Serum 25-Hydroxyvitamin D and Bone Mineral Density among Children and Adolescents in a Northwest Chinese City

Jing Li, MD¹, Wenqing Ding, PhD¹, Juan Cao, MD¹, Lijia Sun, MD¹, Shanghong Liu, MD¹, Jianjun Zhang, MD, PhD², and Haiping Zhao, MD¹

1. Department of Child and Adolescent Health, School of Public Health and Management, Ningxia Medical University, Yinchuan, Ningxia Hui Autonomous Region, China
2. Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health, Indianapolis, IN, USA

Co-corresponding authors:
Haiping Zhao, MD, Department of Child and Adolescent Health, School of Public Health and Management, Ningxia Medical University, 1160 Shengli Street, Yinchuan 750004, China. Phone: 0951-6980016. Email: zhpgj@163.com

Jianjun Zhang, MD, PhD, Department of Epidemiology, Fairbanks School of Public Health, Indiana University, 1050 Wishard Boulevard, RG5118, Indianapolis, IN 46202, USA. Phone: 317-274-4287. Fax: 317-274-3443. Email: JZ21@iu.edu

Funding: This work was supported by the National Key Research and Development Program of China (2016YFC1300101) and the National Natural Science Foundation of China (81660565).

This is the author's manuscript of the article published in final edited form as:
Abstract

Although vitamin D is essential for bone health, little is known about prevalence of vitamin D deficiency and low bone mineral density (BMD) among children, especially those in developing countries. It also remains unclear whether serum 25-hydroxyvitamin D [25(OH)D] is associated with BMD among children. We investigated these questions among children and adolescents in Yinchuan (latitude: 38° N), Ningxia, an economically underdeveloped province in Northwest China. A total of 1582 children (756 boys and 826 girls), aged 6-18 years, were recruited from schools using the stratified random sampling method in fall 2015. Serum 25(OH)D concentrations were measured by enzyme-linked immunosorbent assay, and BMD was quantified by dual-energy X-ray absorptiometry. Vitamin D deficiency (defined as serum 25(OH)D \(\leq 37.5\) nmol/L) was present in 35.5% of study subjects. There were no clear patterns of differences in serum 25(OH)D concentrations across the four age groups compared (6-9 years, 10-13 years, 14-16 years, and 17-18 years). The prevalence of low total body less head (TBLH) BMD (defined as a Z-score of \(\leq -2.0\) standard deviations away from the mean BMD values of the Chinese pediatric reference population) among children examined was 1.8% and was not significantly different among the four age groups considered. Linear regression analysis revealed that age, weight, and height were significantly and positively associated with TBLH BMD and that the strongest determinant of TBLH BMD was age in boys and weight in girls. There were no significant correlations between serum 25(OH)D concentrations and BMD obtained for total body and at various skeletal sites (r ranged from -0.005 to 0.014) regardless of whether children evaluated were sufficient, insufficient, or deficient in vitamin D. In conclusion, more than one-third of
children and adolescents in a Northwest Chinese city were deficient in vitamin D but only less than 2% of them developed low BMD.

**Key Words:** Serum 25-hydroxyvitamin D; bone mineral density; prevalence; Chinese; children and adolescents
Introduction

Vitamin D is essential for bone health as one of its major functions is to promote calcium absorption in the intestine. Calcium and phosphorus are required for bone mineralization, a critical process for bone growth among children and adolescents [1]. There are three sources of vitamin D for human subjects: foods (e.g. fatty fish, egg yolk), supplements [primarily D2 (ergocalciferol)], and skin synthesis by exposure to ultraviolet B [D3 (cholecalciferol)] [2]. Of these sources, sunlight exposure is the primary contributor to the vitamin D nutritional status for most free-living individuals worldwide [2].

Vitamin D undergoes two hydroxylation reactions before it becomes functional. Vitamin D is first converted to 25 hydroxyvitamin D [25(OH)D] in the liver and then further converted to 1,25 dihydroxyvitamin D [1,25(OH)2D] in the kidney [3, 4]. Although 1,25(OH)2D is the biologically active hormone form, circulating concentrations of 25(OH)D are commonly measured as a biomarker of vitamin D nutritional status as it is more abundant and has a longer half-life (2-3 weeks) [5]. Serum 25(OH)D is an integrated reflection of intake of the vitamin from foods, supplements, and skin synthesis. It has been estimated that vitamin D deficiency is present in approximately one-sixth of the world population [6]. Vitamin D deficiency is more pronounced among adolescents and elderly people in developing countries with limited consumption of vitamin D-rich foods and infrequent use of vitamin supplements [6, 7], with a reported global prevalence of 30%-80% in children [2]. China is a developing country with a huge number of school children. However, data regarding vitamin D status in Chinese populations, especially children and adolescents, are scarce.

Childhood and adolescence are critical periods of time for bone growth. In a prospective cohort study of Canadian school children, 35% of total body bone mineral accrued during four peripubertal years [8]. A growing body of evidence indicates that subjects with higher peak bone mass acquired by early adulthood experience a lower risk of osteoporotic fractures.
later in life [9, 10]. This protective effect of early-life origin is biologically plausible as osteoporosis risk is determined by the level of peak bone mass accrued during childhood and adolescence and the rate of bone loss during aging [11]. It is thus important to measure bone mineral density (BMD) for the identification of children and adolescents with low BMD who are at increased risk of developing osteoporosis and subsequent fractures.

Despite the role of vitamin D in bone mineral deposition, it remains inconsistent across previous studies whether circulating 25(OH)D concentrations are associated with BMD among free-living individuals, particularly children and adolescents [12, 13]. Furthermore, the determinants of BMD are not well understood among these subjects at the population level. Therefore, the present study sought to investigate these research questions and to estimate the prevalence rates of vitamin D deficiency and low BMD among school children in Yinchuan, the capital city of the Ningxia Hui Autonomous Region, an economically underdeveloped province in Northwest China.

Materials and Methods

Study subjects

A total of 1582 school children, aged 6-18 years (including 617 Han ethnic males, 612 Han ethnic females, 139 Hui ethnic males, and 214 Hui ethnic females) were recruited from Yinchuan (latitude: 38° N) from September to November 2015, using the stratified random sampling method. Children were eligible if they had lived in Yinchuan with their parents or guardians for more than six months prior to the start of the study, were free from metabolic bone disease, and did not use hormone preparations or therapeutic doses of vitamin D. One elementary school, one middle school, and one high school were randomly selected from all schools in each of these three levels of schools in Yinchuan, respectively. Each grade was considered as a single sampling stratum. Two classes were randomly selected from each of 12 grades. However, one more class was chosen from the first, sixth, ninth, and twelfth grades to
ensure that at least 50 boys and 50 girls were enrolled to the study from each of 13 age
groups considered. After exclusion of ineligible students from 28 selected classes, 1582
children were finally enrolled to the study. The study protocol was approved by the Research
Ethics Committee of Ningxia Medical University, and written informed consent was obtained
from the parents or guardians of all recruited children.

**Questionnaire data and anthropometric measurements**

In-person interviews with selected school children were conducted by trained research
staff using a risk factor questionnaire. If students were 6-9 years of age, the questionnaire was
completed by their parents or guardians. Students who were 10-18 years of age responded to
the questionnaire by themselves. The information solicited through the questionnaire
included age, gender, race, school, grade, cigarette smoking (never, attempted, and current),
alcohol consumption (never, attempted, and current), time spent on outdoor activity
(day/week), nocturnal emission (yes or no), regular menstruation (yes or no), and medical
history. Height without shoes were measured to the nearest 0.1 cm and weight with light
clothes were determined to the nearest 0.1 kg, using a portable weighing scale with height rod
(TXRGZB-200-RT). Both height and weight were quantified twice for each subject and two
measurements were averaged and used in data analysis.

**Serum 25(OH)D measurements**

After blood samples were collected from study subjects, serum was separated and stored
in −20°C freezers until analysis. Serum 25(OH)D concentrations (nmol/L) were measured by
enzyme-linked immunosorbent assay (ELISA) according to the instructions of commercially
available kits purchased from the Shanghai KeShun Biotechnology Co. Ltd. The same batch
of the ELISA kits were used to determine serum 25(OH)D for all study subjects. The intra-
and inter-assay coefficients of variation were 10% and 15%, respectively.

**BMD measurements**
BMD (g/cm²) was measured by dual-energy X-ray absorptiometry (DXA) (Hologic Discovery Fan Beam Densitometer, Bedford, MA), a simple, safe, and precise method recommended by the International Society for Clinical Densitometry (ISCD) [14]. The skeletal sites that were scanned for BMD included total body, head, left arm, right arm, left ribs, right ribs, thoracic spine, lumbar spine, pelvis, left leg, and right leg. Total body less head (TBLH) BMD was calculated as the head accounts for a substantial proportion of total bone mass but changes little with physiological growth and physical activity [15]. Quality assurance measures, including the calibration of the DXA machines with standard phantom every day, were implemented to ensure the accuracy and validity of BMD data. The coefficients of variation of total body BMD measurements was 0.47%.

**Statistical analysis**

Demographical, anthropometrical, physiological, and lifestyle characteristics of study subjects were compared between sexes with t-test for continuous variables and chi-square test for categorical variables. Serum 25(OH)D concentrations and BMD were analyzed separately for all subjects and four age groups (6-9 years, 10-13 years, 14-16 years, and 17-18 years). Differences in serum 25(OH)D and BMD for total body and at various skeletal sites among the four age groups were evaluated with analysis of variance. Vitamin D insufficiency and deficiency were defined as serum 25(OH)D concentrations of 37.5-50 nmol/L and ≤37.5 nmol/L, respectively, which was recommended by both the Society of Pediatrics, Chinese Medical Association [16] and the Pediatric Endocrine Society [17]. The Z-score is routinely used for children as it compares the BMD of a given child to the average BMD of children of the same age and sex [18, 19]. Low BMD was defined as a Z-score of less than or equal to -2.0 standard deviations (SD) [14] away from the mean BMD values of the Chinese pediatric reference population [20]. Prevalence rates of vitamin D insufficiency, vitamin D deficiency,
and low TBLH BMD were calculated for all subjects and the four age groups as well as compared among the age groups.

Pearson correlations between serum 25(OH)D concentrations and BMD for total body and at different skeletal sites were carried out for all subjects and those with vitamin D sufficiency, insufficiency, or deficiency. In this analysis, serum 25(OH)D concentrations were log-transformed to improve the normality of distribution. Sex-specific multiple linear regression was used to examine the associations of TBLH BMD with serum 25(OH)D, age, race (Nan vs. Hui), height, weight, regular menstruation (yes/no; for girls only), nocturnal emission (yes/no; for boys only), and time spent on outdoor activity. These independent variables were examined because they are potential BMD predictors. Standardized regression coefficients were computed to evaluate the relative contributions of these variables to variations in TBLH BMD. In addition, the relations between age and TBLH BMD were plotted separately for all boys and all girls. Statistical analysis was performed using SPSS version 23 (Armonk, NY). A $p$-value of <0.05 was considered statistically significant.

**Results**

The mean ages (SD) of boys and girls were 12.4 (3.6) and 12.9 (3.6) years, respectively. Boys were overall taller and heavier than girls. While 24.6% of girls underwent regular menstruation, 16.5% of boys experienced nocturnal emission (Table 1).

The median concentrations of 25(OH)D were 50.5 nmol/L, with an interquartile range (IQR) of 30.5-94.9 nmol/L. Vitamin D deficiency was present in 35.5% of this study population (Table 2). There were no clear patterns of differences in serum 25(OH)D concentrations across the four age groups compared, with the highest concentrations observed in the youngest age group (6-9 years). Mean concentrations of 25(OH)D among boys (50.5 nmol/L) were almost identical to those among girls (50.7 nmol/L) (data not shown).

As expected, there was a significant, monotonic increase in BMD with age for total body
and all skeletal sites measured (all *p* values <0.0001) (Table 3). For example, mean values (SDs) of TBLH BMD were 0.60 (0.05), 0.70 (0.07), 0.86 (0.07), and 0.93 (0.07) g/cm² among boys aged 6-9 years, 10-13 years, 14-16 years, and 17-18 years, respectively. The corresponding mean values (SDs) for girls were 0.58 (0.06), 0.72 (0.07), 0.81 (0.06), and 0.84 (0.06) g/cm². Of note, 1.8% of children and adolescents examined were classified as low TBLH BMD, with no significant difference in low TBLH BMD prevalence between girls (1.7%) and boys (2.0%) (data not shown). Differences in the prevalence rates of low TBLH BMD among the four age groups considered were not statistically significant in both sexes. A strong correlation existed between age and TBLH BMD (*r*=0.89 for boys and *r*=0.85 for girls, all *p*<0.0001) (Figure 1).

Multiple linear regression analysis revealed highly significant, positive associations of age, height, and weight with TBLH BMD in both boys and girls. Time spent on outdoor activity was inversely but weakly associated with TBLH BMD in boys (*p*=0.025). There were no significant associations of serum 25(OH)D, race, and nocturnal emission with TBLH BMD, but a marginally significant association between regular menstruation and TBLH BMD was observed (*p*=0.06). All variables included in the models accounted for 86.7% and 86.1% of variation in TBLH BMD in boys and girls, respectively. Standardized regression coefficients indicated that age, weight, and height were the main predictors of TBLH BMD in both sexes (Table 4). In addition, body mass index (BMI) was also positively associated with TBLH BMD in both boys and girls (all *p*<0.0001) after adjustment for all other relevant variables in Table 4. A gradient increase in TBLH BMD across BMI quartiles were found, with mean values (SDs) of TBLH BMD being 0.61±0.08, 0.73±0.1, 0.81±0.1, and 0.84±0.1 for the first, second, third, and fourth quartiles, respectively (*p*<0.0001). No significant differences in TBLH BMD existed among normal weight, overweight, and obese school children (data not shown).
There were no associations between log-transformed serum 25(OH)D concentrations and BMD values of total body and different skeletal sites (r ranged from -0.005 to 0.014; all p values >0.05) (Table 5). Similar weak or null associations were observed when analyses were performed separately for subjects with vitamin D sufficiency, insufficiency, and deficiency, although some correlations were statistically significant primarily due to large sample size.

**Discussion**

In the present study, we found that vitamin D deficiency and low TBLH BMD were present among 35.5% and 1.8% of school children examined, respectively. Serum 25(OH)D concentrations were overall not associated with BMD for total body and at various skeletal sites. The major determinants of TBLH BMD were age, height, and weight for both sexes.

The median (IQR) 25(OH)D level of children evaluated in our study was 50.5 (30.5-94.9) nmol/L, which is similar to that of children of similar ages in the Chinese National and Health Survey [median (IQR) serum 25(OH)D: 48.2 (35.4–63.4) nmol/L] [21]. Serum 25(OH)D concentrations among our study subjects were overall higher than those of children in Northern European countries and Canada but lower than those of children in the U.S., UK, and some Western and southern European countries [22]. Differences in vitamin D status among children and adolescents in different parts of the world are largely ascribed to differences in latitude, air pollution, skin pigmentation, and dietary habits [4]. In addition, assays used for serum 25(OH) measurement and seasons of blood collection might have also contributed to differences in serum 25(OH)D concentrations reported in previous studies [12, 17, 23].

There are some controversies over the healthy level of serum 25(OH)D for children and adolescents. Vitamin D deficiency was defined as a serum 25(OH)D level of <30.0, 25.0, and 37.5 nmol/L by the Institute of Medicine in 2001 (insufficiency: 30-50 nmol/L), the Canadian Pediatric Society in 2007, and the Pediatric Endocrine Society in 2008, respectively [24]. In
2008, the American Academy of Pediatrics recommended that serum 25(OH)D concentrations in infants and children should be ≥50 nmol/L [5]. We identified that vitamin D deficiency was present in 35.5% of our study subjects using the Pediatric Endocrine Society criteria that have been formulated on the basis of bone-related biomarkers (e.g. alkaline phosphatase, bone density, calcium absorption) and rickets risk [17]. The prevalence of vitamin D deficiency (defined as serum 25(OH)D of <50 nmol/L) was 53.2% in a national study of Chinese school children (aged 6-17 years) [21] and 45.9% in a regional study of Italian children and adolescents (2-21 years) [25]. As different definitions of vitamin D deficiency have been used in previous studies [21], it is impossible to compare the vitamin D deficiency rates of their study populations. A recent commentary indicated that vitamin D deficiency prevalence was overestimated in most published studies because a serum level of 25(OH)D of 50 nmol/L was considered by the Institute of Medicine (IOM) to be appropriate for 97.5% of healthy people [26]. Such a circulating level is achievable with vitamin D intake of 600 IU/day for healthy free-living U.S. and Canadian children and adults aged 1-70 years, which is the IOM Recommended Dietary Allowance (RDA) for persons of this age group [26]. The serum 25(OH)D cut-off values proposed by the IOM were primarily determined in consideration of bone health indicators (e.g. bone mineral density and risk of fracture and rickets) [27]. However, a study [28] found that one third of infants and toddlers with a serum 25(OH)D level of 50 nmol/L developed bone demineralization, which suggests that the optimal level of serum 25(OH)D for children and adolescents merits further investigation. Finally, it should be pointed out that a considerable proportion of U.S. children and adults (especially African-American teenagers and adults) participating in the National Health and Nutrition Examination Survey (2007-2010) had 25(OH)D levels of <30 nmol/L [29].

Epidemiologic studies have revealed that BMD is a risk factor for osteoporotic fracture [30]. It is important to measure BMD among school children as early detection of those with
low BMD could lead to timely interventions through modifying dietary and/or other lifestyle factors during this period of time that is critical for accrual of optimal peak bone mass. The total body and lumbar spine are the preferred anatomical sites for pediatric densitometry [31]. The total body, TBLH, and/or lumbar spine BMD values of our study children in Northwest China was only slightly different from those of children of the same ages in other regions of China [20]. Conversely, some or all of these BMD values of children in the present study were substantially lower than those of American, Dutch, and Swedish children of comparable ages [32-34]. Although caution should be exercised for the BMD comparisons between our study and the Dutch and Swedish studies due to use of different DXA models that vary in calibration and bone edge detection algorithms, the discrepant BMD values between Chinese and Western children might be primarily attributable to differences in dietary habits (e.g. intake of dairy products and meat) and genetic constitution [20].

In the present study, 1.8% of children and adolescents evaluated were classified as low TBLH BMD in comparison to the Chinese reference pediatric population. However, there is a paucity of data on BMD determinants among children in China. We found that age, height, and weight were significantly and positively associated with TBLH BMD in both sexes and that the strongest determinant of TBLH BMD was age in boys and weight in girls. Rapid bone growth occurs throughout childhood and adolescence, with a concomitant gradual accretion of bone volume and mass [32, 35-37]. This physiological phenomenon accounts for the strong correlations between age and TBLH BMD in both boys (r=0.89, p<0.0001) and girls (r=0.85, p<0.0001) in the present study. Our observed positive associations of height and weight with BMD were confirmed in some other studies [38-40]. The effect of weight on BMD is consistent with the mechanostat theory that bone growth and loss are primarily driven by changes in mechanical load [41]. Weight is the major determinant of mechanistic load of weight-bearing bones. As expected, BMD and bone strength have been found to be
greater in overweight children than in healthy weight children [42]. Other nutrients (e.g. calcium, phosphorous, protein, vitamin K), hormones, and genetic factors are also associated with BMD [34, 43-45]. We are not able to evaluate the influence of these factors on BMD in the present study due to lack of data but intend to investigate these associations among Chinese children in future studies.

We did not find a significant correlation between serum 25(OH)D concentrations and BMD for total body and at measured skeletal sites among school children in Northwest China, which was consistent with the results of most previous studies conducted among children or adults in China, India, Saudi Arabia, and the U.S [12, 13, 45-48]. In addition, this null correlation observed in our study existed regardless of whether children and adolescents were sufficient, insufficient, or deficient in vitamin D. However, some epidemiological studies have reported a significant association between serum 25(OH)D levels and BMD or changes in BMD among postmenopausal women and elderly men. This finding suggests that vitamin D reduces the loss of bone mass among the adults who are at risk of developing osteoporosis [4]. A study of 171 Finnish girls aged 9-15 years revealed that baseline serum 25(OH)D concentrations were not associated with baseline BMD at the lumbar spine but were positively associated with 3-year change in BMD at the lumbar spine and the femoral neck [49]. The 3-year BMD accrual was substantially smaller in the girls with serum 25(OH)D of <20 nmol/L than in those with serum 25(OH)D of ≥37.5 nmol/L, which indicates that pubertal girls with vitamin D deficiency may have an elevated risk of not reaching their maximal peak bone mass. Taken together, our observed null association between serum 25(OH)D and BMD was in agreement with the findings of most, but not all, previous studies. Given the essential role of vitamin D in bone health and disease, it is worthwhile to further investigate the associations of serum 25(OH)D prospectively measured at multiple points in time with BMD and its longitudinal changes among children, adolescents, and adults in
diverse populations. Of note, some randomized trials have shown that vitamin D supplementation significantly increased BMD in peripubertal girls [50] and overweight elderly individuals [51].

There are some strengths in the present study. Our study subjects were recruited from children and adolescents in Yinchuan, an understudied population with distinct dietary habits characterized by high intake of mutton in Northwest China. It is important to investigate the association between dietary intake of protein (especially animal protein) and BMD in this Chinese pediatric population as both beneficial and detrimental effects of protein intake on bone health have been observed in epidemiological studies primarily conducted among adult populations in Western countries [52]. We evaluated vitamin D nutritional status of study subjects by measuring serum 25(OH)D, a reliable biomarker commonly used in epidemiological studies [4]. BMD for total body and at all major skeletal sites was quantified using a standard method (DXA) with implementation of various quality assurance measures.

The present study has several limitations. Its cross-sectional design does not allow us to investigate the longitudinal influence of vitamin D nutritional status on BMD accumulation of the children examined during years of rapid growth and development. Serum 25(OH)D was measured only once, and seasonal variations in the circulating levels of this vitamin could not be considered in our data analysis. The ELISA kit used in our study was designed to measure serum concentrations of total 25(OH)D and thus could not differentiate between 25(OH)D2 and 25(OH)D3. However, a validation study has demonstrated a good correlation between serum 25(OH)D concentrations obtained from ELISA and those from high-performance liquid chromatography (r=0.88) [53]. Physical activity was analyzed as time spent on outdoor activity (day/week) in our study, and it is preferable to have more accurate and extensive data on physical activity to better evaluate its effect on BMD. Pubertal maturation normally assessed with Tanner Staging is a determinant of BMD but was not
included in our statistical analysis due to lack of data. Parathyroid hormone is vital to bone
remodeling as it regulates serum concentrations of calcium and phosphorus. Our inability to
evaluate the effect of parathyroid hormone on BMD among Chinese school children is
another limitation of the present study.

In summary, the present study revealed that more than one third of school children in a
Northwest Chinese city are deficient in vitamin D and that a small proportion of children
have developed low BMD. Dietary enhancement or supplementation of vitamin D should be
considered for these children and adolescents to ensure that they achieve optimal peak bone
mass. More studies are warranted to investigate the associations of demographic,
antropometric, and particularly modifiable lifestyle factors (e.g. diet, alcohol consumption,
cigarette smoking, physical activity) with BMD and other bone health parameters among
children and adults with different dietary habits, socioeconomic status, and genetic
background.
Disclosures

The authors declare that they have no conflict of interest.

Acknowledgements

We would like to thank Drs. Jie Mi, Jun-Ting Liu, and Xiao-Yuan Zhao from the Capital Institute of Pediatrics of Beijing, China for their administrative and technological support for the study.

Authors’ Contributions

WQD and HPZ designed and directed the study; JL conducted the field work, drafted the manuscript, and analyzed data; JC, LJS, and SHL performed laboratory measurements; JJZ supervised data analysis and revised the manuscript. All authors participated in the interpretation of data and the preparation of the manuscript as well as read and approved its final version. HPZ and JJZ take responsibility for the integrity of the data and the accuracy of the data analysis.
References


[10] Hansen MA, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass and bone


Table 1. Characteristics of study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Boys (n = 756)</th>
<th>Girls (n = 826)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>12.4 ± 3.6</td>
<td>12.9 ± 3.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Han</td>
<td>617 (81.6)</td>
<td>612 (74.1)</td>
<td></td>
</tr>
<tr>
<td>Hui</td>
<td>139 (18.4)</td>
<td>214 (25.9)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.3 ± 18.0</td>
<td>151.7 ± 13.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.4 ± 17.0</td>
<td>45.9 ± 13.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Regular menstruation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>–</td>
<td>203 (24.6)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>–</td>
<td>623 (75.4)</td>
<td></td>
</tr>
<tr>
<td>Nocturnal emission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>125 (16.5)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>631 (83.5)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Never</td>
<td>630 (83.3)</td>
<td>797 (96.5)</td>
<td></td>
</tr>
<tr>
<td>Attempted</td>
<td>108 (14.3)</td>
<td>27 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>18 (2.4)</td>
<td>2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Never</td>
<td>507 (67.1)</td>
<td>640 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Attempted</td>
<td>193 (25.5)</td>
<td>164 (19.9)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>56 (7.4)</td>
<td>22 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Time spent on outdoor activity (day/week)</td>
<td>2.29 ± 2.63</td>
<td>2.30 ± 2.58</td>
<td>0.878</td>
</tr>
</tbody>
</table>

* Data shown are mean ± SD for continuous variables and n (%) for categorical variables.
Table 2. Serum 25-hydroxyvitamin D concentrations and vitamin D nutritional status among study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015

<table>
<thead>
<tr>
<th></th>
<th>All Subjects (n = 1582)</th>
<th>6-9 Years (n = 385)</th>
<th>10-13 Years (n = 424)</th>
<th>14-16 Years (n = 504)</th>
<th>17-18 Years (n = 269)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-hydroxyvitamin D (nmol/L)</td>
<td>Median (IQR†)</td>
<td>50.5 (30.5-94.9)</td>
<td>58.6 (34.5-108.1)</td>
<td>46.3 (29.5-84.0)</td>
<td>48.7 (28.9-94.8)</td>
<td>52.4 (31.3-105.1)</td>
</tr>
<tr>
<td>Vitamin D sufficiency [n (%)]</td>
<td>800 (50.6)</td>
<td>223 (57.9)</td>
<td>191 (45.0)</td>
<td>247 (49.0)</td>
<td>139 (51.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Vitamin D insufficiency [n (%)]</td>
<td>220 (13.9)</td>
<td>52 (13.5)</td>
<td>67 (15.8)</td>
<td>68 (13.5)</td>
<td>33 (12.3)</td>
<td>0.57</td>
</tr>
<tr>
<td>Vitamin D deficiency [n (%)]</td>
<td>562 (35.5)</td>
<td>110 (28.6)</td>
<td>166 (39.2)</td>
<td>189 (37.5)</td>
<td>97 (36.0)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Vitamin D sufficiency, insufficiency, and deficiency are defined as 25(OH)D concentrations >50, 37.5-50, and ≤37.5 nmol/L, respectively.

* p value for differences in serum 25-hydroxyvitamin D concentrations among the four age groups compared.
† IQR: interquartile range.
Table 3. Bone mineral density (BMD) and low BMD prevalence by age group among study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015

| Skeletal site          | All Subjects (n = 1582) | 6-9 (n = 194) | 10-13 (n = 223) | 14-16 (n = 227) | 17-18 (n = 112) | p-value*       | 6-9 (n = 191) | 10-13 (n = 201) | 14-16 (n = 277) | 17-18 (n = 157) | p-value*       |
|------------------------|-------------------------|--------------|----------------|----------------|----------------|----------------|--------------|----------------|----------------|----------------|----------------|----------------|
| BMD (g/cm²) (Mean ± SD) |                         |              |                |                |                |                |              |                |                |                |                |                |
| Total body             | 0.89 ± 0.12             | 0.76 ± 0.05  | 0.83 ± 0.06    | 0.97 ± 0.08    | 1.05 ± 0.07    | <0.0001        | 0.73 ± 0.06  | 0.84 ± 0.07    | 0.96 ± 0.07    | 1.00 ± 0.08    | <0.0001        |
| Total body less head   | 0.75 ± 0.12             | 0.60 ± 0.05  | 0.70 ± 0.07    | 0.86 ± 0.07    | 0.93 ± 0.07    | <0.0001        | 0.58 ± 0.06  | 0.72 ± 0.07    | 0.81 ± 0.06    | 0.84 ± 0.06    | <0.0001        |
| Head                   | 1.67 ± 0.29             | 1.48 ± 0.13  | 1.50 ± 0.17    | 1.70 ± 0.24    | 1.90 ± 0.21    | <0.0001        | 1.43 ± 0.14  | 1.55 ± 0.19    | 1.89 ± 0.26    | 2.03 ± 0.25    | <0.0001        |
| Left arm               | 0.58 ± 0.10             | 0.47 ± 0.06  | 0.55 ± 0.05    | 0.68 ± 0.05    | 0.73 ± 0.04    | <0.0001        | 0.43 ± 0.05  | 0.55 ± 0.05    | 0.62 ± 0.04    | 0.65 ± 0.04    | <0.0001        |
| Right arm              | 0.60 ± 0.11             | 0.49 ± 0.05  | 0.57 ± 0.05    | 0.71 ± 0.06    | 0.76 ± 0.05    | <0.0001        | 0.45 ± 0.05  | 0.57 ± 0.05    | 0.64 ± 0.04    | 0.68 ± 0.05    | <0.0001        |
| Left rib               | 0.52 ± 0.08             | 0.46 ± 0.05  | 0.48 ± 0.04    | 0.58 ± 0.06    | 0.63 ± 0.05    | <0.0001        | 0.44 ± 0.04  | 0.48 ± 0.05    | 0.55 ± 0.05    | 0.58 ± 0.05    | <0.0001        |
| Right rib              | 0.53 ± 0.08             | 0.46 ± 0.04  | 0.49 ± 0.04    | 0.59 ± 0.06    | 0.64 ± 0.06    | <0.0001        | 0.44 ± 0.04  | 0.49 ± 0.05    | 0.56 ± 0.05    | 0.58 ± 0.06    | <0.0001        |
| Thoracic spine         | 0.62 ± 0.11             | 0.50 ± 0.05  | 0.55 ± 0.06    | 0.69 ± 0.07    | 0.75 ± 0.07    | <0.0001        | 0.50 ± 0.06  | 0.59 ± 0.08    | 0.70 ± 0.07    | 0.73 ± 0.08    | <0.0001        |
| Lumbar spine           | 0.82 ± 0.17             | 0.63 ± 0.06  | 0.69 ± 0.08    | 0.90 ± 0.11    | 1.00 ± 0.11    | <0.0001        | 0.65 ± 0.08  | 0.78 ± 0.11    | 0.94 ± 0.10    | 0.99 ± 0.11    | <0.0001        |
| Pelvis                 | 0.94 ± 0.20             | 0.71 ± 0.09  | 0.86 ± 0.12    | 1.07 ± 0.14    | 1.16 ± 0.13    | <0.0001        | 0.70 ± 0.10  | 0.91 ± 0.13    | 1.06 ± 0.12    | 1.09 ± 0.12    | <0.0001        |
| Left Leg               | 0.87 ± 0.16             | 0.68 ± 0.07  | 0.83 ± 0.10    | 1.01 ± 0.09    | 1.08 ± 0.09    | <0.0001        | 0.66 ± 0.08  | 0.84 ± 0.09    | 0.94 ± 0.07    | 0.97 ± 0.07    | <0.0001        |
| Right Leg              | 0.88 ± 0.16             | 0.69 ± 0.07  | 0.84 ± 0.09    | 1.03 ± 0.09    | 1.10 ± 0.09    | <0.0001        | 0.67 ± 0.08  | 0.85 ± 0.09    | 0.96 ± 0.07    | 0.99 ± 0.08    | <0.0001        |

Prevalence of low total body less head BMD† (%)  
|              | 1.8 | 3.1 | 2.7 | 1.3 | 0  | 0.21 | 1.0 | 2.5 | 1.8 | 1.3 | 0.70 |

SD = standard deviation.  
* p-value for differences in BMD among the four age groups compared.  
† Defined as a Z-score of ≤-2.0 standard deviations (SD) away from the mean BMD values of the Chinese pediatric reference population [20].
Table 4. Multivariable linear regression of total body less head bone mineral density with serum 25-hydroxyvitamin D concentrations and other variables among study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015

<table>
<thead>
<tr>
<th>Variables</th>
<th>Boys (n = 756)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Girls (n = 826)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>95% (CI)</td>
<td>p</td>
<td>β</td>
<td>B</td>
<td>95% (CI)</td>
<td>p</td>
<td>β</td>
<td>B</td>
</tr>
<tr>
<td>25-hydroxyvitamin D (nmol/L)</td>
<td>0.000047</td>
<td>-0.00002, 0.0001</td>
<td>0.22</td>
<td>0.016</td>
<td>0.000033</td>
<td>-0.00003, 0.00009</td>
<td>0.27</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.017</td>
<td>0.014, 0.019</td>
<td>&lt;0.0001</td>
<td>0.419</td>
<td>0.011</td>
<td>0.010, 0.013</td>
<td>&lt;0.0001</td>
<td>0.333</td>
<td></td>
</tr>
<tr>
<td>Race (Han vs. Hui)</td>
<td>0.003</td>
<td>-0.001, 0.008</td>
<td>0.16</td>
<td>0.019</td>
<td>-0.0002</td>
<td>-0.004, 0.003</td>
<td>0.91</td>
<td>-0.002</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.002</td>
<td>0.001, 0.003</td>
<td>&lt;0.0001</td>
<td>0.249</td>
<td>0.002</td>
<td>0.001, 0.002</td>
<td>&lt;0.0001</td>
<td>0.210</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.003</td>
<td>0.002, 0.003</td>
<td>&lt;0.0001</td>
<td>0.309</td>
<td>0.004</td>
<td>0.003, 0.004</td>
<td>&lt;0.0001</td>
<td>0.439</td>
<td></td>
</tr>
<tr>
<td>Regular menstruation (yes vs. no)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.007</td>
<td>-0.0005, 0.015</td>
<td>0.06</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Nocturnal emission (yes vs. no)</td>
<td>0.005</td>
<td>-0.006, 0.016</td>
<td>0.34</td>
<td>0.014</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Time spent on outdoor activity (day/week)</td>
<td>-0.002</td>
<td>-0.003, -0.0003</td>
<td>0.02</td>
<td>-0.031</td>
<td>0.0003</td>
<td>-0.001, 0.002</td>
<td>0.63</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

R² for the model = 0.867
R² for the model = 0.861

B = partial regression coefficient; CI = confidence interval; β = standardized regression coefficient.
Table 5. Correlations between log-transformed serum 25-hydroxyvitamin D concentrations and bone mineral density among study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015.

<table>
<thead>
<tr>
<th>Anatomic Site</th>
<th>All Subjects (n = 1582)</th>
<th>Vitamin D Sufficiency (n = 800)</th>
<th>Vitamin D Insufficiency (n = 220)</th>
<th>Vitamin D Deficiency (n = 562)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Total body</td>
<td>-0.005</td>
<td>0.83</td>
<td>0.072</td>
<td>0.041</td>
</tr>
<tr>
<td>Total body less head</td>
<td>-0.018</td>
<td>0.47</td>
<td>0.060</td>
<td>0.09</td>
</tr>
<tr>
<td>Head</td>
<td>0.014</td>
<td>0.57</td>
<td>0.092</td>
<td>0.009</td>
</tr>
<tr>
<td>Left arm</td>
<td>-0.034</td>
<td>0.18</td>
<td>0.048</td>
<td>0.18</td>
</tr>
<tr>
<td>Right arm</td>
<td>-0.037</td>
<td>0.14</td>
<td>0.041</td>
<td>0.25</td>
</tr>
<tr>
<td>Left rib</td>
<td>-0.008</td>
<td>0.75</td>
<td>0.075</td>
<td>0.033</td>
</tr>
<tr>
<td>Right rib</td>
<td>-0.014</td>
<td>0.58</td>
<td>0.072</td>
<td>0.040</td>
</tr>
<tr>
<td>Thoracic spine</td>
<td>-0.009</td>
<td>0.71</td>
<td>0.092</td>
<td>0.009</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>-0.005</td>
<td>0.84</td>
<td>0.098</td>
<td>0.005</td>
</tr>
<tr>
<td>Pelvic</td>
<td>-0.025</td>
<td>0.32</td>
<td>0.062</td>
<td>0.08</td>
</tr>
<tr>
<td>Left Leg</td>
<td>-0.016</td>
<td>0.54</td>
<td>0.041</td>
<td>0.24</td>
</tr>
<tr>
<td>Right Leg</td>
<td>-0.017</td>
<td>0.51</td>
<td>0.044</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Vitamin D sufficiency, insufficiency, and deficiency are defined as 25(OH)D concentrations of >50, 37.5-50, and ≤37.5 nmol/L, respectively.
Figure 1: Sex-specific associations between age and total body less head bone mineral density (BMD) among study subjects in Yinchuan, Ningxia Hui Autonomous Region, China, 2015. Data shown are regression lines with 95% confidence intervals and 95% prediction intervals.