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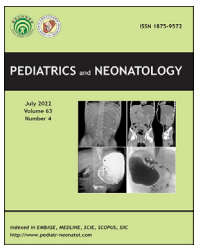

Original Article

The cut-off values of vitamin D deficiency in early infancy

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Key Words
25-hydroxyvitaminD;
broken line regression;
cut-off value;
inflection point;
vitamin D deficiency

Background: Several cut-off points for 25-hydroxyvitamin D (25(OH)D) levels have been proposed to determine vitamin D deficiency or insufficiency. However, the level for 25(OH)D deficiency in early infancy remains unclear. The serum 25(OH)D value at which parathyroid hormone level plateaus, called the "inflection point," is considered the most appropriate criterion for defining an adequate vitamin D status.
Methods: This was a single-center retrospective study involving 305 1-month-old and 252 2-month-old Japanese infants. Nonlinear segmented regression analysis was performed based on the correlation between 25(OH)D and parathyroid hormone levels to determine vitamin D deficiency cut-off points.
Results: Inflection points were 7.90 ng/mL for 1-month-old (95% confidence interval, 6.31–9.49) and 6.74 ng/mL for 2-month-old (95% confidence interval, 5.80–7.68) Japanese infants, which were lower than previously reported. Cut-off values were also lower in the high-body mass index (BMI) group than in the low-BMI group for both 1-month and 2-month-old infants.
Conclusion: These results imply the need for nutritional rickets prevention via policy recommendations in most full-term newborns in Japan. Although validation studies are required, these results can still be used to guide vitamin D insufficiency treatment options in early infancy.

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1. Introduction

The standard method for assessing vitamin D status is to measure the circulating levels of 25-hydroxyvitamin D (25(OH)D), the primary circulating vitamin D metabolite.[1] However, numerous 25(OH)D cut-off thresholds have been reported for vitamin D insufficiency or deficiency, which remain under debate.[2–4] Until 1998, vitamin D deficiency was defined as a serum 25(OH)D level of less than 10 ng/mL (25 nmol/L).[5] Following this, cut-off values at 12, 15, and 20 ng/mL have been used for vitamin D deficiency based on the relationship between 25(OH)D and the parathyroid hormone (PTH), wherein PTH decreases after a vitamin D load.[6–8] These thresholds were based on data obtained from adults or older children.[9] However, a consensus on the optimal serum 25(OH)D concentration is still lacking, especially in early life. With the lack of early infancy guidelines and the absence of criteria due to insufficient evidence, further studies are needed to establish specific guidelines for optimal serum 25(OH)D levels in infants.

Vitamin D deficiency increases PTH production,[10] increasing bone turnover rate and promoting bone mineral loss. Inverse associations between serum 25(OH)D and PTH concentrations have been established in adults and older children.[11] Evidence suggests that PTH is more responsive to 25(OH)D in children than in the elderly.[12] Therefore, measuring 25(OH)D and PTH levels during critical growth and development periods may be essential. Furthermore, if 25(OH)D levels are within the optimal skeletal health range, then the PTH levels are expected to be minimal.[13] This threshold is defined as the 25(OH)D concentration that maximally suppresses PTH and minimizes bone loss. In this context, the association between serum 25(OH)D and PTH was modeled using nonlinear regression analysis.[1,14] This was because most authors attempting to establish vitamin D requirements focused on the 25(OH)D threshold where serum PTH starts to increase or decrease based on vitamin D, assuming that PTH reaches a low plateau as the 25(OH)D level increases.

Specifically, the serum 25(OH)D level at which PTH level plateaus is called the "inflection point," which is widely considered the most appropriate criterion for defining adequate vitamin D.[12,15] However, cut-off point (i.e., the inflection point value) variations may be attributed to the age and body mass index (BMI) of individuals during growth and development. As such, the 25(OH)D cut-off value for vitamin D deficiency in early infancy remains unclear, with limited infant studies. Herein, we investigated the inflection points of 1-month and 2-month-old infants based on the relationship between 25(OH)D and PTH to determine the cut-off points for vitamin D deficiency in infants.

2. Methods

2.1. Data collection and group classification of the study population

This retrospective cohort study was conducted between August 2018 and December 2020 in Saitama City Hospital, which lies 35.9° north (geographically, 25 km north) of Tokyo, Japan. All infants who were born at this hospital, whose blood was drawn for any reason at 1 or 2 months of age in the well-baby outpatient clinic, and who had serum 25(OH)D and intact PTH (iPTH) data were enrolled in the study. This was possible since there had been many opportunities to collect blood samples for various reasons, but primarily for jaundice examination.

Infants were excluded from the study if they were born at less than 36 weeks of gestation, had a low Apgar score, were born to a mother who was treated with medications known to affect calcium or magnesium metabolism in the perinatal period (such as magnesium sulfate), or were born to a mother with any clinical conditions known to affect calcium metabolism. Demographics, health information, and laboratory data of the included infants, including perinatal history, weight, height, and nutrition, were collected from the electronic medical records for each age group (i.e., 1-month-old group and 2-month-old group). BMI was calculated as weight in kilograms divided by height in meters squared.

This study was approved by the Research Ethics Committee of Saitama City Hospital.

2.2. Determination of 25(OH)D and iPTH levels

Since 2016, when DiaSorin Inc. stopped manufacturing 25(OH)D ^{125}I-RIA kits and CLIA/ECLIA-based assays were made available on the National Medical Insurance list, immunoassays using CLIA or ECLIA have been widely performed to measure 25(OH)D levels in Japan. In this study, the total serum 25(OH)D level was assessed via chemiluminescence immunoassay (CLIA) using the Liaison® 25 OH Vitamin D Total Assay with Precision and a Liaison® XL Analyzer (DiaSorin Inc., MN, USA). The package insert states that serum bilirubin levels below 20 mg/dL do not affect the results. The intra-assay coefficient of variation of CLIAs ranged from 12.1% at 9.6 ng/mL to 2.1% at 103.7 ng/mL, and the lower limit of quantitation for 25(OH)D was 4.0 ng/mL. On the other hand, iPTH levels were measured via electrochemiluminescence immunoassay (ECLIA, Elecsys PTH, Roche Diagnostics).

2.3. Measurement of serum calcium, magnesium, inorganic phosphorus, and alkaline phosphatase

Serum calcium, magnesium, and inorganic phosphorus levels were measured using the Aqua-auto Kainos Ca, Aqua-auto Kainos Mg-II (Kainos Laboratories, Inc., Tokyo, Japan), and Acuras-auto IP (Shino-Test Corporation, Kanagawa, Japan) test kits, respectively. Until June 2020, serum alkaline phosphatase levels were analyzed using the Quick Auto Neo ALP-JS method, which is considered the standard method by the Japan Society of Clinical Chemistry (JSCC). Thereafter, the Cygnus Auto ALP IF method was performed, as recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Serum ALP (JSCC) values were then converted to ALP (IFCC) values using the following formula: ALP (IFCC) = ALP (JSCC)/2.84.

2.4. Statistical methods

Data are expressed as means ± standard deviations (SD) or medians (interquartile range [IQR]). Means between the groups were compared using Student's *t*-test, whereas Fisher's exact test was performed to compare categorized data.

If the serum 25(OH)D level was <4.0 ng/mL (CLIA lower limit of quantification), then 4.0 ng/mL was converted to 2.0 ng/mL. To obtain the initial 25OHD-iPTH inflection point estimates, the scatterplots were visually inspected, and a locally weighted linear regression (LOESS) was fitted for each group.[6,16] Once the smooth lines obtained from LOESS supported the nonlinear shape, a broken-line methodology was applied to determine the 25(OH)D threshold.[17] All statistical analyses were two-sided, and a p-value of <0.05 denoted statistical significance. All analyses were performed using JMP 11.2.0 for Windows and the SAS software (version 9.4; SAS Institute, Cary, NC, USA).

3. Results

3.1. Study population demographics

During the study period, a total of 557 blood samples from 315 Asian infants (309 Japanese, 2 Nepalese, 1 Bangladeshi, 1 Chinese, 1 Filipino, and 1 Sri Lankan) were available. There were 305 samples from 1-month-old infants, 252 from 2-month-old infants, and 242 infants had data at both ages (Table 1). Among 1-month-old infants, 149 were breastfed, 154 were mixed formula-fed, and 2 were formula-fed. Among 2-month-old infants, 151 were breastfed and 101 were mixed formula-fed. Among the 242 infants with data at both ages, 115 were breastfed throughout. Furthermore, three of the 315 infants took vitamin D-containing supplements between 1 and 2 months of age.

Table 1 summarizes the infants' baseline clinical characteristics and laboratory data for each month. Weight and height data showed that growth was almost average for Japanese infants each month. Median BMI values at birth, 1 month of age, and 2 months of age corresponded to the 50th percentile, 50th percentile, and 50–75th percentile, respectively (Table 2).

3.2. Serum 25(OH)D levels

Serum 25(OH)D levels were 10.6 ± 6.0 and 12.0 ± 7.1 ng/mL in 1-month and 2-month-old infants, respectively. Moreover, 487 of the 557 samples (87.4%) had serum 25(OH)D levels of less than 20 ng/mL (Table 1). Among all subjects, only three cases in the 1-month-old group had serum bilirubin levels above 20 mg/dL, and they were included in the analysis since they did not substantially deviate from 20 mg/dL: 20.1 mg/dL, 20.3 mg/dL, and 22.4 mg/dL, respectively.

3.3. Vitamin D cut-off point with PTH

Fig. 1 illustrates the correlation between serum 25(OH)D and iPTH levels based on age (months). Initial visual inspection of the scatterplots confirmed an inverse relationship between 25(OH)D and iPTH. LOESS smoothed lines[12] also supported a two-phase linear regression for both the 1-month and 2-month-old age groups. The inflection points were 7.90 ng/mL (95% CI, 6.31–9.49) for 1-month-old infants and 6.74 ng/mL (95% CI, 5.80–7.68) for 2-month-old infants (Fig. 1). There was no difference in the 25(OH)D-iPTH correlation between the two. Furthermore, the inflection point for all subjects (315 infants) after elimination of duplication (242 samples) was 7.58 ng/mL (95% CI, 6.46–8.71).

We then classified infants into two subgroups according to the Japanese BMI standards. Specifically, infants were categorized as in the high-BMI group if their BMI was ≥50 percentile and the low-BMI group if their BMI was <50 percentile (Table 3). The inflection point of serum 25(OH)D

Table 1 Subject demographics by age in months.

	1-Month Infants (n = 305)	Missing value	2-Month Infants (n = 252)	Missing value	p-value
Age, days	31.0 ± 2.1	0	63.6 ± 2.7	0	<0.001
Gestational age, weeks	38.5 ± 1.2	0	38.4 ± 1.2	0	0.54
Male gender, *n* (%)	182 (59.5)	0	153 (60.5)	0	0.86
Birth weight, grams	2952 ± 328	0	2939 ± 327	0	0.64
Birth height, cm	48.4 ± 1.8	0	48.3 ± 1.8	0	0.53
Weight, grams	4028 ± 478	0	5303 ± 607	0	<0.001
Height, cm	52.4 ± 1.9	1	56.5 ± 2.0	2	<0.001
Body mass index, kg/m^2	14.66 ± 1.18	1	16.59 ± 1.41	2	<0.001
25(OH)D, ng/mL	10.6 ± 6.0	0	12.0 ± 7.1	0	0.012
<20 ng/mL, *n* (%)	278 (91.1)		209 (82.9)		0.005
Calcium, mg/dL	10.25 ± 0.31	1	10.34 ± 0.33	0	0.001
Phosphate, mg/dL	6.5 ± 0.4	1	6.3 ± 0.5	0	<0.001
Magnesium, mg/dL	2.0 ± 0.1	291	2.2 ± 0.1	246	0.11
Alkaline phosphatase[a], IU/L (U/L)	1154 ± 328 (406 ± 116)	1	1183 ± 349 (416 ± 123)	0	0.39

Data are presented as the mean (±SD) or *n* (%) for categorized data.

[a] Upper column is expressed as IU/L using the standard method by the Japan Society of Clinical Chemistry (JSCC). Lower column corresponds to values (U/L) for the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Table 2 Body mass index (kg/m^2) of study subjects grouped by age and gender. The *italic numbers* indicate Japanese standards.

	Male	Female
at birth	**12.57 (11.84, 13.13**	**12.60 (12.08, 13.21**
(*n* = 315)	(n = 185)	(n = 130)
	12.62 (11.86, 13.40)	*12.57 (11.82, 13.33)*
1-month	**14.73 (14.01, 15.51**	**14.37 (13.76, 15.21**
(*n* = 304)	(n = 181)	(n = 123)
	14.71 (13.84, 15.61)	*14.37 (13.52, 15.23)*
2-month	**16.89 (15.99, 17.55**	**16.14 (15.41, 17.25)**
(*n* = 250)	(n = 152)	(n = 98)
	16.01 (15.07, 16.98)	*15.56 (14.66, 16.49)*

Data are presented as medians (interquartile range).

level for maximal suppression of PTH was lower in the high-BMI group [6.5 ng/mL (95% CI, 5.5—7.5) for 1-month-old infants; 6.9 ng/mL (95% CI, 6.1—7.8) for 2-month-old infants] than in the low-BMI group [10.1 ng/mL (95% CI, 7.0—13.2) for 1-month-old infants; 10.0 ng/mL (95% CI, 6.9—13.1) for 2-month-old infants] in each month (Fig. 2).

4. Discussion

The vitamin D cut-off level for indicating insufficiency in early infants remains undetermined owing to the lack of data. Thus, this study examined the association between serum 25(OH)D and PTH to determine the clinically relevant 25(OH)D levels in early infancy. On assessment, the serum 25(OH)D thresholds for stimulating PTH secretion in 1-month and 2-month-old infants were 7.90 and 6.74 ng/mL, respectively. To the best of our knowledge, this is the first study to determine the threshold serum 25(OH)D level for vitamin D insufficiency using serum PTH in 1-month and 2-month-old infants. While the vitamin D levels of newborns at birth are entirely dependent on their mothers' 25(OH)D levels,[18,19] an infant's 25(OH)D level at 1 month of age is derived from breastmilk, formula, or sunlight exposure, since serum 25(OH)D has a half-life of 2—3 weeks.[20] Additionally, our study subjects represented an ethnically homogenous population with a narrow age distribution. There have been few reports on infant vitamin D deficiency in East Asia compared to those from Europe and North America.[21]

The study subjects were healthy infants, none of whom showed growth impairment (Table 2). However, 487 (87.4%) exhibited threshold levels of less than 20 ng/mL, which is a frequently used cut-off value in the literature (Table 1). This finding is primarily attributed to forgoing vitamin D supplements in Japanese infants and the Japanese cultural practice of having fewer outdoor activities in early infancy.

Moreover, reports showed that BMI during growth and development influenced the interrelationship between vitamin D and PTH.[6,22] In this study, the point of inflection was lower in the high-BMI group than in the low-BMI group of both 1-month and 2-month-old infants (Fig. 2). These results were consistent with the report by Amini et al.[22]

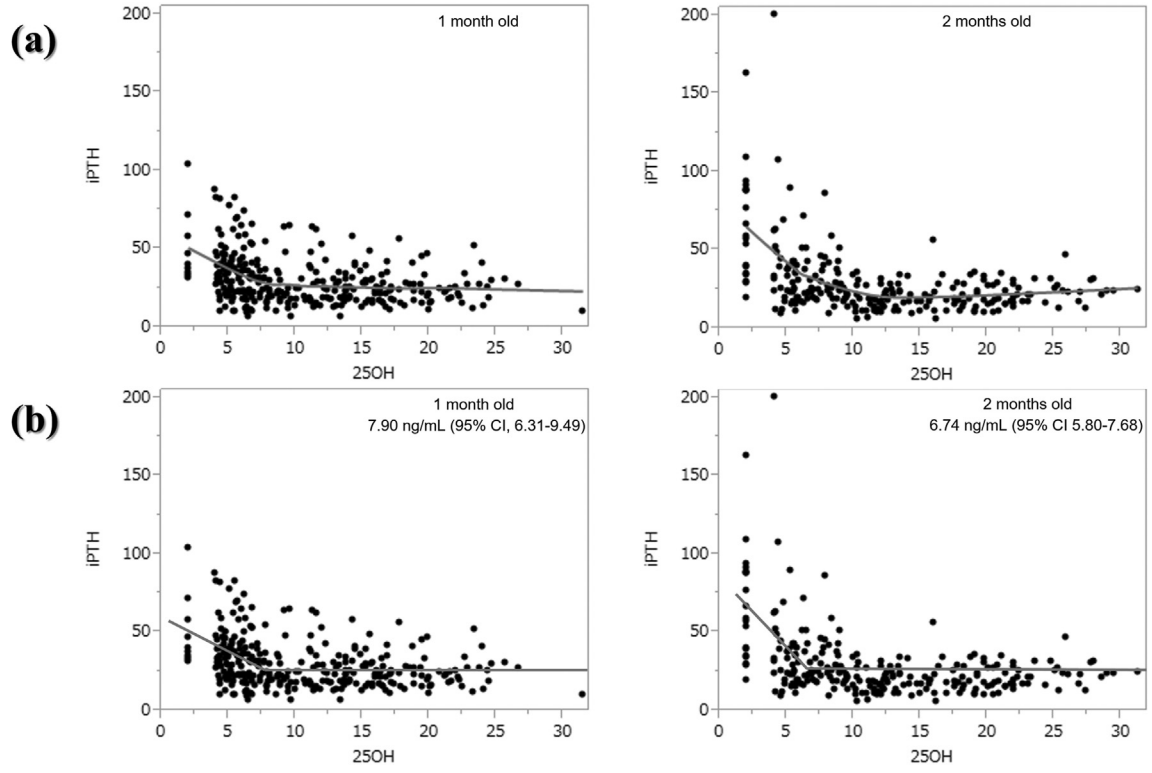

Figure 1 Relationship between serum 25-hydroxyvitamin D (25(OH)D) and intact parathyroid hormone (iPTH) concentrations at 1 and 2 months of age. (a) Scatter plots with a spline curve show a nonlinear relationship. (b) A two-phase linear regression shows inflection points of each age group.

Table 3 Subject demographics classified by age and BMI.

	1-Month Infants		2-month Infants	
	Lower BMI (n = 152)	Higher BMI (n = 152)	Lower BMI (n = 73)	Higher BMI (n = 177)
Age, days	30.7 ± 1.7	31.3 ± 2.4	63.3 ± 2.7	63.7 ± 2.7
Gestational age, weeks	38.2 ± 1.2	38.7 ± 1.1	38.4 ± 1.3	38.4 ± 1.2
Male gender, %	89 (58.6)	92 (60.5)	41 (56.2)	111 (62.7)
Birth weight, grams	2824 ± 291	3081 ± 315	2839 ± 302	2981 ± 330
Birth height, cm	48.0 ± 1.8	48.7 ± 1.7	48.1 ± 1.6	48.4 ± 1.8
Weight, grams	3735 ± 371	4322 ± 384	4750 ± 508	5533 ± 489
Height, cm	52.1 ± 1.9	52.6 ± 1.9	56.3 ± 2.0	56.6 ± 1.9
Body mass index, kg/m^2	13.7 ± 0.7	15.6 ± 0.7	15.0 ± 0.8	17.3 ± 1.0
Calcium, mg/dL	10.24 ± 0.32	10.26 ± 0.29	10.28 ± 0.36	10.37 ± 0.31
Phosphate, mg/dL	6.4 ± 0.4	6.5 ± 0.4	6.2 ± 0.5	6.3 ± 0.5
Alkaline phosphatase[a], IU/L (U/L)	1134 ± 339 (399 ± 119)	1174 ± 318 (413 ± 112)	1201 ± 402 (423 ± 142)	1177 ± 327 (414 ± 115)

Data are presented as the mean (±SD) or n (%) for categorized data.
[a] Upper column is expressed as IU/L using the standard method by the Japan Society of Clinical Chemistry (JSCC). Lower column corresponds to values (U/L) for the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Similar to other studies, our results demonstrated that iPTH significantly decreased with increasing 25(OH)D for lower 25(OH)D levels, whereas iPTH remained nearly constant for higher 25(OH)D levels. These trends were seen in the whole group and subgroup analyses (Figs. 1 and 2). Several methods have been developed to analyze the relationship between 25(OH)D and PTH levels to validate the 25(OH)D threshold. It has been speculated that the 25(OH)D level associated with a rise in PTH may be important during critical growth and development periods, including infancy and the peripubertal period.[23] To that end, the nonlinear segmented regression model[21] was widely adopted in previous observational studies to identify the 25(OH)D sufficiency limit using the deflection point beyond where serum PTH started to increase. However, few studies observed no serum PTH plateau regardless of 25(OH)D concentration.[8] In our study, the model was fit to identify the inflection point for the relationship between 25(OH)D and iPTH, since the smooth lines obtained from LOESS supported the nonlinear shape for both groups, and

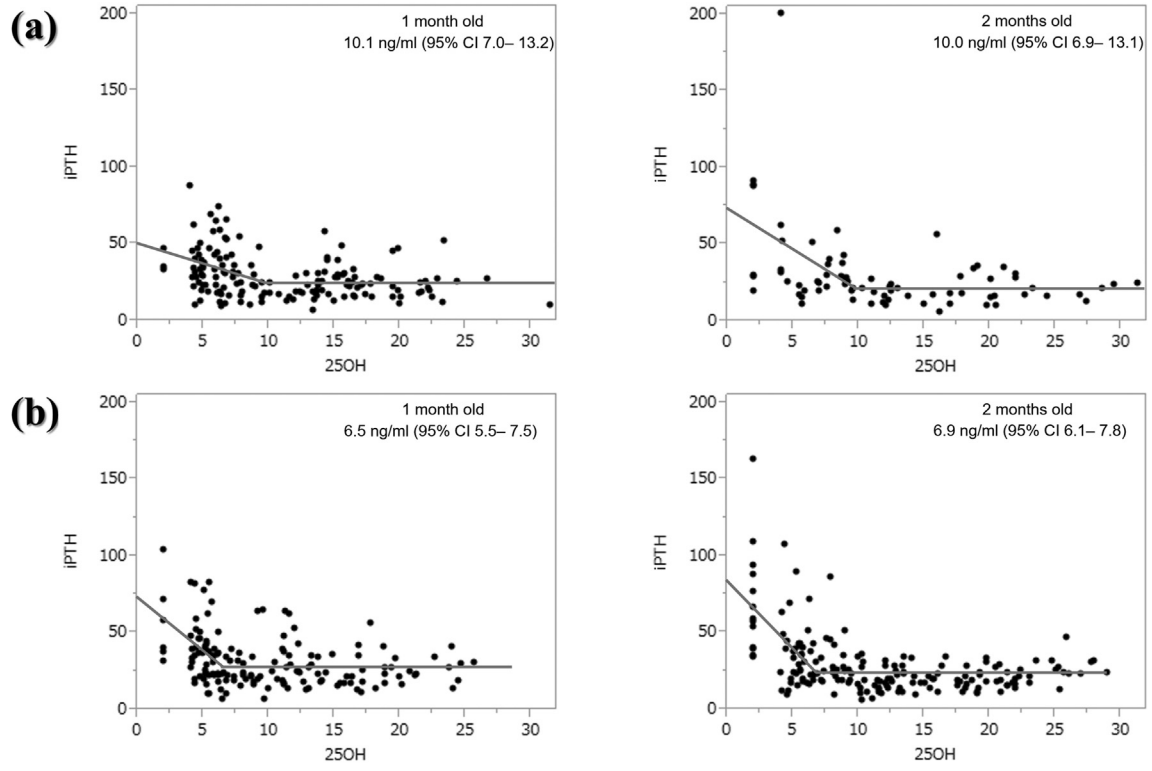

Figure 2 A two-phase linear regression classified by BMI in each age group. (a) the low-BMI group (b) the high-BMI group.

the residual error for the sum of least squares was less than the linear regression analysis (Fig. 1a).

Previous studies indicated that the 25(OH)D concentration resulting in PTH elevation varied depending on age,[16] BMI,[22] and assay method, resulting in a wide range of serum 25(OH)D levels that were reported to maximally suppress serum PTH in children. In our study, the 25(OH)D cut-off levels for 1-month and 2-month-old infants were 7.90 and 6.74 ng/mL, respectively. These values were lower than those reported in numerous studies, including 12 ng/mL, as suggested by Docio; 16 ng/mL, which was suggested for Finnish female adolescents; 35.8 ng/mL, which was reported for healthy post-menarcheal girls from Ohio; and 37 ng/mL, as reported by Hill in children aged 7—18 years from three areas of the USA.[22]

Conversely, our results were comparable with the recently reported findings by Tomimoto et al., who examined the relationship between iPTH and 25(OH)D levels in 155 Japanese infants aged 3—4 months using the same statistical method as our study.[24] They measured 25(OH)D levels using the RIA kits, reporting 9.79 ng/mL of serum 25(OH)D (95% CI, 6.65—12.93) as the iPTH inflection point. In our study, the mean inflection point of all the included subjects was 7.58 ng/mL (95% CI, 6.46—8.71). Considering that both Tomimoto et al.'s study and ours indicated 25(OH)D levels of less than 12 ng/mL among 1- to 2-month-old Japanese infants, these values do not always indicate serious vitamin D deficiency and did not negatively affect bone formation.

Some limitations should be considered when interpreting our study results. First, the study was retrospective, resulting in a lacking number of paired mother-infant sets with serum 25(OH)D and sufficient magnesium data. Additionally, bone turnover markers other than alkaline phosphatase were not assessed. Therefore, we cannot determine an optimal 25(OH)D level based on actual skeletal outcomes. Second, our study was a single hospital-based observational study with relatively small sample size, limiting the generalizability of our results. As such, our recommendations may not be directly applicable to infants beyond the Tokyo area. Larger prospective multi-center studies are thus necessary to validate the current 25(OH)D threshold in correlation with clinical outcomes.

In conclusion, this study reports the vitamin D cut-off points based on PTH in 1-month and 2-month-old Japanese infants. These results may be used to reference nutrition policy recommendations to prevent nutritional rickets in most full-term newborns. Although the results may not be generalizable, this study has clinical implications for vitamin D insufficiency treatment options in early infancy.

Declaration of competing interest

None.

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List of abbreviations

BMI	Body mass index
CI	Confidence interval
CLIA	Chemiluminescence immunoassay
ECLIA	Electrochemiluminescence immunoassay
iPTH	Intact parathyroid hormone
IQR	Interquartile range
LOESS	Locally weighted [regression]
25(OH)D	25-hydroxyvitamin D
PTH	Parathyroid hormone
RIA	Radioimmunoassay
SD	Standard deviation