alkaline phosphatase; iPTH = intact parathyroid hormone.

^aVitamin D was supplemented at a dose of <1000 IU/d during pregnancy.

^bValues are mean ± standard deviation or number (percentile).

surprising because maternal vitamin D levels correlated significantly with infant vitamin D levels at birth (Pearson $r = 0.816$, $p < 0.001$; $N = 92$) (Fig. 2). The 25(OH)D levels were not significantly associated with the weight, length, head circumference, serum Ca, P, ALP, and iPTH levels in the infants at birth (Table 2). The 25(OH)D levels in infants at birth and maternal 25(OH)D levels in the vitamin D deficiency groups, that is, the 62 infants with 25(OH)D <20 ng/mL at birth, also showed a significant correlation (Pearson $r = 0.700$, $p < 0.001$; $n = 62$).

In total, 14 newborns had severe vitamin D deficiency at birth. They received oral vitamin D3 supplementation at a dose of 2000 IU/d, and they were excluded from the study. Two infants were not breastfed exclusively, and four were lost to follow-up during the study. In total, 72 infants completed the study and

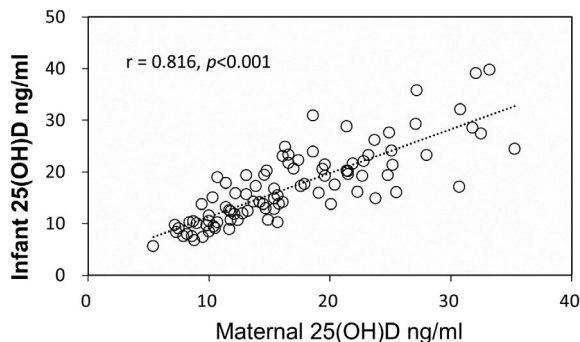


Fig. 2. Correlation between maternal and infant 25-hydroxy-vitamin D (25(OH)D) levels (ng/mL). The serum 25(OH)D levels of pregnant women were assessed before delivery, and those of their newborns were examined on day 1 of birth.

Table 2. Correlation Between 25(OH)D Levels of the Infants at Birth and Vitamin D-Related Parameters

Variables	25(OH)D Pearson coefficient r (95% confidence intervals)	p Value
Maternal BMI	-0.009 (-0.213-0.196)	0.929
Maternal 25(OH)D	0.816 (0.733-0.874)	<0.001
Neonatal body length	-0.074 (-0.274-0.132)	0.485
Body weight	-0.034 (-0.2372-0.172)	0.748
Head circumference	0.043 (-0.163-0.245)	0.685
Ca	0.094 (-0.113-0.293)	0.373
P	-0.009 (-0.213-0.196)	0.929
ALP	-0.124 (-0.320-0.082)	0.238
iPTH	-0.163 (-0.355-0.043)	0.120

BMI = body mass index; Ca = calcium; P = phosphorus; ALP = alkaline phosphatase; iPTH = intact parathyroid hormone.

were assessed. Maternal and infant baseline characteristics did not differ between the two groups (Table 3).

At 4 months of age, the vD-400 group had a significantly higher mean 25(OH)D level than the placebo group (38.6 ± 9.2 versus 13.6 ± 8.8 ng/mL; $p < 0.001$) (Table 4). The mean difference in 25(OH)D levels from birth to 4 months of age was 19.9 ng/mL in the vD-400 and -6.0 ng/mL in the placebo group. The 25(OH)D level of all infants at 4 months of age was significantly negatively correlated with iPTH levels (Pearson $r = -0.420$, $p < 0.001$) (Fig. 3). The growth patterns of infants differed between groups during the study period: the vD-400 group had a significantly larger weight and head circumference than the placebo group ($p = 0.014$ and 0.020 , respectively). The vD-400 group had a significantly higher phosphate level and a significantly lower iPTH level than the placebo group ($p = 0.035$ and $p = 0.001$, respectively). BMD and BMC did not differ between the two groups. However, compared with

Table 3. Characteristics of Mothers and Infants at Baseline

Characteristics	vD-400	Placebo	p Value
	(n = 37)	(n = 35)	
Maternal data			
Preconception BMI (kg/m ²)	21.1 ± 2.4 ^a	20.7 ± 2.3	0.463
25(OH)D (ng/mL)	17.8 ± 6.3	19.7 ± 7.1	0.227
Neonatal data			
Male, n (%)	16 (43.2)	11 (31.4)	0.338
Body length (cm)	50.1 ± 1.4	49.9 ± 1.4	0.473
Body weight (kg)	3.2 ± 0.3	3.1 ± 0.3	0.205
Head circumference (cm)	33.8 ± 1.4	33.5 ± 1.3	0.408
25(OH)D (ng/mL)	18.7 ± 7.1	19.6 ± 6.6	0.559
Ca (mg/dL)	9.5 ± 0.5	9.5 ± 0.4	0.742
P (mg/dL)	5.2 ± 0.6	5.2 ± 0.6	0.848
ALP (IU/L)	166.9 ± 50.4	164.0 ± 46.5	0.801
iPTH (pg/mL)	8.7 ± 10.9	9.3 ± 12.1	0.822

BMI = body mass index; Ca = calcium; P = phosphorus; ALP = alkaline phosphatase; iPTH = intact parathyroid hormone.

^aValues are mean ± standard deviation or number (%).

Table 4. Comparisons Between vD-400 and Placebo Groups at 4 Months of Age

Characteristics	vD-400	Placebo	p Value
	(n = 37)	(n = 35)	
Body length (cm)	63.5 ± 2.0 ^a	63.3 ± 2.1	0.653
Body weight (kg)	7.0 ± 0.8	6.6 ± 0.6	0.014
Head circumference (cm)	41.2 ± 1.3	40.5 ± 1.1	0.020
25(OH)D (ng/mL)	38.6 ± 9.2	13.6 ± 8.8	<0.001
VD severe deficiency	0	16 (45.7)	
VD deficiency	1 (2.7)	11 (31.4)	
VD insufficiency	7 (18.9)	7 (20)	
VD sufficiency	29 (78.4)	1 (2.9)	
Ca (mg/dL)	10.3 ± 0.3	10.2 ± 0.3	0.078
P (mg/dL)	5.8 ± 0.5	5.6 ± 0.4	0.035
ALP (IU/L)	249.1 ± 45.3	259.8 ± 68.3	0.431
iPTH (pg/mL)	19.8 ± 9.9	31.6 ± 18.7	0.001
BMD (g/cm ²)	0.23 ± 0.02	0.24 ± 0.02	0.515
BMC (g)	2.9 ± 0.4	2.8 ± 0.3	0.357

VD = vitamin D; Ca = calcium; P = phosphorus; ALP = alkaline phosphatase; iPTH = intact parathyroid hormone; BMD = bone mineral density; BMC = bone mineral content.

^aValues are mean ± standard deviation or number (%).

participants whose 25(OH)D levels were ≥10 ng/mL, those with severe vitamin D deficiency had a significantly higher iPTH level (40.5 ± 22.5 [n = 16] versus 21.3 ± 10.2 pg/mL [n = 56], *p* < 0.001) and a lower BMC (2.7 versus 2.9 g, *p* = 0.042) (Table 5). None of the infants had hypocalcemia, hypercalcemia, or adverse events related to vitamin D supplementation during the study.

Discussion

Our study showed that daily oral vitamin D3 supplementation at a dose of 400 IU increased vitamin D concentrations among exclusively breastfed infants at 4 months of age by 19.9 ng/mL of 25(OH)D (from 18.7 to 38.6 ng/mL). A Cochrane systematic review in 2020 showed that infants receiving vitamin D supplementation had a 9.1 ng/mL higher 25(OH)D level than those receiving placebo from the start of treatment to 6 months of

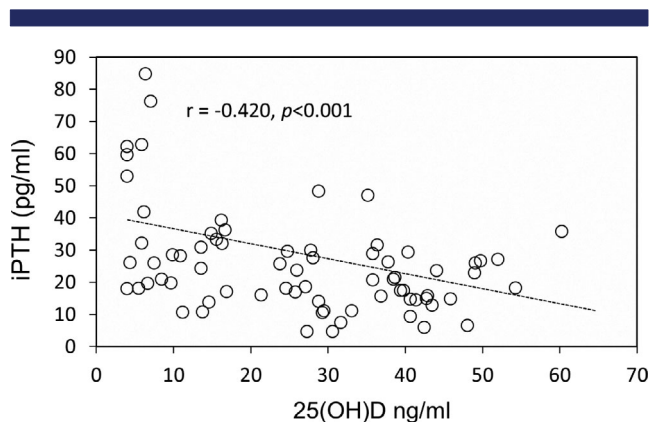


Fig. 3. Correlation between serum 25(OH)D and iPTH of the infants at age 4 months.

Table 5. Outcomes Between Infants of 25(OH)D Levels of ≥10 ng/mL and Those With Severe Vitamin D Deficiency (25(OH)D Levels <10 ng/mL) at 4 Months of Age

Characteristics	25(OH)D	25(OH)D	p Value
	≥10 ng/mL	<10 ng/mL	
	(n = 56)	(n = 16)	
Body length (cm)	63.6 ± 1.8 ^a	63.0 ± 2.7	0.332
Body weight (kg)	6.9 ± 0.8	6.6 ± 0.7	0.182
Head circumference (cm)	40.9 ± 1.3	40.4 ± 0.8	0.121
25(OH)D (ng/mL)	32.2 ± 12.4	6.2 ± 2.0	<0.001
iPTH (pg/mL)	21.3 ± 10.2	40.5 ± 22.5	<0.001
BMD (g/cm ²)	0.24 ± 0.02	0.23 ± 0.02	0.535
BMC (g)	2.9 ± 0.3	2.7 ± 0.3	0.042

iPTH = intact parathyroid hormone; BMD = bone mineral density; BMC = bone mineral content.

^aValues are mean ± standard deviation.

age.⁽¹⁹⁾ In the present study, the mean 25(OH)D concentrations in the placebo group decreased by 6.0 ng/mL (from 19.6 to 13.6 ng/mL). Other studies have reported similar results.⁽²⁰⁻²⁴⁾ Vitamin D levels in human milk are low (average 15.9 ± 8.6 IU/L)⁽²⁾ and UV exposure is negligible at this age.⁽²⁵⁾ Hence, the placebo group had lower and even decreased 25(OH)D levels at 4 months of age. Grant and colleagues showed increased 25(OH)D concentration in the placebo group at 4 months of age. This might be attributed to the concomitant intake of vitamin D from the milk formula.⁽²⁶⁾ However, in our study, infants with severe vitamin D deficiency at birth were excluded from the study because of safety concerns. Hence, we did not assess the actual vitamin D status of exclusively breastfed infants at 4 months of age, regardless of vitamin D supplementation. Hoogenboezem and colleagues showed that in most infants with normal vitamin D status at birth, the levels were depleted approximately 8 weeks after delivery, if there is no vitamin D supplementation.⁽²⁷⁾ Our study showed that the placebo group also had a significant decrease in vitamin D levels, and vitamin D3 supplementation at a dose of 400 IU/d remarkably improved vitamin D levels. This result is consistent with those of previous studies, and the importance of vitamin D supplementation in exclusively breastfed infants is reinforced.

The high prevalence of vitamin D deficiency and insufficiency among newborns in the present study is similar to that of a study conducted in Taiwan in 2016.⁽²⁸⁾ The median maternal 25(OH)D concentration was reported as 15.18 ng/mL, and the median cord blood 25(OH)D was 14.80 ng/mL. Approximately 75.8% of infants had vitamin D deficiency, and 15% had vitamin D insufficiency. Meanwhile, 75% of mothers had vitamin D deficiency, and 19% had vitamin D insufficiency.⁽²⁸⁾ The high prevalence of vitamin D deficiency in the present study might be attributable to inadequate vitamin D supplementation and low sun exposure among pregnant women. Furthermore, we observed a significant correlation between maternal and newborn vitamin D levels. This finding is consistent with those of previous studies.^(16,23,26,28-32) Vitamin D deficiency is commonly observed among pregnant women who do not receive vitamin D supplementation. Adequate maternal vitamin D supplementation may be included in routine antenatal care. However, current evidence regarding the efficacy of vitamin D supplementation in pregnancy is limited. Hollis and colleagues showed that daily vitamin D supplementation

at a dose of 4000 IU for pregnant women was safe and most effective in achieving sufficiency in their neonates. That is, 78.6% in the 4000 IU group had a cord blood 25(OH)D level >20 ng/mL compared with 39.7% in the 400 IU group.⁽³²⁾ However, current recommendations show no consensus about acceptable vitamin D supplementation dosage during pregnancy. The Institute of Medicine (IOM) Recommended Dietary Allowances is 600 IU/d of vitamin D for pregnant women.⁽³³⁾ The Endocrine Society suggested that at least 1500 to 2000 IU/d of vitamin D may be needed for pregnant women to maintain sufficient vitamin D levels.⁽²⁾ In 2020, the World Health Organization (WHO) recommended that oral vitamin D supplementation, which can improve maternal and perinatal outcomes, is not recommended for all pregnant women.⁽³⁴⁾ The American College of Obstetricians and Gynecologists recommended that vitamin D supplementation at a dose of 1000 to 2000 IU/d was safe for pregnant mothers with vitamin D deficiency.⁽³⁵⁾ To maintain vitamin D sufficiency, regular vitamin D supplementation should be recommended for pregnant women and their breastfed infants. It has been reported that high-dose maternal vitamin D supplementation safely supplies breast milk with adequate vitamin D, that is, to elevate circulating 25(OH)D in both mother and nursing infants, to meet the nursing infants' requirement and provide an alternate strategy for infant supplementation.⁽³⁶⁻³⁸⁾ Daily oral vitamin D3 supplementation at a dose of 400 IU is a reasonable and widely acceptable strategy in exclusively breastfed infants. Therefore, we chose daily 400 IU as supplementation dosage.

In our study, maternal and neonatal 25(OH)D levels did not affect the birth length, weight, and head circumference. Some studies support this finding, while others do not.^(16,29,30,39) Multiple confounding factors included maternal nutritional status, Ca and P intake, body mass index during pregnancy, and socioeconomic status. However, our study revealed that vitamin D supplementation significantly increased the weight and head circumference at 4 months of age. Different studies on vitamin D supplementation during infancy have reported contrasting results. Greer and colleagues and Chandy and colleagues showed that anthropometric measurements at 3 months of age did not significantly differ between infants who received vitamin D supplementation at a dose of 400 IU/d and controls.^(21,40) However, Kumar and colleagues revealed that weekly vitamin D supplementation at a dose of 1400 IU improved growth and prevented stunting in low-birth-weight term infants at 6 months of age, but it had a borderline negative effect on head circumference.⁽¹⁸⁾ Some studies evaluated different doses of vitamin D (200, 400, 800, 1200, and 1600 IU/d) among healthy breastfed infants. Higher doses of vitamin D increased plasma 25(OH)D concentration but had no effect on growth between the groups.^(31,41) Vitamin D promotes infant growth via Ca and phosphate metabolism, parathyroid hormone expression, and insulin-like growth factor regulation.⁽³⁹⁾ Therefore, vitamin D supplementation can improve infant growth. Nevertheless, several confounding factors such as race, daily amount of human milk intake, breastfeeding technique, genetics, and parent socioeconomic status must be considered. Thus, further large-scale studies should be conducted to validate this outcome.

One of the important issues in this study was the evaluation of bone mineralization by vitamin D3 supplementation. A total of 72 infants were successfully assessed using DXA. However, BMD and BMC did not differ between the vD-400 and placebo groups at 4 months of age. Moreover, only a few studies have

addressed this issue. Greer and colleagues revealed that vitamin D3 supplementation at a dose of 400 IU/d increased BMC at 12 weeks in breastfed infants.⁽⁴²⁾ However, a later study found no significant difference in BMC among breastfed infants receiving vitamin D supplementation at 3 and 6 months.⁽²¹⁾ A Korean study showed that daily vitamin D3 supplementation at a dose of 200 IU did not increase BMD and BMC at 6 months of age.⁽⁴³⁾ Our study showed similar results. Vitamin D supplementation did not affect BMD and BMC at 4 months of age. Gallo and colleagues and Ziegler and colleagues found no effect of a wide range of vitamin D doses on bone mineralization. They postulated that bone mineral accretion in breastfed infants is less affected by vitamin D supplementation, unless an underlying deficiency exists.^(41,44) In the present study, the severe vitamin D deficiency group had elevated iPTH levels and relatively low BMC. Thus, severe vitamin D deficiency can be a risk factor for rickets, and further studies should be performed to validate this result.

This study has several strengths. This was a prospective, randomized, double-blind, placebo-controlled trial assessing the effect of oral vitamin D3 supplementation at a dose of 400 IU/d among breastfed infants. DXA was performed to measure bone mineralization, and the results showed that BMD and BMC were not different between infants supplemented with vitamin D3 for 4 months and the placebo group. Moreover, a study nurse frequently followed up the mothers to ensure breastfeeding compliance. However, this study had several limitations. The most important limitation was that 14 infants who had 25(OH)D levels of <10 ng/mL at birth were excluded from the initial study due to safety concerns. Therefore, we did not assess the effect of oral vitamin D3 supplementation at a dose of 400 IU/d among newborns with severe vitamin D deficiency at birth. Moreover, the exact quantities of human milk consumed by each infant, which may be an important factor for anthropometric parameters, were not quantified. The study nurse followed up with the mothers via telephone or communication software. Therefore, the exact doses of vitamin D administered were not validated. Furthermore, although vitamin D supplementation at a dose of 400 IU/d is safe based on the current evidence, the possible toxic effect of hypercalciuria was not assessed. Finally, the major limitation of this study was the relatively small number of participants. Hence, further studies with larger populations should be conducted in the future.

In conclusion, vitamin D deficiency is widespread among newborns in Taiwan. Two-thirds of neonates present with vitamin D deficiency or severe deficiency. Maternal 25(OH)D status at birth is a major determinant of newborn vitamin D status. Thus, daily oral vitamin D3 supplementation at a dose of 400 IU increases vitamin D concentration and improves relevant biological indices and anthropometric measurements among exclusively breastfed infants at 4 months of age. Severe vitamin D deficiency was associated with a lower BMC, which could be prevented by vitamin D supplementation. Daily oral vitamin D3 supplementation at a dose of 400 IU is a feasible method for improving vitamin D status and bone health among exclusively breastfed infants during their first 4 months of life.

Disclosures

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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AUTHOR CONTRIBUTIONS

Chao-Hsu Lin: Conceptualization; data curation; investigation; project administration; validation; writing – original draft. **Chien-Yu Lin:** Data curation; investigation; writing – original draft. **Yi-Hsiang Sung:** Investigation. **Sung-Tse Li:** Data curation; investigation; software. **Bi-Wen Cheng:** Data curation; investigation; software. **Shun-Long Weng:** Investigation. **Shing-Jyh Chang:** Investigation. **Hung-Chang Lee:** Conceptualization; data curation; formal analysis. **Yann-Jinn Lee:** Conceptualization; supervision; writing – review and editing. **Wei-Hsin Ting:** Conceptualization; data curation; formal analysis. **Hung-Yang Chang:** Conceptualization; data curation; formal analysis; validation. **Yi-Lei Wu:** Validation. **Chih-Sheng Lin:** Conceptualization; supervision; writing – review and editing.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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