

Original Article

Longitudinal study on the effectiveness of vitamin D supplements in exclusively breast-fed infants

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Abstract. Vitamin D deficiency is a common health problem in infancy. Breast-fed infants are at a higher risk of rickets than formula-fed infants. We observed fluctuations in vitamin D levels in infancy (phase I, 2009–2010) and considered the benefits of vitamin D supplementation specifically in exclusively breast-fed infants in Japan (phase II, 2015). Infants born at our hospital were enrolled in this study. In phase I, we measured 25-hydroxyvitamin D [25(OH)D] levels at 1- to 6-mo intervals from birth. In phase II, we measured 25(OH)D levels before and after supplementation. Vitamin D deficiency was defined as 25(OH)D levels of < 20 ng/mL. All 38 infants in phase I were deficient at birth, and none of the exclusively breast-fed infants achieved 25(OH)D sufficiency by 5 mo of age. Formula-fed infants achieved 25(OH)D sufficiency earlier. The majority of the 71 infants in phase II were deficient at birth. We recommended an oral vitamin D supplement at a daily dose of 4.0 µg for the 15 exclusively breast-fed infants, starting at 1 mo of age; 14 (93.3%) of them achieved 25(OH)D sufficiency by 5 months of age. Exclusively breast-fed infants are at a high risk of vitamin D deficiency; adequate supplementation is an effective preventative strategy.

Key words: vitamin D, vitamin D supplement, 25-hydroxyvitamin D, breast feeding, rickets

Introduction

Vitamin D is an essential nutrient for bone development; therefore, infants require adequate vitamin D levels. Vitamin D deficiency in infancy

reduces skeletal integrity and increases the risk of developing rickets and hypocalcemic seizures (1). To address these risks, Japan's Ministry of Health, Labour and Welfare has recommended that all Japanese infants should have a daily vitamin D intake of 5.0 µg (2). However, breast milk does not contain sufficient vitamin D and is an insufficient source from which infants can obtain the recommended intake (3). Vitamin D supplementation of all breast-fed infants beginning within a few days of birth has been recommended by the American Academy of Pediatrics (AAP); however, there has been no report on the effectiveness of vitamin D

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supplementation in Japan.

In this study, we observed the fluctuations of vitamin D levels in early infancy in Japanese children and considered the benefits of vitamin D supplementation, particularly in exclusively breast-fed infants.

Subjects and Methods

Study design

This biphasic study was conducted at the Toyama Prefectural Central Hospital (TPCH; 36.7°N, 137.2°E). Phase I was an observational study of factors influencing vitamin D levels at birth and fluctuations of vitamin D levels in early infancy. Phase II was a trial of the effectiveness of vitamin D supplementation in exclusively breast-fed infants.

Phase I

The primary objectives of Phase I were to assess influences on vitamin D levels at birth and the fluctuations in vitamin D levels in early infancy.

All infants born at TPCH between December 2009 and February 2010 (defined as the winter season) or between July and October 2010 (defined as the summer season) were eligible for inclusion in the study. Infants were excluded if they were not in good general health at the time of enrollment or their mother refused to consent for participation in the study.

In all infants, the serum concentration of the circulating precursor hormone 25-hydroxyvitamin D [25(OH)D; calcidiol] was assessed to check for vitamin D sufficiency; this was measured using radioimmunoassay techniques (25-Hydroxyvitamin D ¹²⁵I Radioimmunoassay Kit, SRL, Tokyo, Japan). According to the guideline of the Japanese Society for Pediatric Endocrinology, vitamin D deficiency is defined a priori as total 25(OH)D < 20 ng/mL (< 50 nmol/L). Intact PTH was measured using an electrochemiluminescence immunoassay technique (ECLusys reagent

PTH, SRL, Tokyo, Japan); the reference range was 10–65 pg/mL. Other serum levels were measured by the clinical chemistry laboratory using standard methodology and laboratory normative data. These levels were evaluated using umbilical cord blood samples obtained at birth and venous samples drawn at 1- to 6-mo intervals until serum 25(OH)D had reached sufficiency.

We used demographic and health information data collected from clinical records to analyze influences on 25(OH)D levels at birth. At each visit, we interviewed the mothers regarding nutrition and divided them into two groups: exclusive breastfeeding and mixed formula feeding. If a mother used even a small amount of formula, we grouped her in the mixed formula feeding group.

Each mother provided signed informed consent to participate, and the protocol was approved by the TPCH Ethics Committee (Approval number 4327).

Phase II

The primary objective of Phase II was to assess changes in 25(OH)D levels before and after vitamin D supplementation. The secondary objective was to assess serum concentration of 25(OH)D after vitamin D supplementation.

All singleton infants born at TPCH between June and July 2015 were eligible for inclusion in the study. Infants were excluded if they were of less than 37 wk of gestational age, not singletons, not in good general health at the time of enrollment, or their mother refused to provide consent for participation in the study.

Each infant was evaluated for vitamin D deficiency at birth by assessing the 25(OH)D level in an umbilical cord blood sample. We informed the mother of the results at the 1-mo health examination and guided her with regard to methods for vitamin D supplementation if the infant was vitamin D deficient; we suggested BabyD® (Morishita Jintan Co., Ltd.) as an oral vitamin D supplement at a recommended

daily dose of 4.0 µg. We excluded the mother and infant from the trial if the infant's 25(OH)D at birth was sufficient, the mother did not plan to continue exclusively breast feed, or she wanted to stop participating in the study. We assessed changes in 25(OH)D levels after vitamin D supplementation at 5 mo of age, i.e., before beginning baby food.

We measured the initial 25(OH)D level. We also measured the serum concentration of 25(OH)D at 5 mo of age, just as with Phase I. At the same time, we interviewed the mothers regarding nutrition and compliance with vitamin D supplementation. The methods for grouping the mothers and collecting information were the same as those employed in Phase I.

Each mother provided signed informed consent to participate, and the protocol was approved by the TPCHE Ethics Committee (Approval number 5002).

Statistical analysis

Values are expressed as mean \pm SD (reference range). Subject characteristics were compared using Welch's paired *t* test and the obtained data were compared using the Mann-Whitney *U* test, with significance set a priori at $P < 0.05$. All statistical analyses were performed and plots of data were drawn using BellCurve for Excel 2003 (Social Survey Research Information, Tokyo, Japan).

Results

Phase I

Of the 485 eligible infants (268 born in the summer and 217 in the winter), 38 participated in the study. The mean age of the mothers at birth was 32.6 ± 4.9 yr (23–41 yr), infant gestation was 37.0 ± 3.3 wk (28.1–41.3 wk), and infant birth weight was $2,508 \pm 542$ g (1,134–3,545 g). All were Asian, 24 were boys, 18 were born in the summer, and six were twins. The mean umbilical cord blood serum 25(OH)D level was 9.9 ± 3.7 ng/mL (4.0–17.3 ng/mL). The analysis

of infants' characteristics was stopped, as they were all found to be vitamin D deficient.

The mothers of seven infants withdrew consent for participation after registration; we measured 25(OH)D in infancy for the remaining 31 infants and grouped them according to nutrition (Fig. 1), with 20 exclusively breast-fed and 11 mixed formula-fed. Of the 11 mixed formula-fed infants, seven (78%) achieved 25(OH)D sufficiency by 5 mo of age, and two others dropped out of the study. Of the exclusively breast-fed infants, none achieved 25(OH)D sufficiency by 5 mo old, and five dropped out. Three breast-fed infants were vitamin D deficient, with hyperparathyroidism and extreme hyperphosphatasemia (> 1200 U/L). One underwent wrist joint X-ray examination but was found not to have rickets.

Phase II

Of the 166 singleton infants born in the study period, 71 were included (Fig. 2). The mean age of the mothers at birth was 31.8 ± 4.6 yr (24–42 yr), infant gestation was 39.1 ± 1.1 wk (37.1–41.1 wk), and infant birth weight was $3,044 \pm 326$ g (2,160–3,732 g). All were Asian and 28 were boys. The mean umbilical cord blood 25(OH)D level was 11.2 ± 4.8 ng/mL (6.0–40.0 ng/mL); 69 (97%) were vitamin D deficient.

Of the 71 infants, the consent to continue this study at 1 mo was obtained for 55 infants (Fig. 2), 35 of whom visited again when they were 5 mo old; 28 of them were exclusively breast-fed and seven were mixed formula-fed. Nine of the exclusively breast-fed infants were not administered vitamin D supplementation. Of the other 19 exclusively breast-fed infants, 15 were administered 4.0 µg vitamin D supplementation per day; the remaining four were excluded from analysis as they only received 2.0 µg vitamin D per day.

The mean 25(OH)D level for the nine exclusively breast-fed infants without vitamin D supplementation was 16.0 ± 6.5 ng/mL (5.0–27.0 ng/mL), with six (67%) classified as vitamin D deficient; in comparison, the mean 25(OH)D level

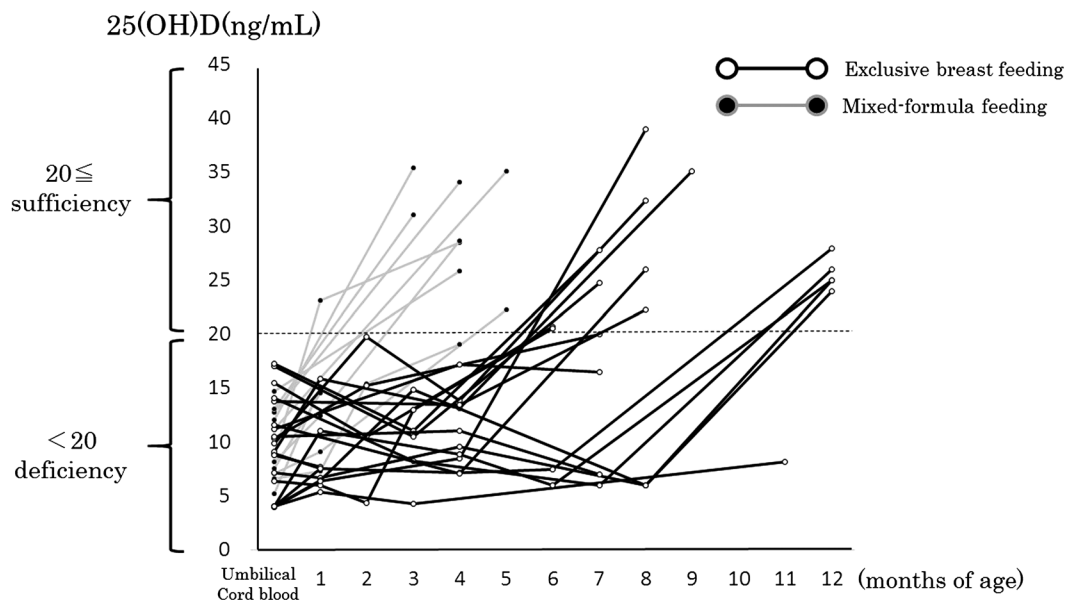


Fig. 1. Fluctuations in 25-hydroxyvitamin D [25(OH)D] levels in infancy. Most mixed formula-fed infants achieved vitamin D sufficiency by 5 mo of age, but exclusively breast-fed infants had prolonged vitamin D deficiency.

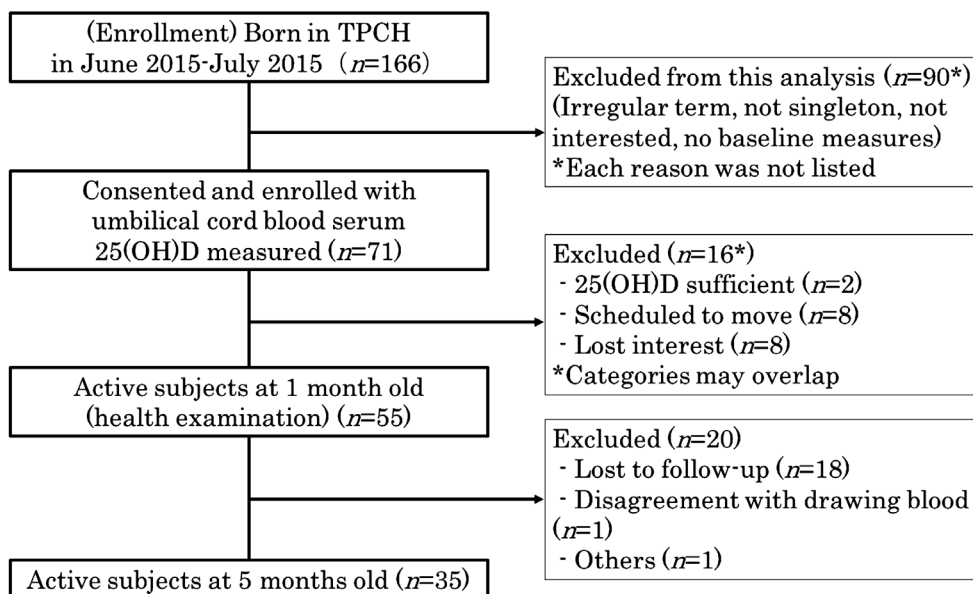


Fig. 2. Participant flow through the study (Phase II). TPCH: Toyama Prefectural Central Hospital.

for the 15 exclusively breast-fed infants receiving 4.0 µg vitamin D per day was 33.1 ± 11.7 ng/mL (8.0–58.0 ng/mL), with only one (6.7%) classified as vitamin D deficient (Fig. 3A). In the supplementation group, 25(OH)D increased by

22.9 ng/mL after the vitamin D supplementation ($P < 0.01$) (Fig. 3A). The mean 25(OH)D level for the mixed formula-fed infants was 44.4 ± 13.2 ng/mL (27.0–63.0 ng/mL). Exclusively breast-fed infants with supplementation had significantly

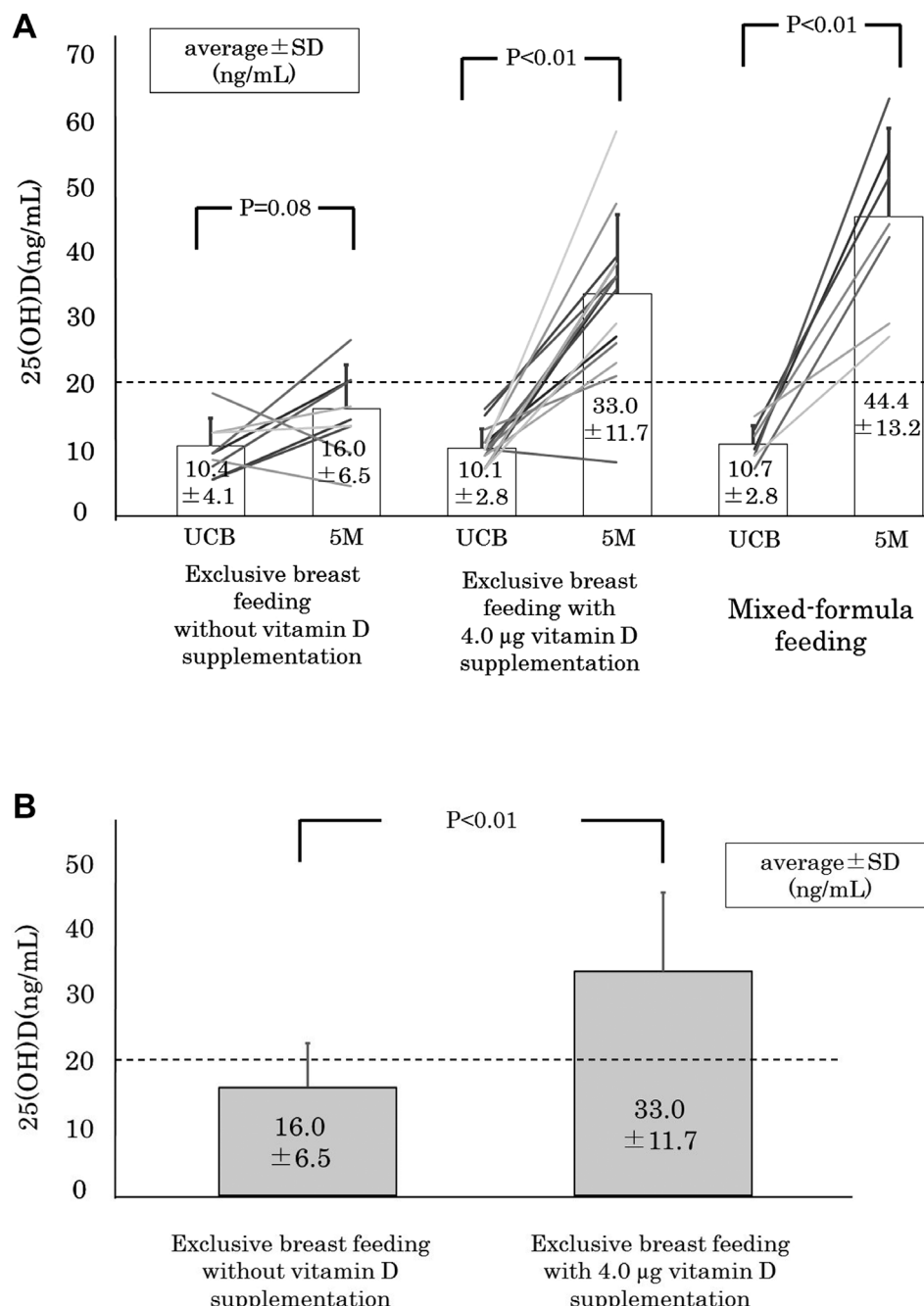


Fig. 3. **A.** Alterations in 25-hydroxyvitamin D [25(OH)D] levels classified as per nutrition and supplementation. Most exclusively breast-fed infants who were vitamin D deficient at birth and subsequently received adequate vitamin D supplementation, achieved vitamin D sufficiency by 5 mo of age. UCB: umbilical cord blood, 5M: at 5 mo of age. **B.** Comparison of the mean 25-hydroxyvitamin D [25(OH)D] level between exclusively breast-fed infants with and without supplementation. Exclusively breast-fed infants with 4.0 μ g vitamin D supplementation achieved significantly higher 25(OH)D levels at 5 mo of age than those without supplementation.

Table 1 Inspection results at 5 mo of age

	Exclusively breast-fed infants		Mixed-formula-fed infants (n = 7)
	With 4.0 µg vitamin D supplementation (n = 15)	Without vitamin D supplementation (n = 9)	
25(OH)D (ng/mL)	33.1 ± 11.7 (8.0–58.0)	16.0 ± 6.5 (5.0–27.0)	44.4 ± 13.2 (27.0–63.0)
Hemoglobin (g/dL)	12.1 ± 0.6 (10.7–13.3)	12.1 ± 0.8 (10.5–13.4)	12.1 ± 0.8 (11.2–13.2)
Alkaline phosphatase (U/L)	934 ± 280 (569–1481)	813 ± 159 (512–1024)	902 ± 319 (459–1280)
Serum calcium (mg/dL)	11.0 ± 0.3 (10.3–11.6)	10.9 ± 0.3 (10.4–11.5)	11.1 ± 0.3 (10.8–11.5)
Serum phosphorus (mg/dL)	5.6 ± 0.2 (5.3–6.1)	5.5 ± 0.3 (5.0–5.8)	5.9 ± 0.4 (5.5–6.4)
Intact PTH (pg/mL)	14.4 ± 2.8 (9.0–20.0)	23.3 ± 11.3 (11.0–47.0)	20.1 ± 6.3 (14.0–29.0)

Average ± SD (reference range).

higher mean 25(OH)D levels at 5 mo of age than those without supplementation (Fig. 3B).

The results of blood test classified according to the nutrition and supplementation groups are summarized in Table 1. None of the infants exhibited hyperparathyroidism, hypocalcemia, hypophosphatemia, or anemia. Of the 35 infants, four (11%) showed extreme hyperphosphatasemia.

Discussion

Vitamin D deficiency, which causes rickets, hypocalcemic seizures, and other hypovitaminosis D-related disorders, is a common problem in infancy (1).

Breast milk provides an excellent diet for infants. However, it contains inadequate vitamin D levels for nursing infants to maintain the recommended minimum circulating levels of 25(OH)D, and exclusively breast-fed infants are at high risk of vitamin D deficiency in early infancy. According to a report of the Japanese Society for Pediatric Endocrinology, 29 of 33 infants observed with vitamin D deficiency from 2003 to 2007 were exclusively breast-fed (4). In contrast, formula contains sufficient vitamin D.

Vitamin D is synthesized in the skin upon solar exposure; thus, living in a high-latitude area and winter result in a high risk of vitamin D deficiency (5). In this study, the majority of the infants born in the summer were vitamin

D deficient. It is difficult to obtain sufficient vitamin D only through solar exposure; therefore, dietary intake of vitamin D is desirable. Of the 109 infants in both phases of the present study, umbilical cord blood serum 25(OH)D was deficient in 107 (98%). Shiraishi *et al.* indicated that most healthy and pregnant Japanese women in the second trimester were vitamin D deficient [25(OH)D < 20 ng/mL] regardless of the season because of the lack of sunlight exposure, especially due to skin protection in the summer, and a low amount of dietary vitamin D intake (6). Therefore, it was considered that low serum 25(OH)D levels in umbilical cord blood were affected by maternal low serum levels. Figure 1 shows that mixed formula-fed infants achieved 25(OH)D sufficiency by the time they were 5 mo old because formula contains sufficient vitamin D. In contrast, all of the exclusively breast-fed infants were vitamin D deficient at 5 mo of age, with some only achieving 25(OH)D sufficiency at 1 yr. Exclusive breast feeding leads to a high risk of vitamin D deficiency in early infancy because of low vitamin D transfer from mothers and low vitamin D intake from feeding.

To address this risk, the AAP has recommended vitamin D supplementation of all breast-fed infants beginning within a few days after birth, and such supplementation is widely accepted. Japan's Ministry of Health, Labour and Welfare has also recommended that all Japanese infants should have a daily vitamin D intake of

5.0 µg; however, no report on the effectiveness of vitamin D supplementation in Japan has yet been published.

In this study, we introduced BabyD® to mothers as a vitamin D supplement. This is a natural formulation of vitamin D₃, which was released for the first time in Japan in 2014; 2.0 µg of vitamin D per drop can be obtained from the supplement. Although most exclusively breast-fed infants without vitamin D supplementation were vitamin D deficient in infancy, most exclusively breast-fed infants who received vitamin D supplementation achieved sufficient 25(OH)D levels by 5 mo of age (Fig. 3A). As all subjects were living near TPCCH during the study, it is difficult to conceive of any geographical or climatic differences between them. Thus, we consider that vitamin D supplementation was the factor that led to the increase in 25(OH)D levels and that it is possible to avoid severe vitamin D deficiency in Japanese infants through supplementation.

Intact PTH is an index of bone metabolism and is inversely proportional to 25(OH)D levels. In Phase I, we observed some vitamin D deficiency with hyperparathyroidism. Although we conducted X-ray examination in only a limited number of cases, enhanced bone metabolism was suggested, which is indicative of rickets. No infant with vitamin D supplementation exhibited hyperparathyroidism.

This study had several limitations. First, it was not a randomized controlled trial. For mixed-formula-fed infants, the amount of formula ingested could have varied greatly and some mothers provided both formula and vitamin D supplements to infants until 5 mo of age. For Phase II, although the infants underwent an intervention, no control group was established and confounding factors were not removed. Second, the study periods were limited. We studied infants born in the summer and winter seasons in Phase I and only in two months, June and July, in phase II. Ideally, all seasons should be investigated. Third, the study consisted of

a relatively small number of subjects, and the resulting lack of statistical power raises the possibility that the results were due to chance. Finally, our results may not be generalizable to other infants living far from Toyama City in Japan because 25(OH)D synthesis is affected by solar exposure. However, the strength of this study is that it is the first report on the effectiveness of vitamin D supplementation in exclusively breast-fed infants in Japan.

In conclusion, our study showed that exclusively breast-fed infants are at high risk of vitamin D deficiency in infancy and that vitamin D supplementation can reduce this risk. This conclusion needs to be tested in studies with larger sample sizes. Further expansion and replication in other studies with sufficient statistical power are needed to confirm our results.

Conflict of Interest: We have no conflicts of interest to declare.

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References

1. 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: 394–415. [[Medline](#)] [[CrossRef](#)]
2. Hishida A, Sasaki S. Overview of dietary reference intakes for Japanese (2015). Tokyo: Daiichi-Shuppan; 2014. p.170–5 (in Japanese).
3. Wagner CL, Greer FR, American Academy of Pediatrics Section on Breastfeeding American Academy of Pediatrics Committee on Nutrition. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008;122: 1142–52. [[Medline](#)] [[CrossRef](#)]
4. Michigami T. An update in vitamin D. *J Jpn Soc*

- Breastfeed Res 2015;9: 33–41 (in Japanese).
5. Miyauchi M, Hirai C, Nakajima H. The solar exposure time required for vitamin D₃ synthesis in the human body estimated by numerical simulation and observation in Japan. J Nutr Sci Vitaminol (Tokyo) 2013;59: 257–63. [\[Medline\]](#)
 6. Shiraishi M, Haruna M, Matsuzaki M, Murayama R. Demographic and lifestyle factors associated with vitamin D status in pregnant Japanese women. J Nutr Sci Vitaminol (Tokyo) 2014;60: 420–8. [\[Medline\]](#) [\[CrossRef\]](#)