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Research article

Establishment of age-specific reference intervals for serum 25-hydroxyvitamin D in a large pediatric population of Nanjing, China

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ABSTRACT

Background: The reference intervals (RIs) is defined as the central 95 % range of reference values from healthy individuals. The establishment of appropriate medical RIs for specific populations is crucial for accurate diagnosis and treatment of disease. However, the RIs for 25-hydroxyvitamin D (25(OH)D) in Chinese pediatric individuals are currently not available. This retrospective study aimed to establish pediatric RIs for serum 25-hydroxyvitamin D (25(OH)D) in the Nanjing area of China.

Methods: After data filtering and deletion of outliers, 133,562 serum 25(OH)D records were finally included in this study. The effects of age, sex, and season on 25(OH)D levels were assessed, and the 2.5 % and 97.5 % percentile points were applied as the lower limit and upper limit of the RIs, respectively.

Results: Age-distribution analysis of serum 25(OH)D levels revealed that children aged 4–12 months old hold the highest 25(OH)D levels, and levels subsequently decreased with age while remaining relatively stable in children aged 7–15 years old. An analysis of sex-specific differences demonstrated that serum 25(OH)D levels in girls were significantly higher than those of boys <4 years old ($P < 0.001$) and dropped to significantly lower than those of boys >7 years old ($P < 0.001$). Season distribution revealed the highest 25(OH)D levels in autumn, followed by summer and winter, and finally spring. Considering the practicability of clinical application and *Z* tests according to CLSI C28-A3 guidelines, age-specific RIs for serum 25(OH)D were established. The calculated RIs for children 0–3 months, 4–12 months, 1–3 years, 4–6 years, and 7–15 years old, respectively, were 18.62–42.18, 22.20–45.60, 21.12–45.20, 17.16–38.20, and 15.56–34.39 ng/mL, respectively.

Conclusions: The levels of serum 25(OH)D exhibited statistically significant age and season variations, and the establishment of age-specific RIs for serum 25(OH)D would be beneficial for clinical diagnosis and treatment.

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1. Introduction

As an essential fat-soluble vitamin, various studies have demonstrated that physiological levels of vitamin D play pivotal roles not only in calcium and phosphorus homeostasis but also in the extra-osseous system, including the cardiovascular system, glucose metabolism, immune system, and cell proliferation and differentiation [1]. The proportion of people with vitamin D insufficiency or deficiency is ever-growing on account of changes in contemporary lifestyles and the depletion of adequate amounts of sunlight exposure [2]. Children are one of the groups traditionally at high risk for vitamin D insufficiency or deficiency. Unfortunately, the incidence of serum vitamin D deficiency or insufficiency observed in Chinese children is also high [3,4]. Inadequate levels of vitamin D contribute to numerous clinical disorders, such as asthma, type 2 diabetes, diabetic kidney disease, cardiovascular disease, and cancer [5–9].

Vitamin D derived from fat-soluble steroids mainly consists of ergocalciferol (D2) and cholecalciferol (D3), both of which are hydroxylated to 25-hydroxyvitamin D (25(OH)D) in the liver, which is further converted to 1,25-hydroxyvitamin D in the kidneys [10]. Among them, 25(OH)D is the major circulating form of vitamin D in the human organism and has been therefore adopted as a credible index to assess the vitamin D status due to a long half-life in circulation. The rapid growth and development of children means they have high vitamin D requirements, which urges us to establish the optimal reference intervals (RIs) and define the specific cutoff point of 25(OH)D levels in children. Although the Institute of Medicine (IOM) and Endocrine Society have suggested that the cutoffs of serum 25(OH) D levels for the definition of insufficient vitamin D concentrations (vitamin D deficiency or insufficiency) be less than 20 ng/mL and less than 30 ng/mL, respectively, the cutoffs are applied for people of all ages without child-specific cutoffs [11,12]. In addition, large-sample studies concerning RIs of 25(OH)D levels for children are also lacking in China. Therefore, it is imperative to establish RIs for serum 25(OH)D levels in children to provide a guide for vitamin D supplementation programs. Due to the fact that vitamin D can be synthesized through sun exposure on the skin, vitamin D levels may fluctuate with the seasons, as sunlight intensity varies throughout the year. Additionally, variations in children's dietary requirements, adherence to vitamin D supplements, outdoor activity time, and physiological requirement may result in age-related changes in vitamin D levels. Accordingly, the present work attempted to investigate the age and season variations and establish pediatric RIs for serum 25(OH)D levels in apparently healthy children to guide appropriate clinical diagnosis and management of vitamin D deficiency.

2. Materials and methods

2.1. Selection of eligible serum 25(OH)D records

This retrospective study was approved by the ethical committee of the Women's Hospital of Nanjing Medical University. In total, 163,908 serum 25(OH)D results processed between April 2019 and December 2022 from children who underwent physical examination in the Child Health Care Department of the Women's Hospital of Nanjing Medical University, together with the details of age, sex, date, and clinical characteristics were gathered. The exclusion criteria were listed as follows: (1) missing critical information such as sex, age, or date information; (2) children seen not for a health examination but for acute and chronic infections, endocrine, heart, liver, and kidney diseases, as well as children who were taking certain special medications that affect vitamin D levels; (3) children suffering from malnutrition, rachitis, or sexual precocity; (4) premature infants, twins, or triplets; (5) and duplicated records within the same time frame (only the first result was retained).

2.2. Samples processing and measurements

Blood samples drawn from children were collected into clear tubes, then centrifuged at 3500 rpm for 10 min. The separated serum was transferred into new tubes for 25(OH)D measurements within 2 h. The iFlash 3000 automated chemiluminescence immunoassay system (Shenzhen YHLO Biotech Co., Ltd.) was applied for 25(OH)D measurements, with the original analytical kits, calibrators, and quality control reagents. To ensure the accuracy of serum 25(OH)D detection, all laboratory procedures were strictly carried out in accordance with the manufacturer's instructions and the standard operating procedure. Moreover, our laboratory attained ISO 15189 accreditation, and both the coefficient variation of the indoor quality control and the bias of the inter-room comparison of serum 25 (OH)D data were <8.33 %.

2.3. Statistical analysis

Data analysis and chart drawing were executed using SPSS 18.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). The Kolmogorov–Smirnov normality test was performed to check the distributions of 25(OH)D data in totality and each subgroup. The outliers were identified and excluded according to Tukey's method, and eligible data were expressed as median with 25th and 75th percentiles for non-normally distributed data. Subgroup analyses were performed according to sex, age (infants: <3 months; infants: 4–6 months; infants: 7–9 months; infants: 10–12 months; toddlers: 1–3 years; preschoolers: 4–6 years; school-age children: 7–9 years; adolescents: 10–15 years), and season (spring: March to May, summer: June to August, autumn: September to November, and winter: December to February). Also, the Mann–Whitney *U* test was applied to compare differences between two groups, the Kruskal–Wallis test was conducted to determine the difference among more than two groups, and pairwise comparisons were executed using Duan's multiple comparison test. The RIs were defined as the 2.5 % and 97.5 % percentile points by the non-parametric rank method. The difference was deemed to be statistically significant when $P < 0.05$.

3. Results

3.1. The selection of eligible serum 25(OH)D records

A total of 163,908 serum 25(OH)D records were collected in the present study, and 25,758 of them were removed from analysis according to the exclusion criteria mentioned above. In addition, 4588 records were identified as outliers and excluded from analysis based on Tukey's method. Finally, 133,562 qualified records, including 70,232 from boys and 63,330 from girls, were enrolled in the statistical analysis. Fig. 1 displays the workflow of the selection of eligible records.

3.2. Age-specific variations in serum 25(OH)D levels

The overall trend of serum 25(OH)D concentrations demonstrated that 25(OH)D levels increase with age, peaking at 4–12 months and then gradually decreasing thereafter (Fig. 2A). The Kruskal–Wallis test confirmed that the difference in serum 25(OH)D concentrations was statistically significant among different age subgroups. Moreover, pairwise comparisons by Dunn's test revealed no statistical difference in serum 25(OH)D levels between children 4–6 months old, 7–9 months old, and 10–12 months old, and between children 7–9 years old and 10–15 years old ($P > 0.05$). However, differences in serum 25(OH)D levels were statistically significant between children 0–3 months old, 4–12 months old, 1–3 years old, 4–6 years old, and 7–15 years old (Table 1).

3.3. Sex-specific variations in serum 25(OH)D levels

The sex-related distributions of 25(OH)D concentrations in different age groups were also evaluated. The results of Mann–Whitney *U* testing indicated that statistically significant differences in serum 25(OH)D concentrations were observed between boys and girls across almost all age groups, except for those aged 4–6 years old (Table 2). In addition, a scatterplot shows the median 25(OH)D concentrations of girls were slightly higher than those of boys aged 0–4 years old, but this trend was reversed among those 7–15 years old (Fig. 2B).

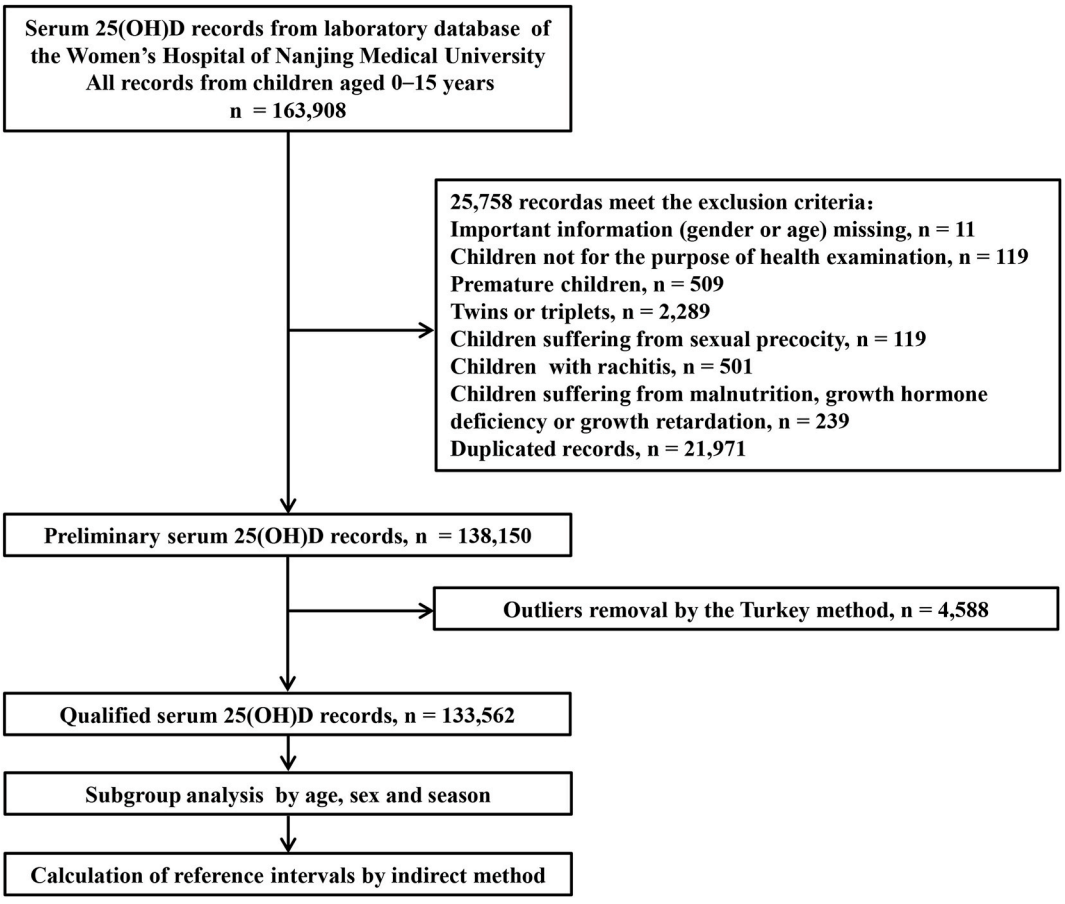

Fig. 1. The workflow of the Establishment of age-specific reference intervals for serum 25-hydroxyvitamin D.

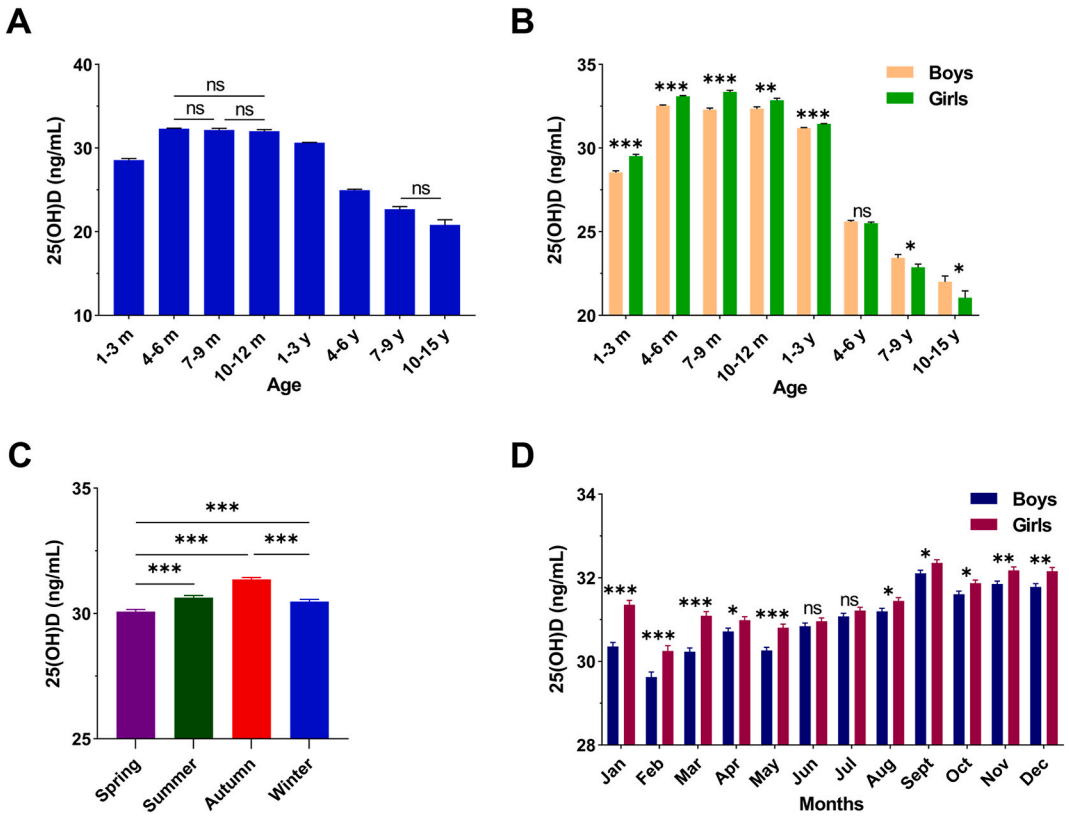

Fig. 2. Variations in median serum 25(OH)D concentrations with (A) age, (B) sex, (C) seasons and (D) months, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns, no significant.

Table 1
Age distribution of serum 25(OH)D [M (P_{25}–P_{75})] levels.[a].

Age groups	No. of cases	25(OH)D (ng/mL)
0–3 m	5383	28.56 (25.08–32.52)
4–6 m	29,297	32.32 (28.64–36.60)[b]
7–9 m	7222	32.16 (28.48–36.77)[b]
10–12 m	5763	32.00 (28.16–36.64)[b]
1–3 y	76,584	30.64 (27.12–34.92)
4–6 y	7981	24.96 (22.20–28.12)
7–9 y	1008	22.69 (20.00–25.56)[c]
10–15 y	324	20.82 (18.29–23.99)[c]
Statistical value		12,710.00
P value		<0.001

[a] Data were expressed as median with 25th and 75th percentiles for non-normally distributed data. M, median; m, months; y, years.
[b] No statistical difference in serum 25(OH)D between children aged 4–6 months, 7–9 months, and 10–12 months.
[c] No statistical difference in serum 25(OH)D between children aged 7–9 years and 10–15 years.

3.4. Season-specific variations in serum 25(OH)D levels

The difference in serum 25(OH)D concentrations between different season groups was statistically significant (Table 3). Two-by-two pairwise comparisons revealed that the levels of 25(OH)D were highest in autumn and lowest in spring; however, no statistically significant difference in 25(OH)D levels was observed between summer and winter ($P > 0.05$) (Fig. 2C). The trends and sex-related distributions of the median concentrations of serum 25(OH)D across all months are displayed in Fig. 2D.

Table 2
Sex distribution of serum 25(OH)D [M (P_{25}–P_{75})] levels.[a].

Age group	Sex	No. of cases	25(OH)D (ng/mL)	*P* value
0–3 m	Boys	2862	28.04 (24.60–32.12)	<0.001
	Girls	2521	29.00 (25.58–33.00)	
4–6 m	Boys	15,366	32.04 (28.48–36.24)	<0.001
	Girls	13,931	32.64 (28.80–37.04)	
7–9 m	Boys	3795	31.76 (27.96–36.08)	<0.001
	Girls	3427	32.76 (29.00–37.48)	
10–12 m	Boys	3003	31.76 (27.96–36.20)	0.0012
	Girls	2760	32.24 (28.36–36.92)	
1–3 y	Boys	40,274	30.64 (27.08–34.76)	<0.001
	Girls	36,310	30.72 (27.20–35.12)	
4–6 y	Boys	4266	24.92 (22.20–28.20)	0.5065
	Girls	3715	24.96 (22.20–28.08)	
7–9 y	Boys	480	22.90 (20.13–26.07)	0.0232
	Girls	528	22.46 (19.85–24.88)	
10–15 y	Boys	186	21.44 (18.31–24.73)	0.0354
	Girls	138	20.14 (18.26–22.82)	

[a] Data were expressed as median with 25th and 75th percentiles for non-normally distributed data. M, median; m, months; y, years.

Table 3
Season distribution of serum 25(OH)D [M (P_{25}–P_{75})] levels1.[a].

Seasonality	No. of cases	25(OH)D (ng/mL)
Spring	33,148	30.08 (26.28–34.56)
Summer	39,831	30.64 (26.76–35.02)[b]
Autumn	36,782	31.36 (27.84–35.64)
Winter	23,801	30.48 (26.72–35.04)[b]
Statistical value		910.10
P value		<0.001

[a] Data were expressed as median with 25th and 75th percentiles for non-normally distributed data. M, median.
[b] There was no statistical difference in serum 25(OH)D levels between summer and winter.

3.5. Establishing RIs of serum 25(OH)D in different age groups

The Kolmogorov–Smirnov normality test indicated that serum 25(OH)D concentrations exhibited skewed distributions in both girls and boys across all age groups. These data were transformed into normal distributions by Blom's normalized rank transformation; then, the *Z* tests were performed on the transformed data to determine the reasonableness of the age-stratified analysis according to CLSI C28-A3 guidelines [13]. The result demonstrated that it is not necessary to establish RIs of serum 25(OH)D based on sex since their *Z* values were smaller than *Z**. Furthermore, considering the practicability of clinical application, the RIs were established by age without season stratification. Ultimately, the ages were split into subgroups aged 0–3 months, 4–12 months, 1–3 years, 4–6 years, 7–15 years, and the corresponding RIs of serum 25(OH)D were 18.62–42.18, 22.20–45.60, 21.12–45.20, 17.16–38.20, and 15.56–34.39 ng/mL, respectively (Table 4).

3.6. Comparison of vitamin D status and RIs of serum 25(OH)D according to different cutoffs by age

For the present study, the 2.5 % percentile points of serum 25(OH) D levels in each age group were applied as the cutoffs for the definition of insufficient vitamin D (vitamin D deficiency or insufficiency) concentrations. As shown in Table 5, the cutoffs of serum 25 (OH) D levels in children aged 0–3 years was 18.62–22.20 ng/mL, which was similar to the IOM cutoffs (<20 ng/mL), but much lower

Table 4
Age-specific pediatric RIs for serum 25(OH)D levels

Age group	No. of cases	Serum 25(OH)D levels (ng/mL)		
		P2.5	P50	P97.5
0–3 m	5383	18.62	28.56	42.18
4–12 m	42,282	22.20	32.24	45.60
1–3 y	76,584	21.12	30.64	45.20
4–6 y	7981	17.16	24.96	38.20
7–15 y	1332	15.56	22.28	34.39

M, median; m, months; y, years.

than the Endocrine Society cutoffs (<30 ng/mL). Moreover, the cutoffs of serum 25(OH) D levels for children aged 4–15 years (15.56–17.16 ng/mL) were lower than both the IOM (<20 ng/mL) and the Endocrine Society cutoffs (<30 ng/mL) (Table 5).

4. Discussion

Vitamin D has pivotal roles in maintaining calcium and phosphorus homeostasis and extra-skeletal health [14]. Total 25(OH)D, consisting of 25(OH)D3 and 25(OH)D2, is the most universally accepted indicator of vitamin D status. Numerous studies have reported that inappropriate levels of 25(OH)D are linked to various human diseases, such as pregnancy complications, metabolic syndrome, and stroke [15–17]. Although vitamin D can be obtained from skin synthesis, food, or vitamin supplements, the incidence of insufficiency and deficiency of vitamin D in Chinese children is relatively high [3,4]. Low levels of 25(OH)D contribute to fracture, rickets, growth and development retardation, lethargy, hypocalcemic seizures, neurological symptoms, and movement disorders in children [18–20]. It is noteworthy that high doses or unnecessary application of vitamin D supplements to children may result in vitamin D intoxication, which leads to metabolism imbalances in calcium, bone, and parathyroid hormone [21,22]. Therefore, the appropriate dose of vitamin D intake and accurate assessment of 25(OH)D levels is essential.

RIs are indispensable for disease diagnosis and efficacy evaluation. Currently, most clinical laboratories can acquire the recommended RIs from instruction manuals, national guidelines, or studies. However, RIs are susceptible to multiple factors, such as geographical regions, lifestyle behaviors, economic levels, and detection systems and methods [23]. Therefore, it is of greater value to establish credible, applicable, and personalized RIs for local laboratories and populations in disease diagnosis, monitoring, and assessment of prognosis.

The establishment of pediatric RIs using a direct method is one of the major challenges clinical laboratories face since it is relatively difficult to acquire a sufficient ethical sample size and sample volume from children. However, with the rapid development of computer storage technology, the laboratory information system can store large amounts of data, which creates favorable conditions for establishing RIs by an indirect method. Therefore, the establishment of RIs for serum 25(OH)D in apparently healthy children of the Nanjing region of China was conducted on the basis of laboratory information system and statistical analysis using an indirect method in the present study. For children in Nanjing aera of China, our data revealed that the prevalence of 25(OH)D < 30 ng/mL (vitamin D deficiency or insufficiency) was 45.40 %, indicating the serum 25(OH)D levels in children of the Nanjing region were slightly higher than those reported from other areas of China and worldwide [4,24,25]. In Hangzhou aera of China, the prevalence of 25(OH)D < 30 ng/mL was 64.00 % with the mean of 25(OH)D of 28.01 ng/mL. Lower vitamin D status was also prevalent in African, such as Gambia and South Africa where about 58.20 % and 49.80 % of children aged 0–8 years were 25(OH)D < 30 ng/mL, respectively. Differences in diet, latitude, and lifestyle may contribute to the higher serum 25(OH)D levels in our study. Also, easy access to medical services makes it possible for infants and preschoolers to undergo regular check-ups at community hospitals or maternal and child health centers, which helps their parents identify vitamin deficiency and supply plenty of solar exposure, vitamin D-rich diet, or extra vitamin D to their children in time.

In accordance with other studies, statistically significant age and season variations in serum 25(OH)D concentrations were observed in our study (Tables 1 and 3) [3,26]. The levels of serum 25(OH)D initially increased with age before the age of 1 year and then gradually decreased thereafter, and a reversed trend was observed in the percentage of serum 25(OH)D insufficiency or deficiency (Fig. 2A and Table 5). According to the judgment standard of Endocrine society, the rate of serum vitamin D deficiency or insufficiency amounted to 45.40 % among all children, and the rate reached 84.06 % and 93.99 % in children aged 4–6 years and 7–15 years, respectively (Table 5). The reasons for this may be that Chinese parents often take their children outside in the first year of their life, which contributes to more ultraviolet light exposure and skin synthesis of 25(OH)D. Also, children in this age group have better adherence to varied diet including breast milk or formula, which allows them to attain adequate vitamin D levels. However, as their age increases, the increased physiological requirement of vitamin D and inadequate vitamin D supplementation leads to lower serum 25(OH)D concentrations, especially in school-aged children (6–15 years old). Moreover, the increase in academic tasks and reductions in time spent outdoors among school-aged children also lead to less skin synthesis of 25(OH)D and age variations in serum 25(OH)D concentrations. Therefore, plenty of time for outdoor activity, regular monitoring of 25(OH)D levels, and appropriate supplementation of vitamin D preparations are necessary for school-aged children to protect them from insufficiency or deficiency of vitamin D. For seasonal variation, we found that levels of 25(OH)D were highest in autumn compared to in other seasons, due to increased outdoor activities, more sunlight, greater ultraviolet exposure, and increased skin synthesis in autumn. Although the median serum 25(OH)D concentrations were comparable between boys and girls in the same age group, statistically significant differences were observed, which were inconsistent with those in other studies [3,26]. We speculated that the large sample size in our study might lead to a significant statistical difference in serum 25(OH)D concentrations between boys and girls in the same age group.

Unfortunately, there were still several limitations in the retrospective study. Firstly, serum 25(OH)D records were unevenly distributed across age subgroups, so more serum 25(OH)D data for apparently healthy children aged 7–15 years should be collected for analysis. Secondly, serum 25(OH)D data obtained from some individuals suffering from underlying diseases or subclinical state may be enrolled for analysis even though the exclusion criteria were performed in this study. For improvement, stricter inclusion and exclusion criteria should be adopted in further research. Thirdly, the external validation studies by cooperating with more medical centers from distinct geographical areas are needed to evaluate the clinical application value of our results in the future. Lastly, some mixed factors, such as vitamin D supplements, diet, sunlight exposure, exercise intensity, and particularly body mass index (BMI) should be explored in the future.

Table 5
Vitamin D status and RIs (ng/mL) of 25(OH)D according to different cutoffs by age.[a].

Age	IOM			Endocrine society			Present study		
	RIs	VDD + VDI	VDS	RIs	VDD + VDI	VDS	RIs	VDD + VDI	VDS
0–3 m	≥20	245 (4.55)	5138 (95.45)	≥30	3227 (59.95)	2156 (40.05)	≥18.62	135 (2.51)	5248 (97.49)
4–12 m	≥20	414 (0.98)	41,868 (99.02)	≥30	14,606 (34.54)	27,676 (65.46)	≥22.20	1060 (2.51)	41,222 (97.49)
1–3 y	≥20	1140 (1.49)	75,444 (98.51)	≥30	34,846 (45.50)	41,738 (54.50)	≥21.12	1943 (2.54)	74,641 (97.46)
4–6 y	≥20	898 (11.25)	7083 (88.75)	≥30	6709 (84.06)	1272 (15.94)	≥17.16	204 (2.56)	7777 (97.44)
7–15 y	≥20	392 (29.43)	940 (70.57)	≥30	1252 (93.99)	80 (6.01)	≥15.56	36 (2.70)	1296 (97.30)

[a] Data are expressed as n (%).IOM, Institute of Medicine; VDD, Vitamin D deficiency; VDI, Vitamin D insufficiency; VDS, Vitamin D sufficiency.

5. Conclusions

In summary, a large sample size cross-sectional study including >130,000 serum 25(OH)D records of apparently healthy children aged 0–15 years in one of the biggest cities in China was conducted to establish pediatric RIs for serum 25(OH)D. Finally, the age-specific RIs for serum 25(OH)D of children were established using an indirect approach in Nanjing, China, which could guide appropriate clinical diagnosis and management of vitamin D insufficiency or deficiency for local children.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethical committee of the Women's Hospital of Nanjing Medical University, and the ethics approval number is 2023KY-09. Informed consent was not required for this study because this study was a retrospective analysis and used non-identified data.

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Data availability statement

Data associated with this study has not been deposited into a publicly available repository. The datasets will be made available on request.

CRediT authorship contribution statement

Chenchen Xu: Writing – original draft, Methodology, Data curation. **Xun Chen:** Writing – review & editing, Validation, Data curation. **Yajun Chen:** Visualization, Software. **Zhifa Wen:** Writing – review & editing, Supervision, Project administration. **Feng Cheng:** Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] T. Ciarambino, P. Crispino, G. Minervini, M. Giordano, Vitamin D: can gender medicine have a role? Biomedicines 11 (6) (2023) https://doi.org/10.3390/biomedicines11061762.
[2] K. Amrein, M. Scherkl, M. Hoffmann, S. Neuwersch-Sommeregger, M. Köstenberger, A. Tmava Berisha, et al., Vitamin D deficiency 2.0: an update on the current status worldwide, Eur. J. Clin. Nutr. 74 (11) (2020) 1498–1513, https://doi.org/10.1038/s41430-020-0558-y.
[3] Y. Wu, F. Wang, A. Li, J. Gao, B. Li, H. Sheng, et al., Vitamin D status among infants and children in Shanghai, China: a hospital-based study, Food Sci. Nutr. 11 (6) (2023) 3111–3120, https://doi.org/10.1002/fsn3.3292.
[4] Z. Chen, X. Lv, W. Hu, X. Qian, T. Wu, Y. Zhu, Vitamin D status and its influence on the health of preschool children in hangzhou, Front. Public Health 9 (2021) 675403, https://doi.org/10.3389/fpubh.2021.675403.
[5] A.A. Litonjua, Vitamin D and childhood asthma: causation and contribution to disease activity, Curr. Opin. Allergy Clin. Immunol. 19 (2) (2019) 126–131, https://doi.org/10.1097/aci.0000000000000509.
[6] X. Chen, Z. Wan, T. Geng, K. Zhu, R. Li, Q. Lu, et al., Vitamin D status, vitamin D receptor polymorphisms, and risk of microvascular complications among individuals with type 2 diabetes: a prospective study, Diabetes Care 46 (2) (2023) 270–277, https://doi.org/10.2337/dc22-0513.

[7] C.S. Souza, A.L. Deluque, B.M. Oliveira, A.L.D. Maciel, C. Giovanini, P.A. Boer, et al., Vitamin D deficiency contributes to the diabetic kidney disease progression via increase ZEB1/ZEB2 expressions, Nutr. Diabetes 13 (1) (2023) 9, https://doi.org/10.1038/s41387-023-00238-2.
[8] B. Thompson, M. Waterhouse, D.R. English, D.S. McLeod, B.K. Armstrong, C. Baxter, et al., Vitamin D supplementation and major cardiovascular events: D-Health randomised controlled trial, Bmj 381 (2023) e075230, https://doi.org/10.1136/bmj-2023-075230.
[9] H. Kim, M. Lipsyc-Sharf, X. Zong, X. Wang, J. Hur, M. Song, et al., Total vitamin D intake and risks of early-onset colorectal cancer and precursors, Gastroenterology 161 (4) (2021) 1208, https://doi.org/10.1053/j.gastro.2021.07.002, 17.e9.
[10] G.D. Carter, Accuracy of 25-hydroxyvitamin D assays: confronting the issues, Curr. Drug Targets 12 (1) (2011) 19–28, https://doi.org/10.2174/138945011793591608.
[11] M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, et al., Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline, J. Clin. Endocrinol. Metab. 96 (7) (2011) 1911–1930, https://doi.org/10.1210/jc.2011-0385.
[12] A.C. Ross, J.E. Manson, S.A. Abrams, J.F. Aloia, P.M. Brannon, S.K. Clinton, et al., The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know, J. Clin. Endocrinol. Metab. 96 (1) (2011) 53–58, https://doi.org/10.1210/jc.2010-2704.
[13] D. Yang, Z. Su, M. Zhao, Big data and reference intervals, Clin. Chim. Acta 527 (2022) 23–32, https://doi.org/10.1016/j.cca.2022.01.001.
[14] R. Marino, M. Misra, Extra-skeletal effects of vitamin D, Nutrients 11 (7) (2019), https://doi.org/10.3390/nu11071460.
[15] N. Karpova, O. Dmitrenko, E. Arshinova, M. Nurbekov, Review: influence of 25(OH)D blood concentration and supplementation during pregnancy on preeclampsia development and neonatal outcomes, Int. J. Mol. Sci. 23 (21) (2022), https://doi.org/10.3390/ijms232112935.
[16] X. Shan, X. Zhao, S. Li, P. Song, Q. Man, Z. Liu, et al., Association of serum 25(OH)D with metabolic syndrome in Chinese women of childbearing age, Nutrients 14 (11) (2022), https://doi.org/10.3390/nu14112301.
[17] H. Shi, H. Chen, Y. Zhang, J. Li, K. Fu, W. Xue, et al., 25-Hydroxyvitamin D level, vitamin D intake, and risk of stroke: a dose-response meta-analysis, Clin Nutr 39 (7) (2020) 2025–2034, https://doi.org/10.1016/j.clnu.2019.08.029.
[18] A. Herdea, A. Ionescu, M.C. Dragomirescu, A. Ulici, Vitamin D-A risk factor for bone fractures in children: a population-based prospective case-control randomized cross-sectional study, Int J Environ Res Public Health 20 (4) (2023), https://doi.org/10.3390/ijerph20043300.
[19] F. Alshahrani, N. Aljohani, Vitamin D: deficiency, sufficiency and toxicity, Nutrients 5 (9) (2013) 3605–3616, https://doi.org/10.3390/nu5093605.
[20] O. Korkut, H. Aydin, Neurological symptoms that may represent a warning in terms of diagnosis and treatment in a group of children and adolescents with vitamin D deficiency, Children 10 (7) (2023), https://doi.org/10.3390/children10071251.
[21] A. Javed, I.J. Kullo, P.B. Balagopal, S. Kumar, Effect of vitamin D3 treatment on endothelial function in obese adolescents, Pediatr Obes 11 (4) (2016) 279–284, https://doi.org/10.1111/ijpo.12059.
[22] C.M. Best, E.K. Pressman, R.A. Queenan, E. Cooper, K.O. O'Brien, Longitudinal changes in serum vitamin D binding protein and free 25-hydroxyvitamin D in a multiracial cohort of pregnant adolescents, J. Steroid Biochem. Mol. Biol. 186 (2019) 79–88, https://doi.org/10.1016/j.jsbmb.2018.09.019.
[23] J.J. Yang, Y.J. Kim, T. Youk, J.M. Byun, J. Yoo, T.S. Park, Hemoglobin concentration reference interval revisited: a nationwide study from korea, Clin. Lab. 65 (6) (2019), https://doi.org/10.7754/Clin.Lab.2018.181033.
[24] K.A. Herrick, R.J. Storandt, J. Afful, C.M. Pfeiffer, R.L. Schleicher, J.J. Gahche, et al., Vitamin D status in the United States, 2011-2014, Am. J. Clin. Nutr. 110 (1) (2019) 150–157, https://doi.org/10.1093/ajcn/nqz037.
[25] R.M. Mogire, A. Morovat, J.M. Muriuki, A.J. Mentzer, E.L. Webb, W. Kimita, et al., Prevalence and predictors of vitamin D deficiency in young African children, BMC Med. 19 (1) (2021) 115, https://doi.org/10.1186/s12916-021-01985-8.
[26] J. Yu, X. He, S. Huang, The establishment of serum 25-hydroxyvitamin D reference intervals in children aged 0-14 years in Zigong area, China, J. Physiol. Anthropol. 40 (1) (2021) 14, https://doi.org/10.1186/s40101-021-00265-x.