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Original Article

Weekly vitamin D supplementation during early infancy as a potential strategy to prevent vitamin D insufficiency: A two-center retrospective study

Kaori Hara-Isono [a,b,*], Kazumi Morisawa [a], Mariko Hida [a], Shintaro Iwamoto [c], Kazushige Ikeda [d]

[a] Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan
[b] Department of Molecular Endocrinology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo, 157-8535, Japan
[c] Department of Data Science, Clinical Research Center, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo, 157-8535, Japan
[d] Division of Neonatology, Department of Pediatrics, Saitama City Hospital, 2460 Mimuro, Midori-ku, Saitama City, Saitama, 336-8522, Japan

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ABSTRACT

Background: For preventing Vitamin D (VD) insufficiency, several VD supplementation guidelines were established worldwide. In Japan, no nationwide guidelines for preventing VD insufficiency have been implemented, whereas guidelines for preventing vitamin K (VK) deficiency-related bleeding recommend weekly supplementation of VK. The aim of this study is to clarify whether weekly VD plus VK supplementation during the early neonatal period prevents VD insufficiency at one month of age.

Methods: We retrospectively analyzed serum 25-hydroxyvitamin D (25(OH)D) levels of 555 one-month-old infants born between 2017 and 2023. Infants were classified into the control group (not supplemented), weekly group (1000 IU/week), and daily group (240 IU/day). We compared serum 25(OH)D levels among the three groups. Multivariable logistic regression analyses adjusted for formula intake and BMI were performed to better estimate the effect of VD supplementation on the prevention of VD insufficiency.

Results: We included 414, 55, and 86 infants in the control, weekly, and daily groups, respectively. All infants received weekly supplementation of VK. Serum 25(OH)D levels in the weekly and daily groups were higher than those in the control group (median (ng/mL): control 9.7 vs weekly 22.2, $P < 0.001$; control vs daily 23.0, $P < 0.001$). The frequencies of VD insufficiency were 370/414 (89.4 %), 11/55 (20.0 %), and 22/86 (25.6 %) in the control, weekly, and daily groups, respectively. Adjusted odds ratios of VD insufficiency compared to the control were 0.038 (95 % confidence intervals (95 %CI): 0.017, 0.085) and 0.036 (95 %CI: 0.019, 0.067) in the weekly and daily groups, respectively. No infant with VD excess was observed.

Conclusion: Our results demonstrated that combined weekly supplementation of VD and VK during early infancy can prevent VD insufficiency at one month of age without causing VD excess. This finding may provide evidence for the development of nationwide prophylaxis for VD insufficiency in regions lacking specific guidelines.

1. Introduction

Vitamin D (VD) insufficiency (25-hydroxyvitamin D (25(OH)D) < 20 ng/mL) is one of the common health problems worldwide, in infants, children, and adolescents [1]. The prevalence of VD insufficiency is affected by dietary intake, geographic location, ethnicity, UVB exposure, and socioeconomic status [2]. In fact, the prevalence of VD insufficiency in healthy infants was 10.8 % in the US [3] and 44.0 % in Northern Taiwan [4]. The VD status of Japanese infants is even more critical. We previously reported that 47/134 (35.1 %) cord blood samples of term infants and 133/282 (47.2 %) blood samples of one-month-old infants showed serum 25(OH)D_3 levels below the lower limit of quantification (4.0 ng/mL) using liquid chromatography tandem mass spectrometry [5,6]. Moreover, we demonstrated that 170/178 (95.5 %) breast-fed infants and 54/200 (27.0 %) mixed-fed infants showed serum 25(OH)D levels below 12 ng/mL at one month of age [7].

To prevent VD insufficiency in infancy, several guidelines for daily VD supplementation have been published worldwide [8]. The American

* Corresponding author. Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan.
E-mail address: kaorihara@keio.jp (K. Hara-Isono).

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Academy of Pediatrics (AAP) and the global consensus group representing eleven international organizations stated that 400 IU/day of VD is recommended for all infants from birth to 12 months of age [1,9]. Despite the presence of these guidelines, actual VD intakes in infants have not reached recommended levels in many countries due to poor adherence [10]; for example, only 27.1 % of infants met VD intake guidelines published by the AAP in the United States [11]. Weekly supplementation of VD is expected to reduce parental burden and improve adherence, compared to the daily supplementation. However, the studies which adopted the weekly regimen of VD supplementation to infants are very limited, and its efficacy and safety remain to be elucidated. Regarding Japan, no VD supplementation guidelines exist and nationwide prophylaxis for VD insufficiency has not been implemented. In addition, VD is not added to infant formula as a standard fortification. According to the dietary reference intakes for Japanese proposed by the Ministry of Health, Labour and Welfare (MHLW) in 2024 [12], adequate intake of VD was still set at 200 IU/day for infants less than one year of age, though internationally recommended intake of VD for infants is set at 400 IU/day. The MHLW determined the recommended dose of VD based on a previous report in which 200 IU/day of VD effectively prevented rickets in breast-fed infants over a six-month period [13], and on the low adherence rates to the 400 IU/day recommendation [14]. Moreover, there was apparently no study which examined the safe and adequate dose of VD supplementation for Japanese infants.

Vitamin K (VK) is essential for the synthesis of coagulation factors II, VII, IX, and X. Infants have a risk of VK deficiency-related bleeding (VKDB) due to poor placental transfer, low VK content in breast milk, and poor intestinal absorption [15]. In 2021, several societies, including the Japan Pediatric Society, Japanese Society of Obstetrics and Gynecology, and Japanese Society of Pediatric Surgeons, established a joint recommendation for prophylaxis of VKDB and strongly recommended the "three-month method" in which VK2 syrup (Kaytwo syrup®, Eisai Co. Ltd., Tokyo, Japan) containing 2 mg of menaquinone-4, is administered weekly for a total of 13 doses until three months of age [16]. At present, the "three-month method" has spread widely throughout Japan and many infants received VK supplementation weekly. In addition, several studies reported a synergic effect between VD and VK [17,18].

We hypothesized that the combined weekly supplementation of VD and VK could prevent VD insufficiency with a high adherence rate. The aim of this study is to clarify whether weekly VD supplementation during the early neonatal period prevents VD insufficiency at one month of age.

2. Method

2.1. Study population and data collection

This retrospective cohort study was conducted in Saitama City Hospital (35.9° North) and Keio University Hospital (35.7° North) located around Tokyo. Almost all infants in both hospitals received VK weekly with a high adherence rate. In both hospitals, infants for whom informed consent was obtained were administered cholecalciferol from January 2022 to January 2023. We obtained informed consent for oral VD supplementation from four days of age, coinciding with the initiation of oral VK, through three months of age, from cases satisfying the following criteria: born at $\geq$ 36 weeks of gestation, Apgar score at 5 min $\geq$ 7, and no evidence of calcium metabolism disorders in either mother or infant. Cholecalciferol was administered weekly (1000 IU/dose) or daily (240 IU/dose), depending on the parents' requests. We enrolled infants born from July 2017 to January 2023 who required blood tests at their one-month regular checkup. The indications for the blood tests were mainly suspected jaundice, along with follow-up testing for newborn screening (with all results confirmed as normal), and requests for blood type confirmation. The serum 25(OH)D levels were assessed using remaining specimens after blood tests. In the weekly group, serum 25(OH)D levels were also assessed at three months if infants required blood tests for any reason at the three-month regular checkup. Infants were excluded from the study if they were twins, born at $<$ 36 weeks of gestation, had low Apgar scores (Apgar score at 5 min $\leq$ 6), showed abnormalities of calcium metabolism, or were born to a mother treated with medication that affects calcium metabolism in the perinatal period (such as magnesium sulfate). Additionally, among VD supplemented infants, we excluded infants with poor adherence, namely, missing more than one and four doses in the weekly and daily group, respectively. Moreover, in the weekly group, we excluded infants who underwent the one-month checkup after only three doses of VD had been administered. Because the half-life of 25(OH)D is approximately two to three weeks, we considered serum levels to be stabilized after at least four doses of weekly supplementation. Demographic, health information, and laboratory data, including weight, height, body mass index (BMI), weight gain at one month, amount of formula intake, and mothers' complications in pregnancy, were collected from electronic medical records.

2.2. VD supplementation

VD was administered using liquid cholecalciferol, BabyD 200® or BabyD 100® (Jintan, Co. Ltd., Japan), the only VD supplements in Japan which can be administered from infancy. BabyD 200® and BabyD 100® contain 200 IU and 80 IU of cholecalciferol per drop, respectively. In the weekly group, infants received one 1000 IU dose (5 drops) weekly, together with the VK syrup, starting at four days of age and continuing to three months of age when VK supplementation is completed. In the daily group, infants received one 240 IU dose (3 drops) every day, starting at four days of age. We determined the dose of VD supplementation at 1000 IU/week because most guidelines defined 1000 IU/day as a tolerable upper limit for infants younger than one month [8]. In addition, because a previous Indian study administered 1400 IU/week of VD for term low birth weight infants without any complications [19,20], we considered that the dose of 1000 IU/week would be definitely safe for healthy term infants. We determined the dose of VD supplementation at 240 IU/day based on the dietary intakes for Japanese (200 IU/day) and recommended doses from the product information of BabyD 100® (160–240 IU/day). Parents administered VD to their infants at home and recorded the number of administrations by themselves.

2.3. Serum 25(OH)D measurement

We evaluated serum 25(OH)D levels using remaining specimens at one and three months for infants who required blood tests. The total serum circulating 25(OH)D level was assessed by chemiluminescence immunoassay (CLIA) using the Liaison® 25 OH Vitamin D Total Assay with Precision and a Liaison® XL Analyzer (DiaSorin Inc., MN, USA). If the serum 25(OH)D level was below the lower limit of quantification, 4.0 ng/mL, the value was converted to 2.0 ng/mL. We defined VD insufficiency as serum 25(OH)D $<$ 20 ng/mL and VD excess as serum 25(OH)D $>$ 100 ng/mL, based on the global consensus recommendations for nutritional rickets [1].

2.4. Statistical methods

Fisher's exact test was used for the comparison of adherence rates between weekly and daily groups. We performed Kruskal-Wallis tests with Dunn's post-hoc tests for the comparison of serum 25(OH)D levels among the three groups. Univariable and multivariable logistic regression analyses were performed to assess the effect of VD supplementation for prevention of VD insufficiency. In multivariable logistic regression analysis, we set the objective variable as the presence or absence of VD insufficiency at one month of age, the explanatory variable as the VD dosing method (control [reference], weekly, daily), and the confounding factors as the amount of formula intake and BMI. The amount of formula intake was converted to semiquantitative variables using a scale of 0 (none) to 10 (more than 900 mL/day). BMI was included in the

confounding factors, based on previous reports [21,22]. The Wilcoxon signed rank test was used for the comparison of serum 25(OH)D levels between one and three months of age in the weekly group. We performed two-sided tests and a P value < 0.05 was considered statistically significant. All the analyses were conducted using R version 4.2.3 for Windows.

2.5. Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Research Ethics Committee of the Saitama City Hospital (committee's reference number: B0460) and Keio University Hospital (committee's reference number: 2023–6021).

3. Results

3.1. Description of the study population

The flowchart of patient selection is shown in Fig. 1. Of 6609 infants discharged from the well-baby nursery, 624 infants, including 377 previously reported infants [7], were eligible for analysis. VD supplementation was administered to 210 infants after obtaining informed consent between 2022 and 2023, during which 1060 infants were discharged from the well-baby nursery. Based on the VD dosing regimens, we classified infants into the control group (not supplemented), weekly group, and daily group. We excluded one infant in the weekly group and 41 in the daily group due to poor adherence. The adherence rate for the weekly group (82/83 [98.8 %]) was significantly higher than that for the daily group (86/127 [67.7 %]) ($P < 0.001$). In the weekly group, 27 infants were excluded from the analysis because their one-month checkup was conducted after only three doses of VD had been administered. However, they had received the required number of doses up to the time of the checkup. Finally, 414, 55, and 86 infants were classified into the control, weekly, and daily groups, respectively. Of 55 infants in the weekly group, 43 infants had their serum 25(OH)D levels measured at three months. All infants in the three groups received weekly VK supplementation until three months of age. All infants in the control and daily groups were born in Saitama City Hospital and infants in the weekly group were born in Keio University Hospital or Saitama City Hospital. The baseline clinical characteristics and laboratory data of the infants in each group are shown in Table 1. Median weight, length, and BMI values at one month of age were within the 25th to 75th percentiles, according to the sex-matched Japanese reference data (http://jspe.umin.jp/medical/taikaku.html), except for the median length of female subjects in the control group (Supplementary Table 1). Weight gain at

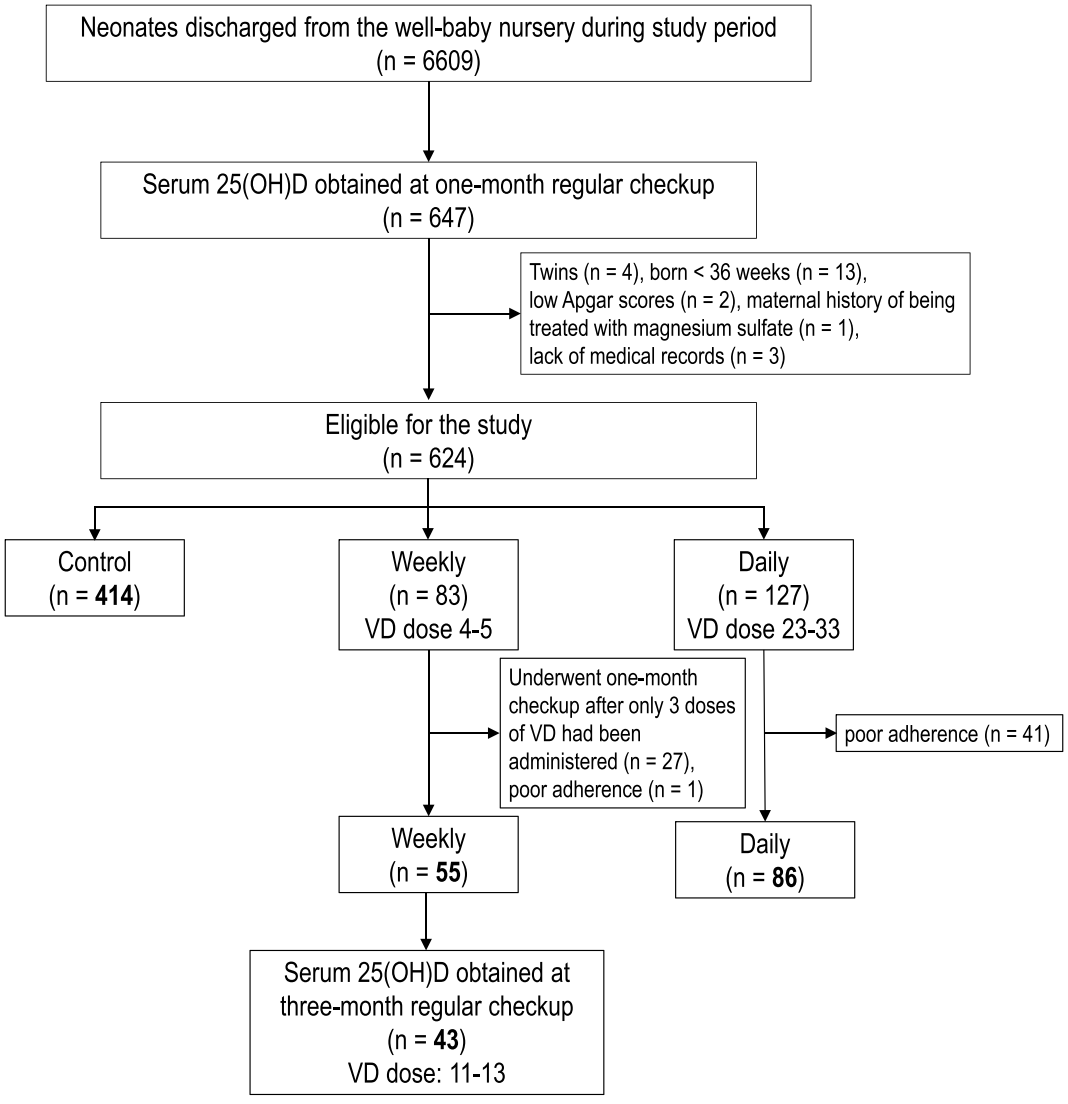

Fig. 1. Flow chart describing patient selection and classification.

Table 1
Baseline characteristics and laboratory data of the study subjects.

	Control (n = 414)		Weekly (n = 55)		Daily (n = 86)	
Clinical characteristics						
<Mothers>	Median	IQR	Median	IQR	Median	IQR
Age, years	33	29, 37	35	32, 38	33	30, 37
Parity	1	0, 1	1	0, 1	1	0, 1
Vaginal/Cesarean	256/158		31/24		52/34	
<Infants>	Median	IQR	Median	IQR	Median	IQR
Male/Female	248/166		21/34		42/44	
Gestational age, weeks	38 2/7	37 4/7, 39 2/7	38 4/7	38 1/7, 40 0/7	38 5/7	38 0/7, 39 5/7
Birth weight, g	2910	2716, 3139	3060	2746, 3268	2993	2745, 3184
Birth length, cm	48.3	47.2, 49.5	48.5	47.5, 49.4	48.8	47.5, 50.0
Age at the examination, days	31	30, 32	34	34, 35	32	31, 33
Weight at the examination, g	4008	3695, 4312	4288	3947, 4671	4169	3849, 4380
Length at the examination, cm	52.2	51.0, 53.5	53.5	52.0, 54.0	53.1	51.8, 54.0
BMI at the examination, kg/m^2	14.7	13.8, 15.5	15.0	14.3, 16.3	14.7	14.0, 15.5
Weight gain, g/day	35.5	29.0, 41.9	37.3	30.9, 42.7	36.3	28.9, 41.3
Amount of formula intake, mL/day	43	0, 200	320	150, 638	105	0, 486
Exclusive breastfeeding, n (%)	176 (42.5)		6 (10.9)		29 (33.7)	
Doses of VD administration	none	none	5	[4, 5]	28	[23, 33]
Laboratory data at one month of age	Median	IQR	Median	IQR	Median	IQR
Serum 25(OH)D, ng/mL	9.7	6.1, 15.8	22.2	20.9, 27.0	23.0	19.6, 27.0
Ca, mg/dL	10.3	10.1, 10.5	10.3	10.1, 10.6	10.3	10.1, 10.5
Phosphate, mg/dL	6.5	6.2, 6.7	6.5	6.2, 6.7	6.5	6.1, 6.7

Abbreviations: IQR, interquartile range; BMI, body mass index; VD, vitamin D.
[] shows the range.

one month of age was adequate in all infants. The formula intake was high in the weekly group and low in the control and daily groups, reflecting the lower rate of exclusive breastfeeding in the weekly group compared to the higher rates observed in the daily and control groups. The median doses of VD administration were five (range: four to five) in the weekly group and 28 (range: 23 to 33) in the daily group. No cases of hypocalcemia in the control group and hypercalcemia in the weekly or daily groups were observed.

3.2. Comparison of the VD status among the three groups

The distributions of serum 25(OH)D levels in one-month old infants in each group are shown in Fig. 2. The serum 25(OH)D levels in the weekly and daily groups were higher than those in the control group (median [interquartile range (IQR)] (ng/mL): control 9.7 [6.1, 15.8], weekly 22.2 [20.9, 27.0], daily 23.0 [19.6, 27.0]; control vs weekly, $P < 0.001$; control vs daily, $P < 0.001$), whereas there were no significant differences in the serum 25(OH)D levels between the daily and weekly groups. The frequencies of VD insufficiency were 370/414 (89.4 %), 11/55 (20.0 %), and 22/86 (25.6 %) in the control, weekly, and daily groups, respectively. No infant showed VD excess in the weekly or daily groups.

We performed multivariable logistic regression analysis adjusting for the amount of formula intake and BMI. Table 2 shows non-adjusted and adjusted odds ratios (ORs) and 95 % confidence intervals (95 %CI) for VD insufficiency in the weekly and daily groups, compared to the control group. Both weekly and daily supplementation regimens of VD were associated with a reduced risk of VD insufficiency (adjusted ORs to the control group: weekly 0.038 [95 %CI: 0.017, 0.085; $P < 0.001$]; daily 0.036 [95 %CI: 0.019, 0.067; $P < 0.001$]).

3.3. VD status in the weekly group at three months of age

Fig. 3 shows the trend of serum 25(OH)D levels in the weekly group from one to three months of age. Because the evaluation at three months of age was conducted only in the weekly group, we assessed the effect at three months as a supplementary analysis limited to this group. All infants from whom we could obtain data at one and three months of age received VD once a week together with VK for a total of 11–13 doses as planned. Serum 25(OH)D level at three months of age was significantly increased compared to that at one month of age (median [IQR]: 28.8 [26.3, 46.9] vs 21.9 [20.9, 44.9]; $P < 0.001$]. At three months of age, five out of 43 (11.6 %) infants had VD insufficiency. No infant showed hypercalcemia or VD excess.

4. Discussion

To our knowledge, this may be the first study to evaluate the effect of weekly VD supplementation for preventing VD insufficiency in healthy Japanese infants. We demonstrated that combined weekly supplementation of VD and VK from early infancy can prevent VD insufficiency at one month of age without causing VD excess.

Comparison of VD status among infants in the control, weekly, and daily groups revealed several notable findings. First, serum 25(OH)D levels in the weekly and daily groups were significantly higher than those of the control group. At Saitama City Hospital, approximately 50 % of infants were breast-fed, compared to about 10 % at Keio University Hospital. As a result, in the control and daily groups, which included only infants from Saitama City Hospital, formula intake was lower than that of the weekly group. Therefore, we performed multivariable logistic regression analysis to more precisely evaluate the contribution of VD supplementation to preventing VD insufficiency. Consequently, weekly and daily supplementation of VD reduced the risk of VD insufficiency without VD excess and hypercalcemia even after adjusting for the amount of formula intake and BMI. These results show that efficacy and safety of weekly VD supplementation for preventing VD insufficiency are equivalent to those of daily supplementation. We previously confirmed that most Japanese parents did not take their infants outside until one month of age and seasonal variation of serum 25(OH)D started to emerge at two months of age [7]. Moreover, the study period included the COVID-19 pandemic and people avoided going out in accordance with the declaration of a state of emergency. Therefore, the frequency of outdoor activities and seasonal variation of VD were not included in the confounding factors for the logistic regression analysis. Second, in the weekly group, about one third of infants still showed VD insufficiency, suggesting that the dose of 1000 IU/week is not adequate to completely prevent VD insufficiency at one month of age. We determined the dose of VD supplementation at 1000 IU/week because most guidelines defined 1000 IU/day as a tolerable upper limit that poses no adverse events for infants younger than one month [8]. In addition, because a previous

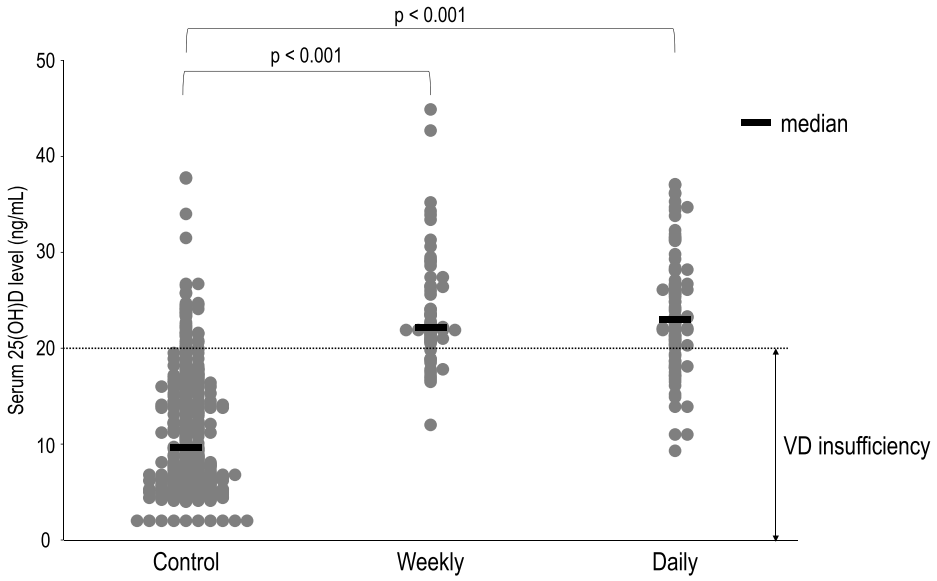

Fig. 2. The distribution of serum 25(OH)D levels in one-month-old infants in each group. Black bars indicate the median serum 25(OH)D levels in each group. VD, vitamin D.

Table 2
Odds ratios of vitamin D insufficiency and deficiency in weekly and daily supplementation groups.

	Non-adjusted			Adjusted[a]		
	ORs	95 %CI	*P*	ORs	95 %CI	*P*
<Vitamin D insufficiency>						
Control	1.0 (reference)			1.0 (reference)		
Weekly	0.030	0.014, 0.062	<0.001	0.038	0.017, 0.085	<0.001
Daily	0.041	0.023, 0.073	<0.001	0.036	0.019, 0.067	<0.001

Abbreviations: ORs, odds ratios; CI, confidence intervals.
[a] Adjusted for the amount of formula intake and body mass index.

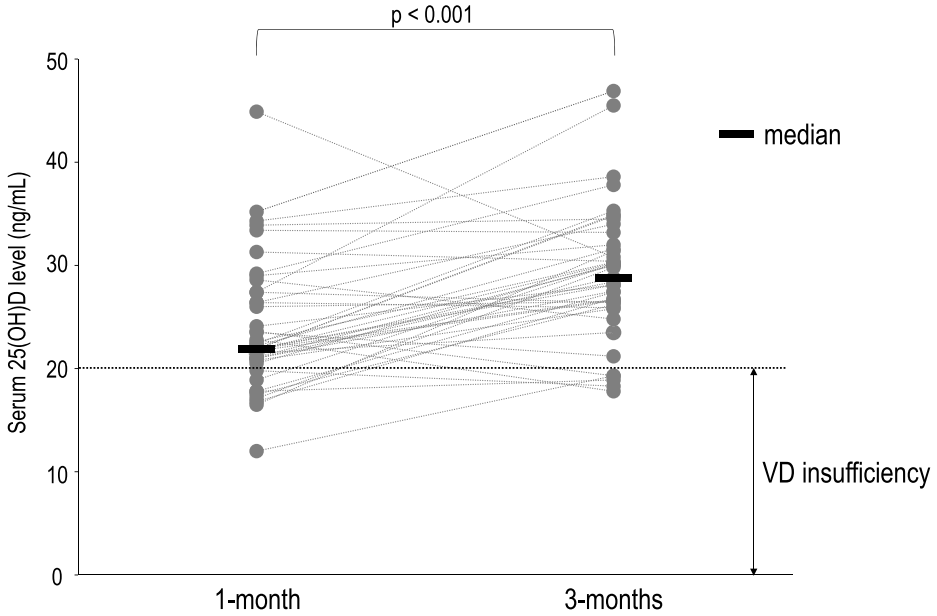

Fig. 3. Distribution of serum 25(OH)D levels in the weekly group at one month and three months of age. Black bars indicate the median serum 25(OH)D levels at one and three months. VD, vitamin D.

Indian study administered 1400 IU/week of VD for term low birth weight infants without any complications [19,20], we considered that the dose of 1000 IU/week would be definitely safe for healthy term infants. From an ethical standpoint, we were forced to set the dose of VD supplementation at lower than the internationally recommended intake, because the recommended doses from product information and dietary

intakes for Japanese are set at 200 IU/day. Regarding iron supplementation, a previous study reported that alternate-day administration results in significantly higher fractional and total iron absorption compared to daily dosing [23]. Although no such studies have been conducted for VD, if weekly dosing improves absorption efficiency, a dose of 1000 IU/week may potentially achieve effects comparable to those of a 1400 IU/week regimen. Further study is required to investigate adequate doses of VD supplementation to prevent VD insufficiency at one month of age.

Several matters should be pointed out regarding VD status in the weekly group at three months of age. First, the serum 25(OH)D level was significantly increased at three months compared to one month, and the frequency of VD insufficiency was decreased from 34.5 % at one month to 11.6 % at three months. Moreover, no infant showed hypercalcemia or VD excess. These results suggest that the weekly VD supplementation has the possibility to reduce the risk of VD insufficiency at three months of age without complications. Because we could not compare the trends in serum 25(OH)D levels between one and three months of the weekly group to that of the control and daily groups, due to the lack of remaining specimens, further studies, including randomized control trials, are required to evaluate the efficacy and safety of weekly VD supplementation until three months of age. Second, as well as the results at one month of age, five infants (11.6 %) had VD insufficiency. Of these five infants, three infants were exclusively breast-fed, and the remaining two infants were mixed-fed consuming less than 120 mL/day of formula. In addition, four infants received 11 doses and one received 12 doses of VD administration. From these results, we suggest that doses higher than 1000 IU/week or more than 12 doses of VD administration are required to completely prevent VD insufficiency, particularly for mainly breast-fed infants.

There are several advantages of administering VD together with VK. First, in the situation where VK supplementation guidelines are properly followed (as in our hospitals), combined supplementation of VD and VK can prevent parents from forgetting to give VD to their children and maintain higher adherence rates than daily administration of VD alone. In fact, the adherence rate in the weekly group was significantly higher than that in the daily group, and all infants from whom we could obtain a blood sample at one and three months of age received VD weekly without missing administration. Second, a synergic effect between VD and VK has been reported [17,18]. The active form of VD, $1,25(OH)_2D$, promotes the reductive recycle of VK, whereas steroid and xenobiotic receptors are activated by VK2, and derange $1,25(OH)_2D$ metabolism through cross interaction with the VD receptor. Moreover, both VD and VK increase the synthesis of functional osteocalcin, which is necessary for bone mineralization. In fact, the combined effect of VD and VK supplementation on skeletal integrity was reported in the general population [24], in particular, postmenopausal women with osteoporosis [25]. Therefore, it is possible that supplementation of VD together with VK enhances the effect of VD in infants.

Our study has some limitations. First, because this was a retrospective cohort study, we could not match the background of each group, and the recruitment periods for the control group and the weekly and daily groups were inconsistent. In particular, the amount of formula intake was high in the weekly group and low in the control group. However, the effect of the weekly VD supplementation for the reduction of VD insufficiency was confirmed by multivariable logistic regression analysis even after adjusting for the amount of formula intake. Moreover, the weekly group included infants from both Keio University Hospital and Saitama City Hospital, whereas the daily group consisted only of infants from Saitama City Hospital. Because Keio University Hospital is located in a central urban area, parents tend to have a higher socioeconomic status. This fact may have led to the higher adherence rate in the weekly group. Second, this was a double-center study conducted around Tokyo using remaining specimens after blood tests mainly for suspected jaundice, our results do not necessarily apply to all healthy Japanese infants. According to the manufacturer's protocol for the Liaison® 25 OH Vitamin D Total Assay, free bilirubin and conjugated bilirubin do not affect the measured values if they are below 20 mg/dL and 30 mg/dL, respectively. Because none of our subjects had total bilirubin levels greater than 20 mg/dL, we believe that bilirubin levels had little impact on serum 25(OH)D levels. Third, we were unable to evaluate baseline serum 25(OH)D levels of infants at birth and maternal serum 25(OH)D levels; we were also unable to obtain information about maternal VD supplement use. Prospective studies are needed to examine the effects of VD supplementation, including the evaluation of cord blood and maternal 25(OH)D levels. Lastly, we could not assess bone density or radiographic findings due to the difficulty in performing these tests for infants. Further studies are required to evaluate the effects of VD supplementation for clinical improvement.

In summary, we demonstrated that weekly supplementation of VD together with VK during early infancy can prevent VD insufficiency at one month of age without causing VD excess. Furthermore, weekly VD administration until three months of age reduced the frequency of VD insufficiency compared to one month of age. The results of this study may provide important evidence for the development of nationwide prophylaxis policy for VD insufficiency in regions lacking specific guidelines.

Study data availability

The data supporting the findings of this study are available on reasonable request from the corresponding author.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2025.08.002.

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