

ORIGINAL ARTICLE

Effect of Vitamin D Supplementation in Healthy Full-Term Infants: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

Objectives: Vitamin D deficiency in infants is a significant global health issue. The aim of this randomized, double-blind, placebo-controlled trial was to assess the effectiveness of vitamin D supplementation in infants at 6 months of age in preventing deficiency or promoting sufficient vitamin D status.

Materials and Methods: Sixty-eight healthy full-term infants were randomly assigned to receive either vitamin D supplementation, 400 international unit (IU)/day (intervention group; n = 34), or placebo (control group; n = 34). Blood samples were collected 48–72 hours after birth to measure serum 25-hydroxyvitamin D (25(OH)D) levels. Supplementation began after blood collection and continued for 6 months. The serum 25(OH)D levels of the two groups were then compared.

Results: After six months, vitamin D supplementation significantly increased mean serum 25(OH)D levels by 7.07 nanograms per milliliter compared to placebo (95% confidence interval (CI): 1.30–12.83; p = 0.016). Among breastfed infants, supplementation with vitamin D 400 IU/day resulted in significantly improved vitamin D status. Notably, 100% of infants in the intervention group achieved vitamin D sufficiency, compared to 77.8% in the control group (p = 0.046). Furthermore, supplemented breastfed infants exhibited significantly higher mean serum 25(OH)D levels at six months (p = 0.019) and a greater increase from baseline (p = 0.010) relative to the control group. In contrast, these effects of supplementation were not observed among formula-fed or mixed-fed infants.

Conclusion: This study highlights the high prevalence of vitamin D insufficiency and deficiency among healthy Thai neonates. Daily supplementation with 400 IU of vitamin D from birth to age 6 months among breastfed infants significantly improved the prevalence of vitamin D sufficiency and elevated serum vitamin D levels by age 6 months without any adverse effects.

Keywords: infant, placebo, randomized controlled trial, vitamin D

INTRODUCTION

Vitamin D deficiency and rickets in infancy are recognized as significant global health problems.^{1,2} The prevalence of vitamin D insufficiency or deficiency in the cord blood of newborns ranges from 20.2% to 69.1%; contributing factors include low maternal serum 25-hydroxyvitamin D (25(OH)D) levels and a lack of vitamin D supplementation during pregnancy.³

Infants are particularly vulnerable to vitamin D deficiency because human breast milk contains low levels of vitamin D (15–50 international unit (IU)/liter (L)), which are insufficient for meeting the daily vitamin D requirements of breastfed newborns.^{4,5} Even formula-fed infants who consume approximately 750 (milliliter) mL/day of vitamin D-fortified formula may receive less than the recommended daily intake of 400 IU/day.^{6–8} In addition, inadequate sunlight exposure during infancy further exacerbates the risk of vitamin D insufficiency or deficiency; thus, supplementation is needed.

Numerous studies have supported the recommendation of supplementing both breastfed and formula-fed infants with 400 IU/day of vitamin D for at least the first 6 months of life.^{9–13} According to international guidelines, 400 IU/day of vitamin D should be administered to all infants, starting within the first few days of life and continuing for 1 year or until the infant consumes at least 1 L per day of vitamin D-fortified formula or whole milk.^{14–16} Despite these global recommendations, routine vitamin D supplementation is not widely supplied to healthy infants in Thailand. The possible reasons include the lack of national guidelines on vitamin D supplementation and the limited availability of studies demonstrating its efficacy in Thai infants.¹⁷

The aim of this study was to evaluate the effectiveness of vitamin D supplementation in preventing deficiency or achieving sufficient levels in breastfed and formula-fed Thai infants by the age of 6 months.

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled study was conducted at Vajira Hospital, Bangkok, Thailand, between July 16, 2020, and September 22, 2021. The study protocol was approved by the Institutional Review Board of the Faculty of Medicine Vajira Hospital (COA 142/2562, October 24, 2019) and was registered retrospectively in the Thai Clinical Trials Registry (thaiclinicaltrial.org; TCTR20240405002)

on April 5, 2024. This study adhered to the Consolidated Standards of Reporting Trials criteria for parallel-group randomized trials.

The sample size was determined to detect a difference in the prevalence of vitamin D sufficiency at 6 months of age between the intervention and placebo groups. At the time the study protocol was developed, no data were available on the prevalence of vitamin D deficiency among 6-month-old Thai infants or from randomized trials evaluating vitamin D supplementation in this population. Therefore, estimates were derived from previously published studies. Data from the South East Asian Nutrition Surveys indicated that vitamin D sufficiency was present in 54.4% of urban Thai children who had not received vitamin D supplementation.¹⁸ For the intervention group, we used data from a study of Turkish infants receiving 400 IU/day of vitamin D supplementation, in which 88% of infants were vitamin D sufficient.¹⁹ This population was considered comparable to our cohort because it included both breastfed and formula-fed infants. Based on these assumptions, a minimum sample size of 28 infants per group was required to achieve 80% power with a two-sided significance level of 0.05. To allow for an anticipated 20% loss to follow-up, the final target sample size was increased to 34 infants per group.

In accordance with the declaration of Helsinki, we required all mothers to give written informed consent to participate before their inclusion in the study. Eligible participants were healthy infants born at Vajira Hospital at full term (gestational age, 37–42 weeks), at normal birth weight (2500–4000 gram), and with no perinatal asphyxia (Apgar score at 5 minutes, > 7), and whose mothers were 18–35 years of age and had no known medical disorders. We excluded newborns receiving medications that affect vitamin D levels (e.g., steroids and antiepileptic drugs) and those with diagnosed intrauterine growth retardation, chromosomal abnormalities, congenital anomalies, or sepsis.

Randomization was performed when infants were 48–72 hours old using a computer-generated block of four by one author (R.P.). Parents and clinicians involved in enrollment, data collection, and analysis (S.T. and T.T.) were blinded to group allocation. At study initiation, no commercially available vitamin D syrup was available in Thailand; therefore, the study medication was compounded in-house by a

hospital pharmacist (R.P.). To maintain double blinding, vitamin D and placebo syrups were prepared in identical 50-mL opaque bottles and were indistinguishable in appearance. The vitamin D syrup (400 IU/mL) was prepared by dissolving calciferol capsules (vitamin D₂, 20,000 IU) in 50 mL of 70% glucose in water, while the placebo consisted of dextrose powder dissolved in the same solution. The compounded preparations were stable at room temperature with an in-use stability of 45 days. As infants were followed up every two months, parents received two bottles at each visit: one prepared by the pharmacist for the first month and pre-measured vitamin D or placebo powder with 70% glucose solution for self-preparation during the second month, following standardized instructions.

We assessed baseline maternal and infant data that could influence serum vitamin D levels, including skin color, duration of sun exposure, sunscreen use, vitamin D intake and supplementation, type of feeding, and season of birth. Skin color in nonexposed areas (e.g., the inner arm or abdomen) of mothers and infants was assessed via the Fitzpatrick skin type classification.²⁰ The duration of sun exposure was assessed by asking mothers to report the average daily time (in minutes) that both the mother and infant were exposed to sunlight between 9:00 a.m. and 3:00 p.m., the period during which cutaneous vitamin D synthesis is most effective. Sunscreen use was evaluated by asking mothers whether they routinely used sunscreen. Maternal vitamin D intake and supplementation included vitamin D obtained from both single-ingredient vitamin D supplements and combined or multivitamin preparations containing vitamin D.

After 1 mL of venous blood was collected from mothers and their infants to measure baseline serum 25(OH)D levels, the infants were given 1 mL of either vitamin D or placebo syrup daily, according to their assigned group, until they were 6 months of age. In accordance with hospital policy, all mothers were encouraged to exclusively breastfeed their infants, as this was the optimal method of feeding. When exclusive breastfeeding was not feasible, mixed feeding or formula feeding was allowed as an alternative. Solid foods were introduced when infants were between the ages of 4 and 6 months.

At 2, 4, and 6 months of age, the infants visited the pediatric outpatient clinic, Vajira Hospital, for routine immunizations, growth assessments

(including weight and length), evaluation of adherence to the supplementation regimen, and monitoring of potential adverse events related to vitamin D toxicity (e.g., symptoms of hypercalcemia, such as nausea, vomiting, constipation, polydipsia, polyuria, muscle weakness, and twitching). At each visit, mothers were provided with two bottles of either vitamin D or placebo syrup. When infants were 6 months of age, we collected 2 mL of their venous blood to assess the serum levels of 25(OH)D, intact parathyroid hormone (PTH), alkaline phosphatase, calcium, and phosphorus to evaluate vitamin D status and adverse events related to supplementation.

Infants who did not complete the study included those who experienced adverse effects from vitamin D supplementation and those whose parents withdrew from the study or did not adhere to the research protocol, such as due to difficulty administering the supplement or inability to attend follow-up visits. To ensure participant safety, an interim analysis was conducted after 50% of participants in each group had been enrolled, and a progress report was subsequently submitted to the Ethics Committee. Serum 25(OH)D and intact PTH concentrations were measured using electrochemiluminescence immunoassays (Elecys Vitamin D total II assay and Elecys PTH assay on the cobas® 8000 e 801 analytical unit; Roche Diagnostics, Basel, Switzerland).²¹ Serum calcium, phosphorus, and alkaline phosphatase levels were measured using photometric methods (cobas® pro integrated solutions c 503 analytical unit; Roche Diagnostics, Mannheim, Germany). Internal quality controls were included in each assay batch to ensure accuracy and precision. Vitamin D status was categorized according to serum 25(OH)D levels as follows: deficiency, < 12 nanograms (ng)/mL (< 30 nmol (nanomoles)/L); insufficiency, 12–20 ng/mL (30–50 nmol/L); and sufficiency, > 20 ng/mL (> 50 nmol/L).¹⁴

The primary outcome of this study was the effect of vitamin D supplementation on the prevalence of vitamin D sufficiency among 6-month-old infants in the intervention and control groups. The secondary outcome was the effect of vitamin D supplementation on serum 25(OH)D level in 6-month-old infants fed breast milk, infant formula and mixed feeding.

We used Stata 13 (StataCorp, College Station, TX, United States) to perform statistical analyses. Data for continuous variables were calculated as means ± standard deviations. To compare the two groups,

we used the independent t test or the Mann-Whitney U test, depending on the data distribution. Data for categorical variables were calculated as counts and percentages, and we compared the groups by using the Chi-square test or Fisher's exact test, as appropriate.

We performed per-protocol analysis to evaluate the effect of vitamin D supplementation on the prevalence of vitamin D sufficiency at age 6 months in comparison with baseline levels in both the intervention and control groups. We conducted multilevel Poisson regression with random intercepts and fixed effects to evaluate and compare the prevalence ratios of vitamin D sufficiency at age 6 months in the two groups. Furthermore, we used multilevel linear regression with random intercepts and fixed effects to analyze the mean differences in serum 25(OH)D levels at baseline between the two groups and from baseline to 6 months within both groups, as well as the mean difference in serum 25(OH)D level changes between the two groups. A p-value of < 0.05 was considered statistically significant.

RESULTS

Among the 68 participants initially enrolled and randomly assigned to receive either vitamin D syrup (intervention group; $n = 34$) or placebo syrup (control

group; $n = 34$), 3 participants from the intervention group and 4 from the control group were withdrawn from the study or lost to follow-up. In addition, the parents of 2 participants from each group did not comply with the supplementation regimen. We therefore analyzed the data of the 57 infants (83.8%) who completed the 6-month study. (**Figure 1**)

The characteristics of mothers and infants in the two groups at baseline did not differ significantly. The baseline serum 25(OH)D levels and vitamin D status of mothers in the two groups were comparable. At 6 months of age, infant feeding practices, duration of sun exposure, type of feeding, growth parameters, and serum levels of calcium, phosphorus, alkaline phosphatase, and intact PTH in the two groups were similar. None of the infants had hypercalcemia or adverse events related to vitamin D toxicity during the study. (**Table 1**)

Table 2 demonstrated the effect of vitamin D supplementation on the prevalence of vitamin D sufficiency and mean serum 25(OH)D of infants at six months. The prevalence of vitamin D sufficiency among the infants at 6 months of age increased significantly from baseline in both the intervention group ($p = 0.002$) and the control group ($p = 0.044$). Although this

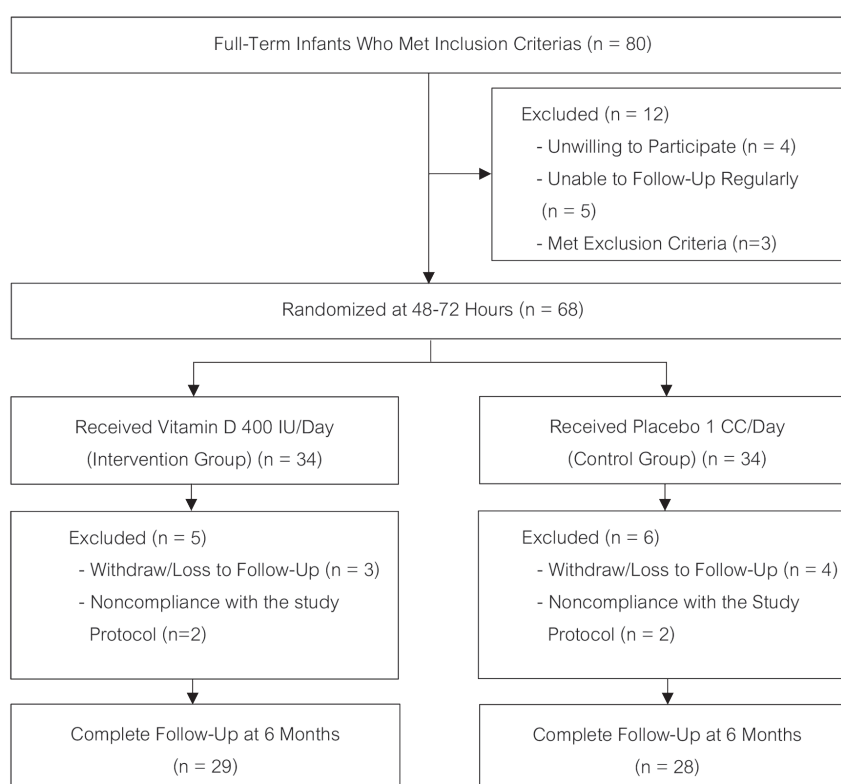


Figure 1 Flow Chart of the Study Participants

Table 1 Characteristics of Participants who Completed the Study (n = 57)

Parameters	Intervention Group n = 29 (%)	Control Group n = 28 (%)	P-value
Maternal Factors at Baseline			
Weight Gain during Pregnancy (kg)	12.28 ± 3.54	13.82 ± 4.06	0.131
Duration of Sun Exposure (min/ day)	96.38 ± 68.44	98.57 ± 68.62	0.904
Sun Exposure per Day			0.760
≤ 15 min	5 (17.2)	4 (14.3)	
> 15 min	24 (82.8)	24 (85.7)	
Skin Color			0.109
Light Brown	5 (17.2)	1 (3.6)	
Medium Brown	19 (65.6)	17 (60.7)	
Dark Brown	5 (17.2)	10 (35.7)	
Sunscreen Use	9 (31.0)	6 (21.4)	0.410
Vitamin D Rich Food Consumption	5 (17.2)	1 (3.6)	0.194
Vitamin D Supplement	0 (0)	0 (0)	NA
Smoking	8 (27.6)	12 (42.9)	0.227
Pregnancy Complications	2 (6.9)	2 (7.1)	1.000
Serum 25(OH)D (ng/mL)	19.80 ± 8.96	19.41 ± 7.77	0.861
Vitamin D Sufficiency	2 (6.9)	1 (3.6)	1.000
Infant Factors at Baseline			
Gestation Age (week)	39.10 ± 0.95	38.89 ± 0.82	0.375
Birth Weight (g)	3126.03 ± 382.51	3063.57 ± 266.69	0.479
Length (cm)	50.62 ± 1.40	50.89 ± 1.37	0.461
Gender			0.689
Male	14 (48.3)	15 (53.6)	
Female	15 (51.7)	13 (46.4)	
Skin Color			0.130
Light Brown	8 (27.6)	3 (10.7)	
Medium Brown	20 (69.0)	21 (75.0)	
Dark Brown	1 (3.4)	4 (14.3)	
Type of Feeding			0.900
Breast Milk	19 (65.6)	18 (64.3)	
Infant Formula	5 (17.2)	4 (14.3)	
Mixed Feeding	5 (17.2)	6 (21.4)	
Season of Birth			0.612
Rainy	9 (31.0)	7 (25.0)	
Winter	20 (69.0)	21 (75.0)	
Serum 25(OH)D (ng/mL)	17.49 ± 4.34	18.69 ± 5.95	0.389
Infant Factors at 6 Months			
Duration of Sun Exposure (min/ day)	14.00 ± 15.97	15.82 ± 18.10	0.688

Table 1 Characteristics of Participants who Completed the Study (n = 57) (cont.)

Parameters	Intervention Group n = 29 (%)	Control Group n = 28 (%)	P-value
Sun Exposure per Day			0.681
≤ 15 min	14 (48.3)	12 (42.9)	
> 15 min	15 (51.7)	16 (57.1)	
Type of Feeding			0.063
Breast Milk	7 (24.1)	15 (53.6)	
Infant Formula	18 (62.1)	10 (35.7)	
Mixed Feeding	4 (13.8)	3 (10.7)	
Complementary Feeding			0.706
Yes	24 (82.8)	25 (89.3)	
No	5 (17.2)	3 (10.7)	
Weight (g)	7597.59 ± 852.77	7669.29 ± 775.02	0.741
Length (cm)	67.90 ± 2.26	67.61 ± 2.10	0.618
Serum 25(OH)D (ng/mL)	39.80 ± 12.69	35.79 ± 11.91	0.224
Serum Calcium (mg/dL)	10.34 ± 0.27	10.43 ± 0.32	0.310
Serum Phosphorus (mg/dL)	5.69 ± 0.42	5.64 ± 0.49	0.677
Alkaline Phosphatase (IU/L)	264.07 ± 77.65	281.00 ± 52.94	0.342
Serum iPTH (pg/mL)	27.96 ± 11.03	25.06 ± 8.58	0.275

Abbreviations: (OH)D, hydroxyvitamin D; cm, centimeter; dL, deciliter; g, gram; iPTH, intact parathyroid hormone; IU, international units; kg, kilogram; L, liter; mg, milligrams; mL, milliliter; n, number; NA, not applicable; ng, nanograms; pg, picograms

Data are presented as n (%) or mean ± standard variation, significant level at $p < 0.05$

Table 2 Effect of Vitamin D Supplementation on the Prevalence of Vitamin D Sufficiency and Mean Serum 25(OH)D of Infants at Six Months

	Intervention Group (n = 29)			Control Group (n = 28)			Multivariable Analysis ^c	
	At Baseline	At 6 Months	P-value ^a	At Baseline	At 6 Months	P-value ^a	Adjusted Change from Baseline between Two Groups	P-value ^b
Infants with Vitamin D Sufficiency (%)	8 (27.6)	28 (96.5)	0.002	11 (39.3)	23 (82.1)	0.044	1.76 (0.56 to 5.48)	0.331
Serum 25(OH)D (ng/mL)	17.49 ± 4.34	39.80 ± 12.69	< 0.001	18.69 ± 5.95	35.79 ± 11.91	< 0.001	7.07 (1.30 to 12.83)	0.016

Abbreviations: (OH)D, hydroxyvitamin D; mL, milliliter; n, number; ng, nanograms

Prevalence of vitamin D sufficiency was shown as frequency (percentage) and serum 25(OH)D as mean ± standard deviation, significant level at $p < 0.05$.

^a Multilevel Poisson regression with random intercept and fixed effect models was used to analyze the effectiveness of vitamin D supplementation on vitamin D sufficiency at 6 months compared to their baseline both intervention and control groups.

^b Multilevel linear regression with random intercept and fixed effect models was used to analyze mean difference in serum 25(OH)D from baseline to 6 months both groups.

^c Multilevel Poisson regression with random intercept and fixed effect models was used to analyze the effectiveness of vitamin D supplementation on vitamin D sufficiency over time (slope) by compared prevalence ratio of intervention group and control group.

^d Multivariable analysis adjusted for potential confounders including maternal factors (sun exposure, sunscreen used, vitamin D rich food consumption and vitamin D status) and infant factors (sun exposure, skin color, type of feeding and complementary feeding).

prevalence was higher in the intervention group than in the control group (96.5% vs. 82.1%), the difference was not statistically significant ($p = 0.331$). In both the intervention and control groups, the mean serum 25(OH)D levels of infants at 6 months of age was significantly higher than baseline levels ($p < 0.001$). After adjustment for potential confounders through multivariate analysis, the intervention group exhibited a mean increase of 22.30 ng/mL over baseline (95% confidence interval (CI), 17.98–26.62) in serum 25(OH)D levels at 6 months, which was 7.07 ng/mL (95% CI, 1.30–12.83 ng/mL) higher than that of the control group ($p = 0.016$).

To compare the effects of vitamin D supplementation on vitamin D status at 6 months of age among breastfed infants with those among formula-fed or mixed-fed infants (Table 3). At baseline, there were no significant differences in maternal or infant vitamin D status, nor in serum 25(OH)D levels between the intervention and

control groups across both feeding cohorts. Among breastfed infants, vitamin D supplementation resulted in significantly better outcomes at six months. Specifically, 100% of infants in the vitamin D group achieved vitamin D sufficiency, compared to 77.8% in the control group ($p = 0.046$). Furthermore, supplemented breastfed infants exhibited significantly higher mean serum 25(OH)D levels at six months ($p = 0.019$) and a greater increase from baseline ($p = 0.010$) relative to the control group. In contrast, these effects of vitamin D supplementation were not observed in formula-fed or mixed-fed infants.

DISCUSSION

In this study, vitamin D insufficiency or deficiency was observed in 66.7% of full-term newborns, which was similar to the high prevalence of these conditions among Thai neonates.³ Supplementation of vitamin D 400 IU/day significantly increased the prevalence of vitamin D

Table 3 Effect of Vitamin D Supplementation on Vitamin D Status of Breastfed Infants Compared to Formula-Fed or Mixed-Fed Infants

	Breastfed Infants			Formula-Fed or Mixed-Fed Infants		
	Intervention Group n = 19 (%)	Control Group n = 18 (%)	P-value	Intervention Group n = 10 (%)	Control Group n = 10 (%)	P-value
Maternal Data						
Vitamin D Status			1.000			NA
Deficiency or Insufficiency	17 (89.5)	17 (94.4)		10 (100.0)	10 (100.0)	
Sufficiency	2 (10.5)	1 (5.6)		0 (0)	0 (0)	
Serum 25(OH)D (ng/mL)	20.08 ± 10.54	19.85 ± 7.75	0.940	19.26 ± 5.22	18.61 ± 8.17	0.834
Infant Data at Baseline						
Vitamin D Status			0.743			0.582
Deficiency or Insufficiency	12 (63.2)	10 (55.6)		9 (90.0)	7 (70.0)	
Sufficiency	7 (36.8)	8 (44.4)		1 (10.0)	3 (30.0)	
Serum 25(OH)D (ng/mL)	18.18 ± 4.74	19.08 ± 6.14	0.620	16.18 ± 3.27	17.99 ± 5.85	0.405
Infant Data at 6 Months						
Vitamin D Status			0.046			1.000
Deficiency or Insufficiency	0 (0)	4 (22.2)		1 (10.0)	1 (10.0)	
Sufficiency	19 (100.0)	14 (77.8)		9 (90.0)	9 (90.0)	
Infant Serum 25(OH)D (ng/mL)	42.41 ± 13.02	32.57 ±11.14	0.019	34.84 ± 10.95	41.60 ± 11.55	0.196
Increment of Infant Serum 25 (OH) D from Baseline (ng/mL)	24.22 ± 12.76	13.59 ± 10.83	0.010	18.66 ± 10.94	23.61 ± 11.37	0.335

Abbreviations: (OH)D, hydroxyvitamin D; mL, milliliter; n, number; NA, not applicable; ng, nanograms

Data are presented as n (%) or mean ± standard variation, significant level at $p < 0.05$.

sufficiency and improved serum vitamin D levels among breastfed infants aged 6 months but not among formula-fed or mixed-fed infants. These findings are consistent with those of a previous study of Thai full-term breastfed infants, in which the mean serum 25(OH)D level was 46.01 ± 16.42 ng/mL and vitamin D sufficiency was present in 93.0% of infants aged 6 months who received vitamin D supplementation.¹⁷ According to our findings, daily supplementation with 400 IU of vitamin D significantly increased serum 25(OH)D levels by an average of 22.30 ng/mL over baseline (95% CI, 17.98–26.62). This mean increase is similar to that observed by Lin et al. in Taiwan (19.9 ng/mL; 95% CI, 18.7–38.6)²² but greater than those reported in a Cochrane review of nine studies involving 743 full-term breastfed infants (9.1 ng/mL; 95% CI, 6.8–11.3)⁶ and in a systematic review and meta-analysis of 25 studies of breastfed and formula-fed infants (13.9 ng/mL; 95% CI, 9.8–18.0).²³ These results suggest that vitamin D supplementation 400 IU/day, beginning in early infancy is effective for achieving serum 25(OH)D levels of > 20 ng/mL. Nearly all infants (96.5%) in our intervention group attained vitamin D sufficiency at 6 months, which confirms the effectiveness of supplementation in improving vitamin D status in healthy full-term infants. Of importance is that no infants in the intervention group exhibited clinical signs of vitamin D toxicity or had serum 25(OH)D levels exceeding 100 ng/mL throughout the study period; thus, the dosage of 400 IU/day was safe and effective, as in previous studies.^{7,10,17,19}

The mean serum 25(OH)D levels of infants at age 6 months were higher than those at baseline; thus, the prevalence of vitamin D insufficiency or deficiency among infants at age 6 months was reduced. These findings are consistent with those of previous studies.^{24,25} Oktaria et al. reported that the prevalence of vitamin D insufficiency or deficiency among Indonesian infants decreased from 90% at birth to 13% at 6 months.²⁴ Similarly, study of Nakano et al. revealed that serum 25(OH)D levels were higher in infants aged 6–15 months, even without supplementation, than in those aged 0–5 months, which indicates that vitamin D deficiency did not persist throughout infancy.²⁵ This increase in vitamin D levels in older infants may be attributed to higher dietary vitamin D intake after weaning and to greater sun exposure. However, our findings emphasize that vitamin D supplementation effectively increased serum vitamin D levels and the

prevalence of vitamin D sufficiency among breastfed infants at 6 months of age, whereas no such effect was observed in formula-fed or mixed-fed infants. Breast milk contains vitamin D at concentrations ranging from 15 to 50 IU/L, significantly lower than the levels found in infant formula (400 IU/L). Consequently, breastfed infants are at a higher risk of vitamin D deficiency, and supplementation is thus particularly beneficial for this group. Furthermore, the relatively high vitamin D levels observed in the control groups at 6 months of age raise the possibility that routine vitamin D supplementation beyond this age may not be necessary; however, this finding should be interpreted with caution and warrants further investigation.

A daily intake of 400 IU of vitamin D is sufficient to prevent rickets in children and is recommended for all infants from birth to 12 months of age, regardless of feeding method.^{14–16} Current evidence supporting the recommendation of vitamin D supplementation to prevent deficiency in Asian infants has been increasing.^{26,27} These guidelines typically advise vitamin D supplementation without prior screening, as the cost of blood testing is considerably higher than that of supplementation.^{14–16,26,27} Despite these recommendations, vitamin D supplementation for healthy full-term infants is not widely practiced in Thailand, possibly because of the absence of national guidelines and the limited availability of studies focusing on Thai infants.¹⁷ This study underscores the benefits of vitamin D supplementation in restoring adequate vitamin D status in full-term breastfed infants.

This study demonstrates a high prevalence of vitamin D deficiency or insufficiency among mothers. Because maternal vitamin D status determines the vitamin D content of breast milk, maternal deficiency may result in inadequate vitamin D intake in exclusively breastfed infants, particularly without supplementation.^{28,29} Current guidelines recommend adequate vitamin D intake during pregnancy and lactation to support maternal and neonatal vitamin D status.³⁰ Our findings highlight the potential benefit of optimizing maternal vitamin D status during pregnancy and breastfeeding to improve infant vitamin D status. Further research is needed to assess the combined effects of maternal and infant vitamin D supplementation

This study had several strengths. To the best of our knowledge, this is the first double-blind, randomized, placebo-controlled evaluation of the effect of vitamin D supplementation on the vitamin D status of healthy full-term Thai infants. While a previous randomized

controlled trial in Thai infants has been reported,¹⁷ that study employed an open-label design despite its larger sample size. The double-blind, placebo-controlled design of the present study minimizes potential bias and strengthens the internal validity of the findings. Baseline characteristics and serum 25(OH)D levels of both infants and their mothers were assessed and confirmed comparability between the intervention and control groups at study entry. In addition, by including both breastfed and formula-fed infants, the findings may be generalizable to a broader infant population.

This study has several limitations. It was conducted at a single hospital with a modest sample size, which may limit generalizability. Lifestyle factors influencing vitamin D status were not comprehensively assessed, potentially introducing recall bias, and the short follow-up limited evaluation of long-term outcomes. Additionally, intention-to-treat (ITT) analysis was not feasible because participants lost to follow-up had no post-baseline serum 25(OH)D data. Given the modest sample size, advanced methods for handling missing data would rely on unverifiable assumptions and could introduce bias. Therefore, the findings should be interpreted cautiously, particularly regarding internal validity.

Future multicenter studies with larger samples and longer follow-up are warranted to enhance generalizability and permit both ITT and per-protocol analyses, thereby providing more robust estimates to inform national vitamin D supplementation guidelines.

CONCLUSION

This study highlighted the high prevalence of vitamin D insufficiency and deficiency among healthy Thai neonates. Daily supplementation with 400 IU of vitamin D from birth to 6 months of age significantly improved the prevalence of vitamin D sufficiency and elevated serum vitamin D levels at age 6 months among the breastfed infants without any adverse effects. However, these benefits were not observed in formula-fed or mixed-fed infants.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

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Formal analysis: T.T., S.T.

Funding acquisition: S.T.

Investigation: T.T., S.T., R.P.

Methodology: T.T., S.T.

Project administration: T.T.

Resources: S.T., R.P.

Software: T.T.

Supervision: S.T.

Validation: T.T., S.T.

Visualization: T.T.

Writing – original draft preparation: T.T.

Writing – review & editing: S.T., R.P.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

REFERENCES

1. Dawodu A, Wagner CL. Prevalence of vitamin D deficiency in mothers and infants worldwide—a paradigm shift. *Paediatr Int Child Health* 2012;32(1):3-13. doi: [10.1179/146532811Y.0000000024](https://doi.org/10.1179/146532811Y.0000000024).
2. Creo AL, Thacher TD, Pettifor JM, Strand MA, Fischer PR. Nutritional rickets around the world: an update. *Paediatr Int Child Health* 2017;37(2):84-98. doi: [10.1080/20469047.2016.1248170](https://doi.org/10.1080/20469047.2016.1248170).
3. Ariyawatkul K, Lersbuasin P. Prevalence of vitamin D deficiency in cord blood of newborns and the association with maternal vitamin D status. *Eur J Pediatr* 2018;177(10):1541-5. doi: [10.1007/s00431-018-3210-2](https://doi.org/10.1007/s00431-018-3210-2).
4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911-30. doi: [10.1210/jc.2011-0385](https://doi.org/10.1210/jc.2011-0385).
5. Leerbeck E, Sondergaard H. The total content of vitamin D in human milk and cow's milk. *Br J Nutr* 1980;44(1):7-12. doi: [10.1079/bjn19800004](https://doi.org/10.1079/bjn19800004).
6. Tan ML, Abrams SA, Osborn DA. Vitamin D supplementation for term breastfed infants to prevent vitamin D deficiency and improve bone health. *Cochrane Database Syst Rev* 2020;12(12):CD013046. doi: [10.1002/14651858.CD013046.pub2](https://doi.org/10.1002/14651858.CD013046.pub2).
7. Jullien S. Vitamin D prophylaxis in infancy. *BMC Pediatr* 2021;21 Suppl 1:319. doi: [10.1186/s12887-021-02776-z](https://doi.org/10.1186/s12887-021-02776-z).

8. Wagner CL, Greer FR, the Section on Breastfeeding and Committee on Nutrition. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008;122(15):1142-52. doi: [10.1542/peds.2008-1862](https://doi.org/10.1542/peds.2008-1862).
9. Specker BL, Ho ML, Oestreich A, Yin T, Shui Q, Chen X, et al. Prospective study of vitamin D supplementation and rickets in China. *J Pediatr* 1992;120(5):733-9. doi: [10.1016/s0022-3476\(05\)80236-7](https://doi.org/10.1016/s0022-3476(05)80236-7).
10. Gallo S, Comeau K, Vanstone C, Agellon S, Sharma A, Jones G, et al. Effect of different dosages of oral vitamin D supplementation on vitamin D status in healthy, breastfed infants. *JAMA* 2013;309(17):1785-92. doi: [10.1001/jama.2013.3404](https://doi.org/10.1001/jama.2013.3404).
11. Choi YJ, Kim MK, Jeong SJ. Vitamin D deficiency in infants aged 1 to 6 months. *Korean J Pediatr* 2013;56(5):205-10. doi: [10.3345/kjp.2013.56.5.205](https://doi.org/10.3345/kjp.2013.56.5.205).
12. Chandy DD, Kare J, Singh SN, Agarwal A, Das V, Singh U, et al. Effect of vitamin D supplementation, directly or via breast milk for term infants, on serum 25 hydroxyvitamin D and related biochemistry, and propensity to infection: a randomised placebo-controlled trial. *Br J Nutr* 2016;116(1):52-8. doi: [10.1017/S0007114516001756](https://doi.org/10.1017/S0007114516001756).
13. Goyal A, Dabas A, Shah D, Malhotra RK, Dewan P, Madhu SV, et al. Sunlight exposure vs oral vitamin D supplementation for prevention of vitamin D deficiency in infancy: a randomized controlled trial. *Indian Pediatr* 2022;59(11):852-8.
14. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, et al. Global consensus recommendations on prevention and management of nutritional rickets. *J Clin Endocrinol Metab* 2016;101(2):394-415. doi: [10.1210/jc.2015-2175](https://doi.org/10.1210/jc.2015-2175).
15. Grossman Z, Hadjipanayis A, Stiris T, del Totso S, Mercier JC, Valiulis A, et al. Vitamin D in European children-statement from the European Academy of Pediatrics (EAP). *Eur J Pediatr* 2017;176(6):829-31. doi: [10.1007/s00431-017-2903-2](https://doi.org/10.1007/s00431-017-2903-2).
16. Saggese G, Vierucci F, Prodam F, Cardinale F, Cetin I, Chiappini E, et al. Vitamin D in pediatric age: consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians. *Ital J Pediatr* 2018;44(1):51. doi: [10.1186/s13052-018-0488-7](https://doi.org/10.1186/s13052-018-0488-7).
17. Ruangkit C, Suwannachat S, Wantanakorn P, Sethaphanich N, Assawawiroonhakarn S, Dumrongwongsiri O. Vitamin D status in full-term exclusively breastfed infants versus full-term breastfed infants receiving vitamin D supplementation in Thailand: a randomized controlled trial. *BMC Pediatr* 2021;21(1):378. doi: [10.1186/s12887-021-02849-z](https://doi.org/10.1186/s12887-021-02849-z).
18. Rojroongwasinkul N, Kijboonchoo K, Wimonpeerapattana W, Purttiponthnee S, Yamborisut U, Boonpradern A, et al. SEANUTS: the nutritional status and dietary intakes of 0.5-12-year-old Thai children. *Br J Nutr* 2013;110 Suppl 3:S36-44. doi: [10.1017/S0007114513002110](https://doi.org/10.1017/S0007114513002110).
19. Mutlu GY, Kusdal Y, Ozsu E, Cizmecioglu FM, Hatun S. Prevention of vitamin D deficiency in infancy: daily 400 IU vitamin D is sufficient. *Int J Pediatr Endocrinol* 2011;2011(1):4. doi: [10.1186/1687-9856-2011-4](https://doi.org/10.1186/1687-9856-2011-4).
20. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol* 1988;124(6):869-71. doi: [10.1001/archderm.124.6.869](https://doi.org/10.1001/archderm.124.6.869).
21. Lim S, Lee K, Woo HY, Park H, Kwon MJ. Evaluation of Cobas 8000 analyzer series module e801 analytic performance. *Ann Clin Lab Sci* 2019;49(3):372-9.
22. Lin CH, Lin CY, Sung YH, Li ST, Cheng BW, Weng SL, et al. Effect of oral vitamin D3 supplementation in exclusively breastfed newborns: Prospective, randomized, double-blind, placebo-controlled trial. *J Bone Miner Res* 2022;37(4):786-93. doi: [10.1002/jbmr.4521](https://doi.org/10.1002/jbmr.4521).
23. Zittermann A, Pilz S, Berthold HK. Serum 25-hydroxyvitamin D response to vitamin D supplementation in infants: a systemic review and meta-analysis of clinical intervention trials. *Eur J Nutr* 2019;59(1):359-69. doi: [10.1007/s00394-019-01912-x](https://doi.org/10.1007/s00394-019-01912-x).
24. Oktaria V, Graham SM, Triasih R, Soenarto Y, Bines JE, Ponsonby AL, et al. The prevalence and determinants of vitamin D deficiency in Indonesian infants at birth and six months of age. *PLoS ONE* 2020;15(10):e0239603. doi: [10.1371/journal.pone.0239603](https://doi.org/10.1371/journal.pone.0239603).
25. Nakano S, Suzuki M, Minowa K, Hirai S, Takubo N, Sakamoto Y, et al. Current vitamin D status in healthy Japanese infants and young children. *J Nutr Sci Vitaminol (Tokyo)* 2018;64(2):99-105. doi: [10.3177/jnsv.64.99](https://doi.org/10.3177/jnsv.64.99).
26. Haq A, Wimalawansa SJ, Pludowski P, Anouti FA. Clinical practice guidelines for vitamin D in the United Arab Emirates. *J Steroid Biochem Mol Biol* 2018;175:4-11. doi: [10.1016/j.jsbmb.2016.09.021](https://doi.org/10.1016/j.jsbmb.2016.09.021).
27. Tuyen le D, Hein VT, Binh PT, Yamamoto S. Calcium and vitamin D deficiency in Vietnamese: recommendations for an intervention strategy. *J Nutr Sci Vitaminol* 2016(1);62(1):1-5. doi: [10.3177/jnsv.62.1](https://doi.org/10.3177/jnsv.62.1).
28. Dawodu A, Tsang RC. Maternal vitamin D status: effect on milk vitamin D content and vitamin D status of breastfeeding infants. *Adv Nutr* 2012;3(3):353-61. doi: [10.3945/an.111.000950](https://doi.org/10.3945/an.111.000950).
29. Hollis BW, Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr* 2004;80(6 Suppl):1752S-8S. doi: [10.1093/ajcn/80.6.1752S](https://doi.org/10.1093/ajcn/80.6.1752S).
30. Palacios C, Kostiuk LL, Cuthbert A, Weeks J. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev* 2024;7(7):CD008873. doi: [10.1002/14651858.CD008873.pub5](https://doi.org/10.1002/14651858.CD008873.pub5).