

Nutritional Status of Vitamin D and Vitamin K in Infants and Young Children in the Liangshan Region and the Impact of Stratified Supplementation on Bone Metabolism Markers

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Abstract: *Objective:* To systematically evaluate the nutritional status of vitamin D and vitamin K (K1, K2) in infants and young children aged 3 months to 3 years in the Liangshan region, explore the impact of a detection-based stratified supplementation regimen on bone metabolism markers, and provide evidence-based support for precise nutritional interventions in infants in minority areas. *Methods:* A cross-sectional survey combined with an intervention study design was employed, involving 3,689 infants and young children aged 3 months to 3 years in Liangshan Prefecture. Among them, serum vitamin D levels were measured in all 3,689 cases, while vitamin K1 and K2 levels were simultaneously measured in 1,172 cases. The composition ratios of nutritional status were analyzed by stratification based on gender and age in months. Individualized stratified supplementation was implemented for those with insufficient/deficient vitamin D or K levels, while those with adequate levels received basic vitamin D supplementation as a control. After 12 weeks of intervention, bone metabolism-related indicators were re-examined and comparatively analyzed. *Results:* In Liangshan Prefecture, the prevalence of vitamin D deficiency among infants and young children was 8.21%, with an insufficiency rate of 10.90%, resulting in a total abnormality rate of 19.11%. The overall prevalence of vitamin K deficiency was 51.11%, with K2 deficiency accounting for a substantial 47.44%, significantly higher than K1 deficiency (3.67%). No statistically significant differences were observed in the composition ratios of nutritional status across different gender and age groups ($p > 0.05$). After the intervention, platelet count, mean platelet volume, serum calcium levels, and bone density significantly increased in those with insufficient/deficient vitamin D or K levels compared to pre-intervention levels ($p < 0.05$), indicating effective regulation of bone metabolism cycles. *Conclusion:* Vitamin D and K nutritional abnormalities are prominent among infants and young children in Liangshan Prefecture, with vitamin K2 deficiency being a core characteristic. Regional dietary patterns are the primary influencing factor. The stratified supplementation strategy based on testing can precisely improve bone metabolism indicators. It is recommended to incorporate joint screening for vitamin D and K and individualized supplementation into routine regional child health care to safeguard the skeletal health of infants and young children.

Keywords: Liangshan Prefecture; Infants and young children; Vitamin D; Vitamin K; Nutritional status; Bone metabolism; Supplementation intervention

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1. Materials and methods

1.1. Study subjects

A total of 3,689 infants aged between 3 months and 3 years, who received pediatric healthcare services or medical treatment at Liangshan Prefecture Maternal and Child Health Hospital and its affiliated collaborative hospitals from January 2023 to May 2024, were selected as the study subjects based on voluntary and random sampling principles. Among them, there were 1,866 males and 1,823 females, with ages ranging from 3 to 36 months and an average age of (7.14 ± 0.42) months, and an average weight of (4.16 ± 0.24) kg. This study was approved by the hospital's ethics committee (Approval No. 2024 Ethical Review 11), and informed consent was obtained from the guardians ^[1].

1.1.1. Inclusion criteria

Complete medical records, absence of jaundice, rickets, or chronic diseases, and no recent diarrhea.

1.1.2. Exclusion criteria

Infants with concurrent blood disorders, congenital metabolic disorders, skeletal malformations, infectious diseases, cardiovascular and cerebrovascular diseases, pulmonary diseases, or renal diseases.

1.2. Sample grouping, testing items, and criteria for judgment

1.2.1. Specimen collection and testing

1.5 mL of fasting fingertip capillary blood was collected from the infants, and serum was separated by centrifugation at 4,000 r/min for 10 minutes. The levels of serum vitamin D (including D2 and D3), K1, and K2 were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), with operations strictly following the “Expert Consensus on Standardization for the Detection of 25-Hydroxyvitamin D by Liquid Chromatography-Tandem Mass Spectrometry (2021 Edition)”.

Blood routine indicators (platelet count, mean platelet volume) were determined using an automated biochemical analyzer. Blood calcium levels were measured using inductively coupled plasma mass spectrometry (ICP-MS). Bone mineral density of the mid-left tibia was assessed using an ultrasound bone densitometer. All testing instruments were regularly calibrated, and reagents were within their expiration dates ^[2-4].

1.2.2. Sample grouping

- (1) Grouped by test items
3,689 cases were included in the vitamin D test group, and 1,172 cases were included in the vitamin K (K1 + K2) test group;
- (2) Grouped by gender
Male group (1,866 cases) and female group (1,823 cases);
- (3) Grouped by age in months
Groups for 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 months;
- (4) Grouped by nutritional status
Based on the evaluation criteria for vitamins D and K, the samples were divided into deficient, insufficient, adequate, and excessive (for vitamin D only) groups for subsequent intervention and comparative analysis.

1.2.3. Nutritional status evaluation criteria

For vitamin D, the WS/T 677—2020 health industry standard was used as a reference: < 30 nmol/L indicates deficiency, 30–50 nmol/L indicates insufficiency, 50–250 nmol/L indicates adequacy, and > 250 nmol/L indicates excess. For vitamin K, the Mayo Clinic standard in the United States was used as a reference: serum vitamin K < 0.5 nmol/L indicates deficiency, 0.5–1.0 nmol/L indicates insufficiency, and ≥ 1.1 nmol/L indicates adequacy. Among those with deficiency,

further differentiation was made between K1 deficiency (43 cases) and K2 deficiency (556 cases).

1.3. Methods for supplementing active vitamin D and vitamin K

1.3.1. Control group receiving vitamin D without vitamin K

For those with adequate levels of vitamins D and K: Follow medical advice and take oral vitamin D drops on an empty stomach in the morning, one capsule (400 IU or 800 IU, adjusted according to age in months), without additional vitamin K supplementation, serving as the basic control group. Regular follow-up visits are conducted to monitor compliance.

1.3.2. Vitamin D and K supplementation group

For individuals with insufficient or deficient levels of vitamins D and K, a stratified and individualized supplementation protocol should be implemented, following the “Expert Consensus on the Clinical Application of Vitamins A and D in Chinese Children (2024)” and the “International Clinical Practice Guidelines for Vitamin K”^[5].

This involves combined supplementation with vitamin D and the corresponding subtype of vitamin K preparation, followed by a reevaluation after 12 consecutive weeks of intervention. For those who do not meet the target levels, dosage adjustments should be made dynamically^[6,7]. The specific protocol is as follows:

- (1) For insufficient vitamin D levels (30–50 nmol/L)
Administer 800 IU (20 µg) of oral vitamin D daily for 12 weeks; concurrently increase the intake of foods such as egg yolks and vitamin D-fortified dairy products, and engage in outdoor activities for 1–2 hours between 9:00 and 11:00 daily (exposing the face and arms while avoiding damage from strong ultraviolet rays at high altitudes)^[8].
- (2) For vitamin D deficiency (< 30 nmol/L)
Administer a therapeutic dose of 2000 IU (50 µg) of vitamin D daily for the initial 4 weeks, followed by a maintenance dose of 1000 IU (25 µg) daily for the subsequent 12 weeks, supplemented by food fortification and scientific sun exposure interventions^[9–15].
- (3) For vitamin K1 deficiency (< 0.5 nmol/L)
Administer 3 drops of oral vitamin K1 drops (containing 10 µg of K1) daily after meals.
- (4) For vitamin K2 deficiency (< 0.5 nmol/L): Administer 3 drops of oral vitamin K2 drops (containing 90 µg of K2) daily after meals.

1.3.3. Supplementation monitoring and follow-up

Supplementation Monitoring Establish a dedicated follow-up system to record medication adherence (excluding those with a medication adherence rate of less than 80% from statistical analysis). After 12 weeks of intervention, reevaluate serum 25-hydroxyvitamin D, vitamin K1/K2, and bone metabolism markers, and adjust the supplementation dosage based on the reevaluation results.

1.4. Quality control

1.4.1. Intervention targets and implementation of the protocol

Supplemental intervention is only implemented for infants and young children with insufficient/deficient serum vitamin D or vitamin K levels, with the specific protocol executed as outlined in Section 1.3. Those with adequate levels of both vitamins D and K do not receive additional vitamin K supplements and only receive routine vitamin D supplements, serving as the control group^[9].

All intervention targets receive continuous and regular supplementation for three months. During this period, guardians are instructed to ensure that infants and young children have daily outdoor activity time, receive appropriate complementary foods, and avoid adjusting dosages or using other nutritional supplements on their own. After the intervention, serum vitamin D and vitamin K levels, as well as bone density indicators, are uniformly re-examined to compare changes before and after the intervention.

1.4.2. Quality control measures

To ensure the accuracy and reliability of the research data, comprehensive quality control measures are implemented throughout the study, as follows:

(1) Personnel and operational standards

All participating researchers receive unified training and strictly adhere to standardized operational procedures and judgment criteria. Senior pediatric healthcare experts conduct regular on-site inspections to review the implementation of the research protocol and the quality of case report form completion, ensuring the authenticity and reliability of the data ^[15].

(2) Sample handling and testing quality control

Blood samples were collected by professional medical staff in accordance with standard procedures, centrifuged within 2 hours after collection, and the separated serum was stored at 2–8 °C to avoid repeated freeze-thaw cycles. High-, medium-, and low-concentration quality control samples were included in each batch of tests, and the standard operating procedures for liquid chromatography-tandem mass spectrometry were strictly followed to ensure accurate and traceable test results ^[4].

(3) Criteria for determining outliers

Vitamin D levels were evaluated based on WS/T 677—2020, “Screening Criteria for Vitamin D Deficiency in Populations”; vitamin K levels were referenced from the “International Guidelines for Standardized Vitamin K Testing” and relevant standards from the Mayo Clinic in the United States; bone metabolism indicators were referenced from the normal reference ranges for pediatric bone metabolism published by the WHO.

(4) Follow-up management

Through a combination of telephone follow-ups and outpatient reviews, guardians were regularly reminded to administer nutrient supplements in accordance with established guidelines, and adverse reactions and adherence were recorded to ensure the effective implementation of intervention measures.

1.5. Statistical methods

Data were analyzed using SPSS 28.0 statistical software. Categorical data were expressed as [n(%)], and comparisons of the constituent ratios of vitamin D and K nutritional status across different genders and age groups in months were conducted using the χ^2 test. Continuous data were expressed as ($\bar{x} \pm s$), and comparisons of indicators before and after intervention were conducted using the paired *t*-test, while comparisons between the intervention group and the control group were conducted using the independent samples *t*-test. A *p*-value < 0.05 was considered statistically significant.

2. Results

2.1. Distribution of vitamin D nutritional status among infants and young children of different genders and ages

Among the 3,689 infants and young children, 303 cases (8.21%) had vitamin D deficiency, 402 cases (10.90%) had insufficient vitamin D levels, 2,969 cases (80.48%) had adequate vitamin D levels, and 15 cases (0.41%) had excessive vitamin D levels. By gender: Among the 1,866 male infants, 151 cases (8.09%) had vitamin D deficiency, 211 cases (11.30%) had insufficient vitamin D levels, 1,496 cases (80.17%) had adequate vitamin D levels, and 8 cases (0.43%) had excessive vitamin D levels; among the 1,823 female infants, 152 cases (8.34%) had vitamin D deficiency, 191 cases (10.48%) had insufficient vitamin D levels, 1,473 cases (80.80%) had adequate vitamin D levels, and 7 cases (0.38%) had excessive vitamin D levels. Comparison of the composition ratios between the male and female groups yielded $\chi^2 = 1.699$, *p* = 0.428, indicating no statistically significant difference.

By age group: The vitamin D deficiency rates among the 3–12 months age groups ranged from 0.32% to 1.95%, the adequate rates ranged from 7.86% to 8.78%, and excessive levels were only observed in the 6–12 months age group

(0.03% to 0.13%). There was no statistically significant difference in comparisons between groups ($p > 0.05$). The specific distribution is shown in **Table 1**.

Table 1. Vitamin D nutritional status among male and female infants and young children [n(%)]

Group	Total (n)	Deficient	Insufficient	Adequate	Excessive
Overall	3689	303 (8.21)	402 (10.90)	2969 (80.48)	15 (0.41)
Male	1866	151 (8.09)	211 (11.31)	1496 (80.17)	8 (0.43)
Female	1823	152 (8.34)	191 (10.48)	1473 (80.80)	7 (0.38)
χ^2 -value				1.699	
p -value				0.428	

2.2. Distribution of vitamin K nutritional status among infants and young children of different genders and ages

Among the 1,172 infants and young children, 599 cases (51.11%) had vitamin K deficiency, 214 cases (18.26%) had insufficient vitamin K levels, 359 cases (30.63%) had adequate vitamin K levels, and 12 cases (1.02%) had excessive vitamin K levels, with an average serum vitamin K level of (10.21 ± 1.42) nmol/L. Among them, 43 cases (3.67%) had K1 deficiency, and 556 cases (47.44%) had K2 deficiency, suggesting that K2 deficiency is the core issue contributing to abnormal vitamin K nutrition in infants and young children in this region. Gender-based grouping: Among 582 male cases, 321 cases (55.15%) had vitamin K deficiency, 102 cases (17.53%) had insufficient levels, 152 cases (26.12%) had adequate levels, and 7 cases (1.20%) had excessive levels, with a mean serum vitamin K level of (10.12 ± 1.61) nmol/L. Among 590 female cases, 278 cases (47.12%) had vitamin K deficiency, 112 cases (18.98%) had insufficient levels, 207 cases (35.08%) had adequate levels, and 5 cases (0.85%) had excessive levels, with a mean serum vitamin K level of (10.31 ± 1.62) nmol/L. There were no statistically significant differences in the composition ratios between the male and female groups ($\chi^2 = 0.046$, $p = 0.977$), nor in the serum levels ($t = 1.532$, $p = 0.126$). Age-based grouping (in months): The deficiency rates ranged from 0.32% to 2.99%, and the adequacy rates ranged from 1.11% to 8.66% across the 3–12 months age groups. Excessive levels were only observed in the 6–12 months age group, with no statistically significant differences among the groups ($p > 0.05$). Specific distributions are shown in **Table 2** and **Table 3**.

Table 2. Vitamin K nutritional status in males and females [n(%)]

Group	Total (n)	Deficient	Sufficient	Excessive	Serum 25(OH)D level (nmol/L)
Overall	1172	599 (51.11)	573 (48.89)	12 (1.02)	10.21 ± 1.42
Male	582	321 (55.15)	261 (44.85)	7 (1.20)	10.12 ± 1.61
Female	590	278 (47.12)	312 (52.88)	5 (0.85)	10.31 ± 1.62
Test statistic			0.046		1.532
p -value			0.977		0.126

Table 3. Vitamin D and K nutritional status by age group (in months)

Age (months)	Vitamin D nutritional status				Vitamin K nutritional status			
	Deficient	Insufficient	Sufficient	Excessive	Deficient	Insufficient	Sufficient	Excessive
3	12	18	364	0	12	11	42	0
4	14	16	415	0	14	10	52	0
5	14	20	328	0	12	8	41	0
6	22	21	346	1	16	24	26	1
7	22	25	265	1	74	26	41	1
8	25	38	208	2	84	19	38	2
9	30	41	216	2	91	20	41	2
10	34	50	268	2	92	34	32	2
11	58	61	269	4	94	28	20	2
12	72	112	290	5	110	34	14	2
Total	303	402	2969	15	599	214	347	12

2.3. Comparison of bone metabolism indicators in infants and young children

2.3.1. Comparison before and after intervention in individuals with vitamin D

Insufficiency/Deficiency: After intervention, among 705 cases with vitamin D insufficiency/deficiency, the platelet count significantly increased from $(171.22 \pm 21.42) \times 10^9/L$ to $(180.37 \pm 50.24) \times 10^9/L$, the mean platelet volume significantly increased from (8.04 ± 0.24) fl to (10.68 ± 1.12) fl, blood calcium significantly increased from (1.42 ± 0.42) mmol/L to (2.08 ± 0.16) mmol/L, and bone density significantly increased from (-0.12 ± 0.08) to (0.18 ± 0.09) . All these indicators showed statistically significant differences ($p < 0.05$) when compared before and after the intervention (Table 4). An increase in platelet indices suggests enhanced bone metabolic activity, providing synergistic evidence alongside improvements in blood calcium levels and bone density^[7].

Table 4. Changes in bone metabolic indices before and after vitamin d supplementation ($\bar{x} \pm s$)

Indicator	Time point	Platelet count ($\times 10^9/L$)	Mean platelet volume (fL)	Serum calcium (mmol/L)	Bone mineral density (Z-score)
Vitamin D insufficiency/ deficiency (n = 705)	Before supplementation	$171.22 \pm 21.42^*$	$8.04 \pm 0.24^*$	$1.42 \pm 0.42^*$	$-0.12 \pm 0.08^*$
	After supplementation	$180.37 \pm 50.24^\#$	$10.68 \pm 1.12^\#$	$2.08 \pm 0.16^\#$	$0.18 \pm 0.09^\#$

2.3.2. Comparison before and after intervention in individuals with insufficient/deficient

Vitamin K: Among 813 individuals with insufficient/deficient vitamin K, after intervention, the platelet counts significantly increased from $(152.41 \pm 21.22) \times 10^9/L$ to $(171.24 \pm 24.24) \times 10^9/L$. The mean platelet volume also significantly increased from (8.11 ± 0.24) fl to (9.12 ± 0.82) fl. Blood calcium levels significantly rose from (2.01 ± 0.14) mmol/L to (2.72 ± 0.24) mmol/L, and bone density significantly improved from (-0.14 ± 0.09) to (0.28 ± 0.11) . All these indicators showed statistically significant differences ($p < 0.05$) when compared before and after intervention (Table 5).

Table 5. Changes in bone metabolic indices before and after vitamin K supplementation in individuals with insufficient/deficient vitamin K ($\bar{x} \pm s$)

Indicator	Time point	Platelet count ($\times 10^9/L$)	Mean platelet volume (fL)	Serum calcium (mmol/L)	Bone mineral density (Z-score)
Vitamin K insufficiency/ deficiency (n = 813)	Before supplementation	152.41 \pm 21.22*	8.11 \pm 0.24*	2.01 \pm 0.14*	-0.14 \pm 0.09*
	After supplementation	171.24 \pm 24.24#	9.12 \pm 0.82#	2.72 \pm 0.24#	0.28 \pm 0.11#

2.3.3. Comparison between intervention group and sufficient group

After the intervention, the mean bone density in the control group with sufficient vitamin D and K (2969 cases) was (0.22 ± 0.10), while in the intervention group (1518 cases), it was (0.23 ± 0.10). The comparison between groups yielded $t = 0.864$ and $p = 0.388$, indicating no statistically significant difference. This suggests that stratified supplementation can effectively narrow the bone density gap between the intervention population and the nutritionally sufficient population, confirming the efficacy of the intervention.

3. Discussion

3.1. Nutritional status and regional characteristics of Vitamins D and K in infants and young children in the Liangshan region

This study, for the first time, focuses on the nutritional status of vitamin K subtypes (K1 and K2) in infants and young children in the ethnic minority areas of Liangshan. It reveals that the overall abnormal rate of vitamin D in infants and young children in this region is 19.11%, which is lower than that in Zhangjiakou (28.7%) and Maoming (62.90%)^[13,14]. It is speculated that this is related to the high intensity of ultraviolet radiation and the high efficiency of skin synthesis of vitamin D in the Liangshan Plateau, which can partially compensate for the short sunshine duration^[5]. The overall deficiency rate of vitamin K reaches 51.11%, with K2 deficiency accounting for 47.44%, significantly higher than that in plain areas (approximately 20%). This characteristic is highly correlated with the regional dietary structure. The diet of ethnic minorities in Liangshan primarily consists of grains and meats, with minimal intake of fermented foods (such as natto and fermented milk), which are the main sources of natural vitamin K2. Additionally, the K2 content in breast milk is extremely low, and the proportion of dark green vegetables (the main source of K1) in complementary foods is insufficient. These dual factors contribute to the high prevalence of K2 deficiency. Compared with the study by Tian Xue et al. on children aged 0–3 years in the Yibin region, this study found a lower abnormal rate of vitamin D but a significantly higher deficiency rate of K2, reflecting the differential impact of dietary patterns in different regions of the southwestern mountainous areas on nutrient intake^[8]. It also highlights the necessity of targeted nutritional interventions in ethnic minority areas^[5]. In this study, no significant differences in nutritional status were observed between different genders and age groups in months, suggesting that the influence of regional dietary structure on the levels of vitamins D and K is greater than individual developmental differences. This conclusion is consistent with the study by Yin Shian et al. on the current status of vitamin nutrition in children in China.

3.2. Mechanism of the impact of active supplementation on bone metabolism indicators

After the intervention, bone metabolism indicators significantly improved in individuals with insufficient/deficient levels of vitamins D and K, which is consistent with the research findings of Zhang Nan et al. on ethnic minority populations, confirming the effectiveness of the stratified supplementation strategy^[5]. Vitamin D promotes intestinal calcium and phosphorus absorption and accelerates bone mineralization by activating vitamin D receptors^[15]. Vitamin K2 specifically activates osteocalcin and matrix Gla protein; the former facilitates calcium deposition in the bone matrix, while the latter inhibits vascular calcification, and together they synergistically regulate bone metabolism balance. This is also the core

mechanism behind the significant increase in bone mineral density observed in this study. This study adopted a precise supplementation plan of “dose stratification + subtype matching”, providing basic/therapeutic doses for individuals with insufficient/deficient vitamin D levels and matching corresponding formulations for those with K1/K2 deficiencies. It also combined food fortification with scientific sun exposure, adhering to the stratification principles outlined in the “Expert Consensus on the Clinical Application of Vitamin A and Vitamin D in Chinese Children (2024)” while taking into account regional characteristics to optimize intervention details ^[15]. This approach is more targeted compared to traditional one-size-fits-all supplementation plans. The increase in platelet count and mean platelet volume suggests that bone metabolism has entered an active repair phase, forming a logical closed loop with the rise in blood calcium levels and improvement in bone mineral density. This association is also supported by relevant bone metabolism research, confirming that supplementation interventions can effectively activate bone repair mechanisms ^[7].

3.3. Research limitations and practical recommendations

This study is a single-center study with samples limited to medical institutions, introducing selection bias. Additionally, the intervention follow-up period was only 12 weeks, lacking long-term data on bone metabolism outcomes. Subsequent recommendations include conducting multi-center studies, incorporating healthy infants and toddlers from the community, extending follow-up to over one year, and dynamically monitoring bone density and skeletal development indicators. Based on the study results, targeted practical recommendations are proposed: first, establish a combined screening mechanism for “Vitamin D + K2” and include it in routine healthcare for infants aged 3 to 12 months, with a particular focus on K2 testing. Second, promote stratified supplementation plans, emphasizing the dietary characteristics of the Liangshan region by reinforcing education on adding fermented foods and dark green vegetables as complementary foods, and guiding scientific outdoor activities based on the high-altitude sunlight advantages ^[8]. Third, explore the inclusion of active vitamin supplementation in regional public health services to improve the coverage of nutritional interventions for ethnic minority families ^[14]. The “screening-stratified supplementation-dynamic monitoring” precision nutrition intervention model proposed in this study can provide a replicable practical paradigm for child healthcare in southwest ethnic minority regions, promoting the transition of child nutrition interventions from “experience-based” to “evidence-based” approaches ^[15].

4. Conclusion

Infants and toddlers in the Liangshan region exhibit prominent abnormalities in vitamin D and K nutrition, characterized by a regional pattern of “predominant vitamin K2 deficiency and mild vitamin D abnormalities”. Nutritional status shows no significant correlation with gender or age, with regional dietary structure being the core influencing factor. The detection-based hierarchical supplementation strategy for active vitamin D and K can significantly improve bone metabolism indicators in individuals with deficiencies or insufficiencies, effectively narrowing the gap with those who have adequate nutrition. It is recommended to incorporate joint screening for vitamins D and K, along with individualized supplementation, into routine pediatric healthcare practices, taking into account regional characteristics. This should be coupled with enhanced dietary education and dynamic monitoring to provide precise protection for the bone health of infants and young children. This study offers evidence-based support for targeted nutritional interventions in children from ethnic minority regions and serves as a reference for the regional application of clinical case studies.

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Disclosure statement

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