Threshold effects of vitamin D status on bone health in Chinese adolescents with low calcium intake

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Context: There is no consensus on the definition of vitamin D deficiency for bone health based on serum 25-hydroxyvitamin D (25OHD) levels.

Objective: To determine whether thresholds exist for associations between 25OHD levels and bone outcomes, below which low 25OHD levels have adverse effects on bone health.

Design: Cross-sectional study.

Setting: Secondary school students in Beijing, China, aged 12–15 years.

Outcome: Measures and Participants: Serum 25OHD, bone mineral density (BMD) of total body, hip and lumbar spine (LS), serum parathyroid hormone (PTH), bone alkaline phosphatase (BAP) and tartrate-resistant acid phosphatase 5b (TRAP5b) in 222 healthy adolescents, (111 girls, 111 boys).

Results: Prevalence of low 25OHD was 61% (<30nmol/L) and 97% (<50 nmol/L) (mean 25OHD 30 nmol/L). Dietary calcium intake was low (294 and 307 mg/d for boys and girls, respectively). In girls, break-points for 25OHD (nmol/L) were: total body BMD 20 (95%CI: 14–27), hip BMD 25 (17–34), LS BMD 22 (14–30), TRAP5b 37 (22–52), and PTH 31 (23–38). In boys: total body BMD 39 (17–55), TRAP5b 33 (20–45), PTH 35 (27–43); no break-points were identified for hip and LS BMD. No break-points were identified for BAP in either gender. Below these break-points, greater 25OHD is associated with increased total body BMD, reduced PTH and TRAP5b, while above them, no such relationship exists.

Conclusions: Vitamin D deficiency and insufficiency is common in healthy Chinese adolescents. Attaining serum 25OHD levels of 20–37 nmol/L in girls and 33–39 nmol/L in boys had positive influences on BMD and bone remodelling markers. However, estimates may be affected by low calcium intake and low serum 25OHD levels with 97% <50 nmol/L.

Causal relationships between vitamin D and bone health are well-established (1). Vitamin D deficiency causes rickets in growing children, and osteomalacia in older adolescents and adults. However, the definition of vitamin D deficiency remains controversial, particularly in healthy children and adolescents because of limited studies. Based on serum 25-hydroxyvitamin D (25OHD) level, the optimal indicator of vitamin D status, the Pediatric Endocrine Society defined vitamin D deficiency and insufficiency in children as a 25OHD level of less than 37.5 and 50 nmol/L, respectively (2). In contrast, a 25OHD level of <50 nmol/L was defined as deficiency, and a 25OHD level of 32.5 to 72.5 nmol/L as insufficiency by the Endocrine Society (3). However, the evidence on which those definitions are based is primarily from data in adults (4, 5).

Previous research suggests that there are threshold effects of vitamin D on bone outcomes (6), with serum vi...
tamin D below a certain range conferring increased risk of adverse outcomes, but conferring no increased risk above the threshold, ie, associations between vitamin D and the outcomes only exist below the threshold. However, only a few studies examined potential threshold effects of vitamin D on bone health in children and adolescents (7–11), and most of them made recommendations for serum 25OHD threshold by comparing differences in outcomes in categories with 25OHD level below and above arbitrary preselected thresholds. Based on these studies, a 25OHD level less than or equal to 37.5 nmol/L has been suggested as an appropriate definition of vitamin D deficiency in healthy children and adolescents (2). Notably, studies have been conducted mainly in Caucasian populations and estimates for the 25OHD threshold may differ across racial groups because of differences in the extent of vitamin D deficiency (12) and the associations between 25OHD levels and bone health (13). To our knowledge, no studies have been done to specifically address thresholds for associations between serum 25OHD, BMD, parathyroid hormone (PTH) and bone turnover biomarkers (bone alkaline phosphatase (BAP) – a measure of bone formation; tartrate-resistant acid phosphatase 5b (TRAP5b) – a measure of bone resorption) in healthy Chinese adolescents, particularly in boys.

Therefore, the aims of this study were to 1) determine whether thresholds for associations between serum 25OHD level, serum PTH, BAP, TRAP5b and BMD exist in healthy Chinese adolescent girls and boys, and if so 2) to estimate associations below and above identified thresholds of 25OHD.

Materials and Methods

Participants

We utilized participants from a randomized controlled trial (RCT) of calcium supplementation in this study. The RCT was a part of a study on calcium requirement and dietary evaluation as a part of a study on calcium requirement and dietary evaluation in a susceptible Chinese population (Trial registration number: ChiCTR-PRC-09 000 580), and was conducted at 3 secondary schools in a rural district in Beijing, China (lat 39°N) in 2009. All first grade students (n = 12 classes) were invited to participate. To be eligible for the trial, participants had to be 10–18 years of age, and to be free of the following conditions for 1 year prior to enrolment: calcium or vitamin D supplement, cancer, hepatic or renal insufficiency or mental diseases, or diseases and conditions known to affect calcium absorption and metabolism or bone health (eg, musculoskeletal diseases, liver or kidney disorders or other metabolic syndromes, such as diabetes, hyperthyroidism). Students were also excluded if they were taking vitamin D or calcium supplements or medicines recently. Finally, 235 students were eligible and were randomized either to a control group (consuming habitual diets) or to receive milk powder containing 400 IU/d vitamin D plus different dosage of calcium per day of 300 (Ca3D), 600 (Ca6D) and 900 (Ca9D) mg. Of the 255 students, 23 declined to continue after randomization but before any measurements were taken or supplementation was given. Of the 232 students retained, 222 (111 of each gender) who had serum 25OHD levels, bone mineral density (BMD) and anthropometric measurements were included in this cross-sectional analysis using baseline data only.

The trial was approved by the Ethic Committee of National Institute for Nutrition and Food Security, Chinese Center for Disease Control and Prevention, and it conformed to the Declaration of Helsinki. Written informed consent was signed by students’ parents prior to the study.

Serum 25OHD, PTH, BAP and TRAP5b levels

One 5 ml overnight fasting venous blood sample (drawn between 0630 and 0900) was collected during the periods between 25 April and 24 May 2009 (spring, n = 175) and 26–27 October 2009 (autumn, n = 47). Serum 25(OH)D concentration was determined by 125I-radioimmunoassay (RIA) kits (IDS Ltd, Boldon, Tyne and Wear, UK). The limit of detection of the assay was 3 nmol/L, and intra-assay and interassay coefficients of variation (CVs) were less than 6% and 9%, respectively. Serum intact PTH levels were measured using immunometric assay (Immulite Intact PTH: Diagnostic Products Corporation, Los Angeles). The intraassay CV was 5.4% and the interassay CV was 5.0% at 86 pg/ml. Serum BAP and TRAP5b were measured using a commercial ELISA kit method (IDS Ltd, Boldon, Tyne and Wear, UK) with an intra-assay CV of 5.5% to 7.3% and 1.7% to 3.4%, respectively. All samples were assayed in duplicate using the same batch of kits.

BMD

BMD of the total body (TB), lumbar spine (LS) and hip was measured by dual-energy X-ray absorptiometry (DXA) (Norland XR-46 densitometer, Norland Medical Systems, Fort Atkinson, WI, USA) during the same time period when blood samples were collected. Measurements were performed by two experienced technicians at the Beijing Sport University, Beijing. All bone data were analyzed by the same technician using Norland enhanced software (version 3.9.4). A daily quality assurance (QA) test was performed with a manufacturer-supplied hydroxyapatite phantom, and the accuracy error of repeated measurements was < 1.0%. Prior to the first scan of each clinic, the densitometer was calibrated in accordance to the manufacturer’s recommendations.

Other measurements

Usual food intake was estimated using the 24-hour dietary recall method for 3 consecutive days (including two week days and Saturday), including meals and snacks (14). The quantity of food items was measured in bowls, plates and spoons of standard size. Nutrient intakes were calculated using the Chinese Food Composition Tables published in 2002 (15) and 2004 (16). Physical activity was measured by a consecutive 3-day physical activity questionnaire (including two week days and Saturday), which was checked after completion by participants. Students were asked to recall their activities for every 15 minutes interval (a total of 96 intervals a day), and activity examples were given. The metabolic equivalents of energy (METS) for each type of physical activity was determined according to the coding scheme developed by Ainsworth et al (17). Average METs per hour was
used to represent overall physical activity. Anthropometric factors include height measured to the nearest 0.1 cm using a stadiometer (Jianmin, Beijing, China), weight by an electronic weight scale (Thinner; Measurement Specialities, Fairfield, NJ, USA) to the nearest 0.1 kg. Participants were asked to wear light clothing and no shoes. Body mass index (BMI) was calculated by the equation: body mass index (BMI) (kg/m²) = Weight (kg) / Height (m)². Pubertal stages were measured using the Tanner grading system (18) based on pubic hair and breasts for girls and pubic hair and testicular volume for boys.

**Statistical analysis**

Characteristics of participants are reported as mean (SD) or median (interquartile range), unless otherwise indicated. Difference in characteristics between girls and boys were tested using unpaired two-sample t-tests or Kruskal-Wallis or χ² test (Fisher’s exact test for those with expected frequency less than 5) as appropriate.

Serum BAP, TRAP5b and PTH levels were log transformed to approximate a normal distribution. To adjust for potential confounders (age, BMI, pubertal status, dietary calcium intake and physical activity), an adjusted sex-specific variable was generated for each outcome using its mean value to plus the residuals resulted from regressing the outcome on those confounding factors. An adjusted sex-specific variable of serum 25OHD levels was also generated in the same way using the same covariates. The adjusted variables were used for following analyses. Locally weighted regression smoothing (LOWESS) was used to explore nonlinear associations between serum 25OHD, BAP, TRAP5b, PTH and BMD measurements, and nonlinear least-squares estimation to estimate the potential break-points within the association. Segmented regression (also known as piecewise regression) was further used to determine the association for participants with 25OHD below and above the identified break-points, to test for threshold effects of 25-OHD on bone health. To take into account differences in season of blood sample collected, nonlinear least-squares estimation and segmented regression were performed again by excluding the 47 participants (n = 24 for boys and 23 for girls) whose blood samples were collected in autumn (26–27 October 2009). All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value < 0.05 was considered statistically significant.

**Results**

Difference in characteristics between boys and girls (n = 111 for each gender; 12–15 years of age) are presented in Table 1. They were comparable in age, height, weight,
Compared to boys, girls had more advanced pubertal stages, higher total body and lumbar spine BMD, but lower serum 25OHD, PTH, BAP and TRAP5b levels. Prevalence of low serum 25OHD was 61% for the definition of < 30 nmol/L, and 97% for < 50 nmol/L. As expected, compared to blood samples collected in autumn, those collected in spring had significantly lower 25OHD levels (mean (SD) 27.2 (6.9) vs. 38.6 (8.9) nmol/L; \( P < .001 \)), while significantly higher PTH (mean (SD) 9.7 (5.5) vs. 7.3 (3.4) pmol/L; \( P = .005 \)). Participants had very low dietary calcium intake, which only reached 29% and 31% of the Chinese recommended daily calcium intake for adolescent boys and girls, respectively (19).

Box plots of differences in BMD by pubertal stages are given in Figure 1. BMD increased with advancing pubertal stage in both boys (\( P = .011, .001 \) and 0.016 for total body, lumbar spine and hip, respectively) and girls (\( P < .001 \) for total body, lumbar spine and hip). Figure 2 and 3 show exploratory views of potential nonlinear associations between 25OHD and PTH and BMD of total body, hip and lumbar spine in boys and girls. After adjustment for confounders, break-points of serum 25OHD (nmol/L) were identified for BMD of total body [20 (95% confidence interval (CI): 14, 27)], hip [25 (17, 34)] and lumbar spine [22 (95% CI: 14, 30)], log-transformed TRAP5b [37 (95% CI: 22–52)] and log-transformed PTH [31 (95% CI: 23, 38)] in girls; while break-points for only total body BMD [39 (95% CI: 24, 55)], log-transformed TRAP5b [33 (95% CI: 20–45)] and log-transformed PTH [35 (95% CI: 27, 43)] were identified in boys (Table 2). No break-points were identified for BAP in either gender.

Associations between serum 25OHD and bone health outcomes in participants with 25OHD above and below previously identified break-points are given in Table 3. Significant associations between serum 25OHD and total body BMD, log-transformed TRAP5b and log-transformed PTH were found in girls and boys with 25OHD below break-points, but not above the break-points. No significant associations between serum 25OHD and BMD of hip and lumbar spine were found in girls with 25OHD either below or above the previously identified break-points.

We performed a sensitivity analysis by season. When participants whose blood samples collected in autumn (\( n = 47 \)) were excluded, we found similar results for break-points and associations between serum 25OHD and all BMI, dietary calcium intake and physical activity. Compared to boys, girls had more advanced pubertal stages, higher total body and lumbar spine BMD, but lower serum 25OHD, PTH, BAP and TRAP5b levels. Prevalence of low serum 25OHD was 61% for the definition of < 30 nmol/L, and 97% for < 50 nmol/L. As expected, compared to blood samples collected in autumn, those collected in spring had significantly lower 25OHD levels (mean (SD) 27.2 (6.9) vs. 38.6 (8.9) nmol/L; \( P < .001 \)), while significantly higher PTH (mean (SD) 9.7 (5.5) vs. 7.3 (3.4) pmol/L; \( P = .005 \)). Participants had very low dietary calcium intake, which only reached 29% and 31% of the Chinese recommended daily calcium intake for adolescent boys and girls, respectively (19).

### Table 1. Characteristics for study boys and girls

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.0 (0.6)</td>
<td>13.0 (0.6)</td>
<td>0.662</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.1 (8.5)</td>
<td>154.7 (6.5)</td>
<td>0.183</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.9 (11.8)</td>
<td>46.6 (10.6)</td>
<td>0.804</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>19.0 (3.4)</td>
<td>19.4 (3.7)</td>
<td>0.507</td>
</tr>
<tr>
<td>Pubertal Stage, ( n (%) )*</td>
<td>14 (13)</td>
<td>3 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum 25OHD (nmol/liter)</td>
<td>30.9 (8.8)</td>
<td>28.2 (8.3)</td>
<td>0.024</td>
</tr>
<tr>
<td>Serum PTH (pmol/liter)</td>
<td>9.8 (6.2)</td>
<td>8.6 (4.0)</td>
<td>0.078</td>
</tr>
<tr>
<td>Serum BAP (µg/liter)</td>
<td>68.9 (26.2)</td>
<td>48.4 (20.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum TRAP5b (U/liter)</td>
<td>5.3 (2.9)</td>
<td>4.0 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total body BMD (g/cm²)</td>
<td>0.737 (0.083)</td>
<td>0.759 (0.078)</td>
<td>0.040</td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>0.739 (0.124)</td>
<td>0.865 (0.131)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip BMD (g/cm²)</td>
<td>0.858 (0.117)</td>
<td>0.836 (0.112)</td>
<td>0.141</td>
</tr>
</tbody>
</table>

**Values are mean (SD) unless otherwise indicated.**

IQR, interquartile range; 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; PTH, parathyroid hormone; BAP, bone alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphatase 5b.

*Pubertal stage was determined by pubic hair and breast development for girls and pubic hair and testicular volume for boys, only 1 person in stage 5 for each gender.

†from dietary food intake only.
outcomes in participants with 25OHD above and below the identified break-points (data not shown).

Discussion

To our knowledge, our study provides the first evidence of thresholds for associations between 25OHD concentrations, PTH, bone turnover markers and BMD in Chinese adolescent boys and girls aged 12 to 15 years, who have poor vitamin D status and low calcium intake. We demonstrate that there are thresholds for serum 25OHD, below which greater 25OHD is associated with increased total BMD, reduced serum PTH and TRAP5b, while above these thresholds, no such relationship exists. This suggests that 25OHD levels of at least 20–37 nmol/L had a positive influence on BMD and markers of bone remodeling in girls, and 33–39 nmol/L in boys. Importantly, we used nonlinear least-squares estimation, which allows for a clear-cut estimation of the threshold break-points for 25OHD in an exploratory approach, as compared to using preselected threshold values (eg, comparing differences in outcomes in categories with 25OHD level below and above 40 nmol/L) or a data-driven approach (eg, using quantiles of the distribution) (20).

Despite the great interest in vitamin D worldwide, few cross-sectional studies have specifically examined potential thresholds for vitamin D deficiency in adolescents (7–10, 21). Two studies in adolescent girls (aged 14–16 (9) and 12–14 years (10)) in Finland suggested that serum 25OHD < 40 nmol/L was associated with poorer BMD, increased serum intact PTH and bone resorption (tartrate-resistant acid phosphatase 5b) (9) (10). A study in Australian boys (aged 16–18 years) suggested that serum 25OHD of ≥ 43–55 nmol/L is required for optimal bone health, based upon minimizing levels of bone turnover markers (bone-specific alkaline phosphatase and urinary

![Figure 2 & 3](#)

**Figure 2 & 3.** Locally weighted regression smoothing (LOWESS) scatter plots for exploratory views of adjusted associations between serum 25OHD levels and BMD of total body, hip and lumbar spine and log-transformed bone alkaline phosphatase (bap), tartrate-resistant acid phosphatase 5b (TRAP5b) and parathyroid hormone (PTH) for boys and girls, respectively (see statistical section in text for details of how adjustment was performed). Vertical lines indicate identified break-points using nonlinear least-squares estimation (no vertical lines for figures where break-points were not identifiable or not statistically significant, see Table 2).
Table 2. Break-points for the associations between 25-hydroxyvitamin D levels and bone mineral density measures, log-transformed parathyroid hormone and bone turnover biomarkers†

<table>
<thead>
<tr>
<th>Boys</th>
<th>Total body BMD (g/cm²)</th>
<th>Hip BMD (g/cm²)</th>
<th>Lumbar spine BMD (g/cm²)</th>
<th>Log-transformed PTH</th>
<th>Log-transformed BAP</th>
<th>Log-transformed TRAP5b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39 (24, 55)*</td>
<td></td>
<td>19 (-3, 40)</td>
<td>35 (27, 43)*</td>
<td>25 (-7, 57)</td>
<td>33 (20, 45)*</td>
</tr>
<tr>
<td>Girls</td>
<td>Total body BMD (g/cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 (14, 27)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NI, not identifiable; 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; PTH, parathyroid hormone; BAP, bone alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphatase 5b.

†Serum 25OHD and outcomes were adjusted for age, weight, height, physical activity, dietary calcium intake and pubertal stage.

*P < 0.001.

pyridinoline) (8). In comparison, two recent studies documented distinct thresholds for 25OHD levels at which PTH starts to increase (34 vs. 75 nmol/L) (7, 21). This may be explained by the fact that levels of PTH fluctuate widely, varying with diet, physical activity and time of day (22).

Threshold estimates generated from our study in Chinese adolescent boys (33–39 nmol/L) and girls (20–37 nmol/L), below which greater 25OHD level was associated with increased BMD, decreased serum PTH and TRAP5b, and above which there was no association are consistent with most studies mentioned above and the conclusion from a meta-analysis of 6 studies of vitamin D supplementation. This found that clinically useful improvements in BMD of the lumbar spine and total body bone mineral content could be only found in female children and adolescents (10 to 17 years of age) with low baseline serum vitamin D status (less than 35 nmol/L) (23). Therefore, adolescents with 25OHD level below those thresholds should be primarily targeted in future interventional studies of vitamin D supplementation.

Measurements of bone turnover biomarkers and PTH allow assessment of bone remodeling (24, 25). Serum BAP is an indicator of bone formation and may be a useful surrogate marker for osteomalacia, which is not optimally captured by DXA. In our study, no clear pattern or break-points were identified for serum BAP concentrations in either gender. This may be due both to skeletal growth and rapid bone turnover during adolescence, which may not be fully accounted for by adjusting for pubertal status. In contrast, we identified similar patterns and break-points for TRAP5b (bone resorption) as PTH. This suggests that BAP alone might not be an appropriate biomarker of osteomalacia, due to vitamin D deficiency during adolescence. Certainly, other authors recommend that more than one bone turnover biomarker should be measured to monitor longitudinal growth and bone mineral accrual (26). Also, interpretation of the results from bone turnover biomarkers is difficult because they depend on many factors, including age, pubertal stage, growth velocity, mineral accrual, hormonal regulation, nutritional status, circadian variation, day-to-day variation, specificity for bone tissue, sensitivity and specificity of assays (27). Therefore, it is critical to take into account those factors in future research.

As expected, we observed gender differences, with higher thresholds identified in boys. This can be explained predominantly by the difference in vitamin D status and BMD as indicated in our study and others (28, 29); specifically, in our study, boys had higher 25OHD level but lower BMD compared to girls. This may be attributable to the more advanced sexual maturation of girls in our study, with 3 in 4 girls but less than 1 in 2 boys having pubertal stage ≥ 3. Indeed, studies have documented that pubertal development is negatively associated with vitamin D status (21, 30, 31) while positively associated with BMD (32). Therefore, while pubertal stage should be taken into account in studies assessing relationships between 25OHD and BMD in adolescents, we found no interaction between 25OHD concentrations and pubertal stages as related to BMD measures in either gender (data not shown), suggesting the association between 25OHD concentrations and BMD measures did not differ by pubertal stages.

Heterogeneity of serum 25OHD in our study was limited, with most our participants (97%) having serum 25OHD ≤ 50 nmol/L. This did not allow exploration of associations between serum 25OHD and bone health in adolescents with higher 25OHD. However, it is unlikely that this would have an influence on the estimation of break-points in our study given the observation that all...
break-points were below 40 nmol/L, but this should be further investigated in adolescents with a broader scope of 25OHD concentrations. Importantly, the low dietary calcium intake (mean (SD) = 300 (141) mg/d) of study participants may have influenced the estimates of the 25OHD threshold. Some authors suggest that calcium intake is an important contributor to various thresholds of vitamin D in previous published papers and guidelines because deficient calcium intake can cause rickets even in individuals with normal 25OHD levels (7, 33). In older Korean adults, maintaining serum PTH within the normal range, and to achieve the optimal BMD, high dietary calcium intakes (≥ 668 mg/d) are necessary even in individuals with high serum 25(OH)D level (mean = 48 nmol/L) (34). Therefore, studies are needed in adolescent populations to assess influences of different calcium intakes on vitamin D requirements for bone health. Given the high prevalence of low vitamin D in our setting, vitamin D supplementation and correction of calcium deficiency may be critical in the population as a whole, but particularly in those with 25OHD level below those thresholds.

Limitations of these analyses include the cross-sectional data used, which does not allow causal inferences to be made. However, causal relationships between vitamin D and bone health has been well-established (1). Also, again, our sample had low calcium intake and a high prevalence of low 25OHD levels, which may have influenced our estimation of break-points and limited the examination of adolescents with high 25OHD level. Therefore, our results may not be generalizable to adolescents with higher calcium intakes and/or 25OHD levels. The break-points in our study should not be interpreted as a hard and fast cut-points, but a region of transition between high risk and normal risk, as can be visualized in Figure 2 and 3. Transitions between these states is likely to be gradual rather than a sharp transition as assumed in the nonlinear least-squares estimation used in our study. Therefore, it may be rigorous and rational to consider our break-points as some indicative values above which the initial linear relationship may be not optimal. Furthermore, the sample size was relatively small, which lowered the accuracy of identifying break-points as indicated by the wide confidence intervals. One-fifth of our participants had them blood samples collected in autumn, which might have influenced our results; however sensitivity analyses demonstrated that this did not affect our results. Nevertheless, future studies are required to examine the influences of season on the optimal 25OHD levels for bone health of growing adolescents.

In conclusion, vitamin D deficiency and insufficiency is common in healthy Chinese adolescents. We identified thresholds of 25OHD concentrations below which serum 25OHD was associated with adverse bone outcomes (increased total body BMD, reduced serum PTH and TRAP5b), while above which there was no relationship. This suggests that attaining 25OHD levels of at least 20–37 nmol/L had positive influences on bone health in girls and 33–39 nmol/L in boys. The potential for calcium intake to influence these estimates for 25OHD thresholds

### Table 3. Associations above and below break-points of serum 25-hydroxyvitamin D levels for selected bone health outcomes, in boys and girls†

<table>
<thead>
<tr>
<th>Break-points</th>
<th>Below break-point</th>
<th>Above break-point</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body BMD (g/cm²)</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>Log-transformed PTH</td>
<td>35</td>
<td>84</td>
</tr>
<tr>
<td>Log-transformed TRAP5b</td>
<td>33</td>
<td>76</td>
</tr>
<tr>
<td><strong>Girls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body BMD (g/cm²)</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Hip BMD (g/cm²)</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Lumbar spine BMD (g/cm²)</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Log-transformed PTH</td>
<td>31</td>
<td>81</td>
</tr>
<tr>
<td>Log-transformed TRAP5b</td>
<td>37</td>
<td>98</td>
</tr>
</tbody>
</table>

BMD, bone mineral density; PTH, parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase 5b.

†Serum 25OHD and outcomes were adjusted for age, weight, height, physical activity, dietary calcium intake and pubertal stage.

*β coefficients were not estimated for outcomes when break-points were not identifiable or not statistically significant.

*P < 0.05, **P < 0.01, ***P < 0.001.
needs to be addressed in future studies with a broader range of 25OHD concentrations.

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**Authors’ roles:** FW and QZ were involved in study design. FW and QZ were responsible for data collection and management. FW performed data analysis and drafted the manuscript with input from LLL. All authors revised manuscript content and approved the final manuscript and had access to the data. QZ is the guarantor of the study and accepts full responsibility for the finished article, had access to any data, and controlled the decision to publish.

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