**Vitamin D Insufficiency and Its Association with Biochemical and Anthropometric Variables of Young Children in Rural Southwestern China**

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**Abstract**

Background: With recognition of the important roles of Vitamin D (VitD) in various physiological processes, increasing attention has been drawn to the status of VitD in early life. However, the VitD status of young children and the related factors in rural areas of Southwestern China remain unclear. This study aimed to explore VitD status and its seasonal variation in 18-month-old children living in rural Southwestern China. The association of VitD with biochemical and anthropometric variables was also investigated.

Methods: A total of 177 18-month-old children in a rural area of Yunnan Province, Southwestern China, were enrolled. Serum concentrations of 25-hydroxy Vitamin D (25(OH)D) were measured through high-performance liquid chromatogram-tandem mass spectrometry. Parathyroid hormone (PTH) levels were measured with a chemiluminescence assay. Serum concentrations of calcium, phosphorus, and alkaline phosphatase (ALP) were also measured. Anthropometric data and the outdoor activity time of each participant were collected.

Results: The serum 25(OH)D concentration was 26.61 ± 7.26 ng/ml; concentrations lower than 30 ng/ml accounted for 70.6% of the participants and concentrations lower than 20 ng/ml accounted for 16.4%. The level of serum 25(OH)D was not significantly different among four seasons (\(P > 0.05\)). A positive relationship was found between 25(OH)D concentration and the time of outdoor activities (\(r = 0.168, P < 0.05\)). Serum PTH concentration was negatively correlated with 25(OH)D concentration (\(r = -0.163, P < 0.05\)). A positive relationship was found between the serum concentrations of 25(OH)D and calcium (\(r = 0.154, P < 0.05\)). No significant association was observed between 25(OH)D and ALP, phosphorus, or anthropometric variables.

Conclusions: The prevalence of VitD insufficiency is high among young children in the rural Southwestern China regardless of the seasons. VitD supplementation is still essential to maintain VitD sufficiency for children living in rural area.

Key words: 25-Hydroxy Vitamin D; Infant; Rural Area; Vitamin D; Vitamin D Deficiency

**INTRODUCTION**

Vitamin D (VitD) is an essential nutrient with hormone-like activity that regulates calcium and bone metabolism throughout life. Adequate VitD is required among children for effective bone mineralization and normal growth. Reduced intestinal calcium and phosphate absorption and increased bone resorption might happen when the levels of VitD are too low. Furthermore, VitD insufficiency not only influences the bone formation of children, but it is also related to many other diseases, including respiratory infection, nocturnal enuresis, diabetes, and asthma in children.¹⁻⁸ The serum concentration of 25-hydroxy Vitamin D (25(OH)D) has been routinely used to assess the VitD status of children because it is the primary form that exists in the circulation.

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However, the serum concentration of 25(OH)D has also been reported to be related to parathyroid hormone (PTH), calcium, phosphorus, and alkaline phosphatase (ALP).\[16\,18\]

To keep the adequate serum levels of 25(OH)D, the US Endocrine Committee has suggested the intake of 400–1000 U/d under 1 year of age and 600–1000 U/d from 1 to 18 years of age.\[21\] However, most parents are not aware of VitD supplementation because of a lack of information with the high rates of reported suboptimal VitD levels among children.\[22\] Hence, VitD insufficiency or deficiency remains as an important public health problem in children, with a reported prevalence of 15%–80% worldwide.\[13\,15\]

Many studies observing the VitD status of young children have been conducted in the developed countries.\[16\,18\] Similarly, in the developing countries, almost all studies have been conducted in major cities where the living standard and socio-economic status are as high as developed countries.\[13\,19\,20\] However, few studies investigated the VitD status of young children in poor rural areas where less or no VitD supplementation were given to children. VitD status of young children and the potential factors related to VitD status in rural areas remain unclear.

The VitD status of young children without VitD supplementation in the poor rural area has been investigated in Xichou, which is a poor county in a rural area of Yunnan Province, Southwest China (located at 104°41′E, 23°25′N). The average altitude of this area is higher than 1000 m, and this area receives adequate sunlight. The participants enrolled in this study were all from the countryside and not given any VitD supplements. The serum concentrations of 25(OH)D, calcium, phosphorus, PTH, and ALP were measured to explore the VitD nutritional status of 18-month-old children in the rural Southwestern China. We also investigated the seasonal variation of VitD status and its relationship with biochemical indices and anthropometric data.

**Methods**

**Study design and subjects**

This study is a part of our large study entitled “Development and Health of Rural Chinese Children Fed Meat as a Daily Complementary Food from 6 to 18 Months of Age”, which was conducted in Yunnan Province. This is a cross-sectional study conducted in Xichou County, Yunnan Province of China, from May 2010 to April 2011. The eligibility criteria for infants were as following: healthy-term singleton infants, 17–19-month-old, a birth weight over 2500 g born between 37 and 42 weeks of gestational age, no metabolic or physical problems, breastfeeding for at least 6 months, living in Xichou County, and a willingness to follow the study protocol. The exclusion criteria included any preterm/low birth weight infant, use of VitD supplements, or medication that might affect VitD metabolism. Written informed consent was obtained from the mother of each infant. The study protocol was approved by the Ethics Committee of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. According to the prevalence of VitD deficiency reported in domestic studies and the following equation, the minimum sample size is estimated as 96 cases (equation 1).\[13\]

\[
\begin{align*}
n &= \frac{U^2 \cdot (1 - p_o)}{d^2} \cdot \frac{1}{d/n} \\
\end{align*}
\]

Where, \( U \) is the statistic of confidence level; \( p_o \) is the estimated prevalence of VitD deficiency (22%);\[13\] and \( d \) is the investigation error (20%).

**Biochemical measurements**

Nonfasting blood samples (5 ml) were obtained using venipuncture by a medical doctor. The serum was separated, aliquoted, and stored at −80°C until analysis. Serum 25(OH)D was measured using high-performance liquid chromatography-tandem mass spectrometry (AB Sciex, USA). The serum concentrations of 25(OH)D\(_1\) and 25(OH)D\(_3\) were successively measured. The serum concentration of PTH was assayed with a chemiluminescence assay (automatic chemiluminescence analyzer, Siemens, Germany). Serum concentrations of calcium, phosphorous, and ALP were determined using the ortho-cresolphthalein complexone, phosphomolybdic acid ultraviolet (UV), and French Society for clinical Biochemistry (SFBC) rate method, respectively.

**Anthropometric measurements**

All anthropometric measurements were carried out by two well-trained researchers. Height and weight were measured with a standard clinical Seca stadiometer (Seca, Germany) to the nearest 0.1 cm and a standard body electronic measuring scale (Seca) to the nearest 0.005 kg, respectively, without shoes and in light clothing. Body weight was measured twice consecutively; if the difference between the two measurements was less than 0.01 kg, the first measurement was recorded. Height was also measured twice consecutively, if the difference between the two measurements was <0.4 cm, the mean height of the two measurements was recorded. If the difference was more than 0.01 kg (0.4 cm), continuous measurements were conducted again until the difference between the two measurements was in the permissible range, and then the datum in the last measurement was recorded. The body mass index (BMI) was calculated according to the following equation: \( \text{BMI} = \frac{\text{weight (kg)}}{(\text{height [m]})^2} \). Z-scores were calculated using the WHO Anthro 3.0.1 software program (WHO Anthro, Geneva, Switzerland), including the weight-for-age Z-score (WAZ), weight-for-height Z-score (WHZ), and length-for-age Z-score (LAZ). Underweight, stunting, and wasting were defined as WAZ <−2, LAZ <−2, and WLZ <−2, respectively. Overweight was defined as WLZ >+2. Questionnaire about basic information, dietary intake, and the time for outdoor activities per week were collected in the face-to-face interviews.
Definition of Vitamin D status
In this study, VitD deficiency was defined as a serum 25(OH)D level <20 ng/ml, while VitD insufficiency was in the range of 20–30 ng/ml, and normal values were defined as >30 ng/ml.[14,21]

Statistical analysis
Continuous data were shown as mean ± standard deviation (SD). The frequency of subjects in each group of VitD status was identified. The distributions of all variables were checked for normality. Among the variables with normality, differences between two groups were examined using the independent samples t-test. When more than two groups were considered, one-way analysis of variance (ANOVA) was used. Pearson correlation coefficients were calculated to investigate the correlations between VitD status and other normal variables. A significance level of 0.05 (P value) was selected for the test. Statistical analyses were performed using SPSS 17.0 software (IBM Corp., Chicago, IL, USA).

RESULTS
Sample characteristics
One hundred and eighty-four young children were enrolled in this study. Among these children, seven subjects were excluded for unavailable biochemical data. Totally, 177 children were enrolled into the final data analysis. The participants included 85 boys (48.0%) and 92 girls (52.0%) with a mean age of 18.0 months (range: 17.8–18.1 months). The mean time of outdoor activities for all children was 21.3 h/week, and there was no significant difference between boys and girls (P > 0.05). According to the WHO Child Growth Standards, there were 57 children (32.2%) exhibiting signs of stunting (LAZ <-2), 17 underweight (9.6%) (WAZ <-2), and two wasting (1.1%) (WLZ <-2). In this study, the height and weight of the boys were significantly higher than those of the girls, as same as the BMI (P < 0.05). The difference of the LAZ between the boys and girls was also significant (P < 0.05). However, there was no significant difference in age, WHZ, or WAZ between the two genders [Table 1].

Measurements of 25-hydroxy Vitamin D concentration
25(OH)D exists in two forms in serum, namely 25(OH)D3 and 25(OH)D2. In this study, the levels of both 25(OH)D3 and 25(OH)D2 were measured; however, 25(OH)D3 was detected in only eight cases. The peak serum concentration of 25(OH)D2 measured was 12.60 ng/ml, accounting for 38.2% of the 25(OH)D in one case, whereas 25(OH)D2 only accounted for approximately 10.0% of the 25(OH)D in all of the other seven cases. Serum 25(OH)D levels ranged from 7.70 to 58.50 ng/ml, with a mean concentration of 26.61 ± 7.26 ng/ml. Sufficient concentrations of 25(OH)D were found in 29.4% of all cases. A total of 125 (70.6%) children presented with low blood levels of 25(OH)D, with 29 (16.4%) children exhibiting 25(OH)D concentration of <20 ng/ml. No significant difference in the 25(OH)D concentration was found between the boys and girls (P > 0.05) [Table 1].

Seasonal variation of 25-hydroxy Vitamin D concentration
According to different blood collection times, the subjects were divided into four groups: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). The serum 25(OH)D concentration was 25.28 ± 7.14 ng/ml, 26.71 ± 6.47 ng/ml, 28.51 ± 8.38 ng/ml, and 25.99 ± 6.43 ng/ml in spring, summer, autumn and winter, respectively. The seasonal variations in the serum of 25(OH)D concentrations are shown in Figure 1. There was no significant difference of 25(OH)D concentrations among these four seasons (P > 0.05).

Table 1: Characteristics of anthropometric and biochemical measurements of 177 18-month-old children in a rural area of Yunnan Province, Southwestern China, enrolled in this study

| Items                | Boys (n = 85) | Girls (n = 92) | t    | P     |
|----------------------|---------------|----------------|------|-------|
| Age (months)         | 18.0 ± 0.3    | 18.0 ± 0.2     | 0.715| 0.476 |
| Weight (kg)          | 9.90 ± 0.94   | 9.33 ± 0.91    | 4.076| 0.000 |
| Height (cm)          | 77.69 ± 2.62  | 76.77 ± 2.63   | 2.327| 0.021 |
| BMI (kg/m²)          | 16.37 ± 1.04  | 15.80 ± 1.04   | 3.645| 0.000 |
| WHZ                  | −0.19 ± 0.79  | −0.23 ± 0.76   | 0.323| 0.747 |
| WAZ                  | −0.94 ± 0.86  | −0.80 ± 0.81   | −1.090| 0.277 |
| LAZ                  | −1.70 ± 0.99  | −1.34 ± 0.89   | −2.497| 0.013 |
| 25(OH)D (ng/ml)      | 27.41 ± 7.59  | 25.88 ± 6.90   | 1.407| 0.161 |
| Calcium (mmol/L)     | 2.39 ± 0.10   | 2.41 ± 0.11    | −1.201| 0.231 |
| ALP (U/L)            | 214.98 ± 66.26| 222.99 ± 73.50 | −0.760| 0.449 |
| PTH (pg/ml)          | 15.11 ± 8.12  | 17.47 ± 10.21  | −1.690| 0.093 |
| Phosphorus (mmol/L)  | 1.77 ± 0.24   | 1.74 ± 0.26    | 0.715| 0.476 |
| Outdoor time (h)     | 21.34 ± 0.98  | 20.96 ± 0.92   | 0.430| 0.673 |

Values were shown as mean ± SD. BMI: Body mass index; WAZ: Weight-for-age Z-score; WHZ: Weight-for-height Z-score; LAZ: Length-for-age Z-score; 25(OH)D: 25-hydroxy Vitamin D; PTH: Parathyroid hormone; ALP: Alkaline phosphatase; SD: Standard deviation.

Figure 1: Serum 25-hydroxy Vitamin D concentration among different seasons.
Measurement of biochemical variables
The concentrations of calcium, phosphorus, ALP, and PTH were 2.40 ± 0.11 mmol/L, 1.75 ± 0.25 mmol/L, 219.14 ± 70.03 U/L, and 16.34 ± 9.32 pg/ml, respectively. There was no significant difference in the serum PTH levels between boys and girls (P > 0.05), and neither were the differences of calcium, ALP, or phosphorus between the two genders (P > 0.05 for all).

Relationship of 25-hydroxy Vitamin D concentration with biochemical and anthropometric variables
The correlation coefficients between the 25(OH)D concentration and biochemical variables are presented in detail in Table 2. There was a significant difference among the concentration of PTH in different 25(OH)D status (P < 0.05) [Figure 2]. A significant negative correlation was found between the 25(OH)D concentration and serum PTH level (r = −0.163, P = 0.030) [Figure 3]. The 25(OH)D concentration was positively correlated with the serum calcium concentration (r = 0.154, P = 0.040) [Figure 4]. However, the 25(OH)D concentration was not found to be significantly related to the serum levels of ALP or phosphorus (P > 0.05 for both). No significant correlation was found between the 25(OH)D concentration and several anthropometric variables (WAZ, WHZ, LAZ, and BMI) (P > 0.05 for all) [Table 2].

DISCUSSION
VitD deficiency is regarded as the most common nutritional deficiency and also one of the most common undiagnosed medical conditions worldwide.[22] It has been estimated that one billion people worldwide have VitD deficiency or insufficiency. Though the optimal level of VitD is still controversial, it has been widely adopted in the clinic that VitD insufficiency is defined as a serum 25(OH)D level <30 ng/ml and deficiency is defined as serum levels of 25-(OH)D <20 ng/ml. The prevalence of VitD deficiency varies among different populations and regions.[16] In this study, 70.6% (125 subjects) of all the participants were

| Variables      | Correlation coefficient | P     |
|----------------|------------------------|-------|
| PTH            | −0.163                 | 0.031 |
| ALP            | −0.004                 | 0.956 |
| Calcium        | 0.154                  | 0.041 |
| Phosphorus     | 0.062                  | 0.416 |
| WAZ            | 0.135                  | 0.073 |
| WHZ            | 0.110                  | 0.147 |
| LAZ            | 0.108                  | 0.152 |
| BMI            | 0.085                  | 0.258 |
| Outdoor time   | 0.168                  | 0.029 |

25(OH)D: 25-hydroxy Vitamin D; PTH: Parathyroid hormone; ALP: Alkaline phosphatase; WAZ: Weight-for-age Z-score; WHZ: Weight-for-height Z-score; LAZ: Length-for-age Z-score.
observed to have VitD insufficiency and 16.4% (29 subjects) with VitD deficiency. Compared with domestic studies, the children in our study had a much lower prevalence of VitD deficiency (89.2% in Beijing, 58.6% in Shanghai, and 22% in Hangzhou).\[13,19,20\] In our study, 29 (16.4%) children presented with 25(OH)D concentration <20 ng/ml, which was similar to the study performed by Saintonge et al.\[16\] However, in the study performed in Britain, 35% of all subjects had serum 25(OH)D concentration <20 ng/ml, so were the studies conducted in Belgian and Tehran.\[17,18\] Several factors might account for the relatively lower prevalence rate of VitD deficiency in this study. The present study was conducted in Xichou County, which is at a lower latitude (23°25′N) and higher altitude (more than 1000 m). Children living in this area are exposed to UV irradiation with stronger UV intensity for the latitude- and altitude-influenced solar exposure. Furthermore, participants in our study had long time of outdoor activities with a mean time of 21.25 h. The children were not given proper protection from the sun exposure due to lack of awareness. Thus, the sunlight-induced VitD synthesis was higher among our participants compared with that in other reports.\[13,19,20\] Besides that, this study was conducted in community rather than in hospitals. The children have better general health compared with the hospital-based population.

It is worth noting that the prevalence of VitD insufficiency is still higher compared with other studies in which the children received VitD-fortified diets.\[9\] As we know, there are two main sources of VitD for humans. One source is the cutaneous synthesis of VitD (mainly VitD3) and the other source is dietary intake (mainly VitD2). VitD from either source is efficiently converted to 25(OH)D. In our study, the children took no VitD supplements, and the VitD intake from diet was rare. The lack of VitD supplementation might be the reason for the high prevalence of VitD insufficiency in this rural area, even though the latitude of this area is low and the solar exposure is ample.

In this study, no significant difference in the serum 25(OH)D concentrations among the four seasons was found. However, Kemp et al. reported that the serum 25-OH-D concentrations in summer were significantly higher than those in winter.\[23\] This discrepancy may be mainly caused by the different study sites. Xichou County, where the present study was conducted, is located at 23°25′N, within the plateau area. The UV intensity varies slightly throughout the entire year. Hence, there was no significant difference among four seasons. However, the level of VitD in this plateau area is still varied in the four seasons. In summer, children were exposed to UV radiation less clothes. Therefore, the serum 25(OH)D concentration in autumn reached to the peak after accumulation during summer. In winter, children wore more and exposed less to UV radiation for the coldness. The 25(OH)D level of Spring was the lowest after being consumed during winter. It is worthwhile noting that although the level of 25(OH)D in autumn was the highest among the four seasons, the mean 25(OH)D concentration was still insufficient (28.51 ± 8.38 ng/ml), which implies that insufficient VitD status is common in this area.

The anthropometric characteristics of the participants demonstrated that the growth and development status of the children in this study are lower than the WHO standard. This lower status is mainly because our subjects were all from one rural area in Southwest China where socio-economic levels are low. Razzaghy-Azar and Shakiba reported that the 25(OH)D level in males is significantly greater than that in females.\[24\] However, significant difference in the 25(OH)D concentrations between boys and girls was not observed in this study. This discrepancy might be caused by the age differences of the subjects recruited in the studies. This study enrolled a much younger population with a mean age of 18 months. Whereas, some subjects recruited in other studies were in puberty, which exerts significant differences in development between boys and girls. These developmental status subsequently resulted in different requirements for 25(OH)D. Furthermore, compared with boys, adolescent girls are more reluctant to bask in the sun. VitD is more easily deposited and stored in the fat.\[16,25,26\] However, in this study, there was no difference in the time of outdoor activities between the boys and girls.

The small intestine absorbs no more than 10%–15% of dietary calcium without VitD. However, the absorption efficiency of dietary calcium in the small intestine is 30% on average if the person has sufficient VitD, increasing to as high as 80% during growth, lactation, and pregnancy.\[27\] In the present study, a positive correlation was found between the serum concentration of calcium and 25(OH)D, revealing that VitD deficiency can reduce the absorption of calcium. VitD supplement is required for children in this rural area to increase the calcium absorption. VitD has also been reported to promote the absorption of phosphorous in the gastrointestinal tract.\[28\] However, no significant associations of the 25(OH)D concentration with the levels of phosphorous or ALP was found in this study. The result was consistent with other studies conducted in New Zealand, Tehran, or California.\[29,30\]

The serum calcium concentration decreases when the level of 25(OH)D concentration is low. Meanwhile, the decrease in serum calcium concentration promotes the secretion of PTH to maintain the calcium concentration. The negative correlation between the concentration of 25(OH)D and PTH was found in this study, which is consistent with previous studies.\[27,29\] Weng et al. have estimated the 25(OH)D concentration required for maximal suppression of PTH in children and adolescents according to the inverse relationship between 25(OH)D and PTH.\[25\] The inverse relationship between serum 25(OH)D and PTH concentration reveals that a poor VitD status can be defined as the 25(OH)D concentration below which the PTH concentration will rise. It is presumed that at this concentration of 25(OH)D, the 1,25(OH)2D production is inadequate to meet its requirements for function in the parathyroid gland. A high serum concentration of PTH may have adverse effects on bone health. Therefore, VitD supplementation is requisite for young children in this rural area.
There are some limitations in this study: the sample size in this study was relatively small; however, to the best of our knowledge, it is larger than other studies conducted in this rural area of Southwestern China to assess VitD status. The optimal 25(OH)D concentration for the children in this area was not determined in this study. Great caution should be noted when interpreting the seasonal variation of 25(OH)D because this is a cross-sectional study, where each participant only had one observation.

In conclusion, the majority of young children in the rural Southwest China had VitD insufficiency regardless of the season. VitD supplementation might still be essential to young children in this rural area because ample solar exposure alone does not enable children to maintain VitD sufficiency.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Holick MF, Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. Am J Clin Nutr 2008;87:1080S-6S.
2. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum Vitamin D levels and severe asthma exacerbations in the childhood asthma management program study. J Allergy Clin Immunol 2010;126:52-8.e5. doi:10.1067/j.jaci.2010.03.043.
3. Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. J Allergy Clin Immunol 2011;127:1249-6. doi: 10.1002/jpul.21089.
4. McNally JD, Leis K, Matheson LA, Karuunanyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatr Pulmonol 2009;44:981-8. doi:10.1002/ppul.21089.
5. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. Acta Paediatr 2010;99:389-93. doi:10.1111/j.1651-2227.2009.01594.x.
6. Ashraf A, Alvarez J, Saenz K, Gower B, McCormick K, Franklin F. Threshold for effects of Vitamin D deficiency on glucose metabolism in obese female African-American adolescents. J Clin Endocrinol Metab 2009;94:3200-6. doi: 10.1210/jc.2009-0445.
7. Johnson MD, Nader NS, Weaver AL, Singh R, Kumar S. Relationships between 25-hydroxyvitamin D levels and plasma glucose and lipid levels in pediatric outpatients. J Pediatr 2010;156:444-9. doi: 10.1016/j.peds.2009.09.070.
8. Li L, Zhou H, Yang X, Zhao L, Yu X. Relationships between 25-hydroxyvitamin D and nocturnal enuresis in five to seven-year-old children. PLoS One 2014;9:e99316. doi: 10.1371/journal.pone.0099316.
9. Liang GY, Qin R, Li J, Liang GX, Guan YJ, Gao ZH. Optimal level of 25-(OH)D in children in Nanjing (32°N Lat) during winter. Pediatr Int 2011;53:541-5. doi: 10.1111/j.1442-200X.2010.03309.x.
10. Carpenter TO, Herreros F, Zhang JH, Ellis BK, Simpson C, Torrealba-Fox E, et al. Demographic, dietary, and biochemical determinants of Vitamin D status in inner-city children. Am J Clin Nutr 2012;95:137-46. doi: 10.3945/ajcn.111.018721.
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of Vitamin D deficiency: An endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30. doi: 10.1210/jc.2011-0385.
12. Druzy R, Rehm A, Jodal S, Nadler R. Vitamin D supplementation: We must not fail our children! Medicine (Baltimore) 2015;94:e817. doi: 10.1097/MMD.0000000000000817.
13. Zhu Z, Zhan J, Shao J, Chen W, Chen L, Li W, et al. High prevalence of Vitamin D deficiency among children aged 1 month to 16 years in Hangzhou, China. BMC Public Health 2012;12:126. doi: 10.1186/1471-2458-12-126.
14. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81. doi: 10.1056/NEJMra070553.
15. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: A global perspective of current status. J Nutr 2005;135:310-6.
16. Saintonge S, Bang H, Gerber LM. Implications of a new definition of Vitamin D deficiency in a multicultural us adolescent population: The National Health and Nutrition Examination Survey III. Pediatrics 2009;123:797-803. doi: 10.1542/peds.2008-1195.
17. Sioen I, MouraTidou T, Kauffman JM, Bamann K, Nichols N, Pigeot I, et al. Determinants of Vitamin D status in young children: Results from the Belgian arm of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants) study. Public Health Nutr 2012;15:1093-9. doi: 10.1017/S1368980011002989.
18. Absoud M, Cummins C, Lim MJ, Wassmer E, Shaw N. Prevalence and predictors of Vitamin D insufficiency in children: A Great Britain population based study. PLoS One 2011;6:e22179. doi: 10.1371/journal.pone.0022179.
19. Foo LH, Zhang Q, Zhu K, Ma G, Trube A, Greenfield H, et al. Relationship between Vitamin D status, body composition and physical exercise of adolescent girls in Beijing. Osteopors Int 2009;20:417-25. doi: 10.1007/s00198-008-0667-2.
20. Yu X, Zhang J, Yan C, Shen X. Relationships between serum 25-hydroxyvitamin D and quantitative ultrasound bone mineral density in 0-6 year old children. Bone 2013;53:306-10. doi: 10.1016/j.bone.2012.12.012.
21. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, 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 2011;96:53-8. doi: 10.1210/jc.2010-2704.
22. Balasubramanian S, Dhanalakshmi K, Amperayani S. Vitamin D deficiency in childhood – A review of current guidelines on diagnosis and management. Indian Pediatr 2013;50:669-75.
23. Kemp FW, Neti PV, Howell RW, Wengier P, Louria DB, Bogden JD. Elevated blood lead concentrations and Vitamin D deficiency in winter and summer in young urban children. Environ Health Perspect 2007;115:630-5. doi: 10.1289/ehp.9389.
24. Razaghy-Azar M, Shakiba M. Assessment of Vitamin D status in healthy children and adolescents living in Tehran and its relation to iPTH, gender, weight and height. Am Hum Biol 2010;23:692-701. doi: 10.1111/j.1462-0314.2010.005234.
25. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS. Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. Am J Clin Nutr 2007;86:150-8.
26. Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. Nutr Rev 2006;64:479-86. doi: 10.1038/nr.2006.nov.479486.
27. Holick MF. Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 2004;79:362-71.
28. Abrams SA. In utero physiology: Role in nutrient delivery and fetal development for calcium, phosphorus, and Vitamin D. Am J Clin Nutr 2007;85:6045-7S.
29. Houghton LA, Szymlek-Gay EA, Gray AR, Ferguson EL, Deng X, Heath AL. Predictors of Vitamin D status and its association with...
30. Liang L, Chantry C, Styne DM, Stephensen CB. Prevalence and risk factors for Vitamin D deficiency among healthy infants and young children in Sacramento, California. Eur J Pediatr 2010;169:1337-44. doi: 10.1007/s00431-010-1226-3.

31. Ardawi MS, Sibiani AM, Bakhsh TM, Quri MH, Maimani AA. High prevalence of Vitamin D deficiency among healthy Saudi Arabian men: Relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. Osteoporos Int 2012;23:675-86. doi: 10.1007/s00198-011-1606-1.

32. Carnevale V, Nieddu L, Romagnoli E, Battista C, Mascia ML, Chiodini I, et al. Regulation of PTH secretion by 25-hydroxyvitamin D and ionized calcium depends on Vitamin D status: A study in a large cohort of healthy subjects. Bone 2010;47:626-30. doi: 10.1016/j.bone.2010.06.013.