Original Article

Bone mineral content has stronger association with lean mass than fat mass among Indian urban adolescents

Raman K. Marwaha, M. K. Garg¹, Kuntal Bhadra², Nikhil Tandon³

Senior Consultant Endocrinology and Scientific Advisor (Projects), ILSI-India, New Delhi, ¹Commandant and Consultant, Department of Medicine and Endocrinology, Military Hospital, Shillong, Meghalaya, ²Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences, ³Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India

ABSTRACT

Introduction: There are conflicting reports on the relationship of lean mass (LM) and fat mass (FM) with bone mineral content (BMC). Given the high prevalence of Vitamin D deficiency in India, we planned the study to evaluate the relationship between LM and FM with BMC in Indian children and adolescents. The objective of the study was to evaluate the relationship of BMC with LM and FM. Materials and Methods: Total and regional BMC, LM, and FM using dual energy X-ray absorptiometry and pubertal staging were assessed in 1403 children and adolescents (boys [B]: 826; girls [G]: 577). BMC index, BMC/LM and BMC/FM ratio, were calculated. Results: The age ranged from 5 to 18 years, with a mean age of 13.2 ± 2.7 years. BMC adjusted for height (BMC index and BMC/height ratio) was comparable in both genders. There was no difference in total BMC between genders in the prepubertal group but were higher in more advanced stages of pubertal maturation. The correlation of total as well as regional BMC was stronger for LM (B: Total BMC - 0.880, trunk - 0.715, leg - 0.894, arm - 0.891; G: Total BMC - 0.827, leg - 0.846, arm - 0.815 (all value indicate \( r^2 \), \( P < 0.0001 \) for all) when compared with FM (B: Total BMC - 0.776, trunk - 0.676, leg - 0.772, arm - 0.728; G: Total BMC - 0.781, leg - 0.741, arm - 0.689; all \( P < 0.0001 \)) except at trunk BMC (LM - 0.682 vs. FM - 0.721; all \( P < 0.0001 \)), even after controlling for age, height, pubertal stage, and biochemical parameters. Conclusions: BMC had a stronger positive correlation with LM than FM.

Key words: Bone mineral content, children and adolescents, fat mass, lean mass

INTRODUCTION

Bone mineral accrual during childhood and adolescence depends on genetic factors, hormonal status, growth, sexual maturation, nutritional status including body composition⁴,⁵ and ethnicity.⁶-⁹ Several body composition studies have shown the bone mass to vary significantly among ethnic groups.⁴,⁵ Height has been correlated with bone mineral content (BMC).¹⁰ Since, Asians are shorter than Caucasians,⁴ it was easy to explain the racial difference in BMC by the height difference. However, height adjusted BMC was reported to be lower in Asians.¹⁴ The differences in BMC and bone mineral density (BMD) have been noted among genders,¹⁷ particularly during pubertal progression.¹⁷,¹⁸,¹⁹

There is growing evidence which suggests that tissues such as fat, muscle, and bone are intimately involved in regulation of each other.¹⁰ The bone mass is affected by lean mass (LM) and fat mass (FM). Effect of FM is probably mediated through its weight-bearing effect

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Marwaha RK, Garg MK, Bhadra K, Tandon N. Bone mineral content has stronger association with lean mass than fat mass among Indian urban adolescents. Indian J Endocr Metab 2015;19:608-15.
and other pathways including adipokines, and lean body mass (LM) positively affect the bone accrual by the mechanical strains. Though, bone mass has been found to be positively associated with FM and LM in children and adolescents, but controversy exists in the relative contribution of each on bone mass.

Among pediatric population for analysis of body composition, dual energy X-ray absorptiometry is most widely used as in addition to bone health, it gives precise information about the total and regional distribution of FM and LM. There are few Indian studies which have assessed BMD and BMC but none have assessed the effect of FM and LM on bone health. In the present study, we have assessed the total and regional BMC among children and adolescents, evaluated the gender differences and its relation with pubertal status, and assessed relative contribution of FM and LM on bone health.

**Materials and Methods**

This study was an extension of the analysis from our earlier study. Adolescents were recruited from different schools in the city of Delhi as a part of a project to generate normative data for BMD. There were 1829 apparently healthy children and adolescents who underwent health examination (clinical, biochemical, and densitometric) on a voluntary basis. The data on BMC, LM, and FM, and its distribution were available from 1403 children and adolescents, for the present study. Children and adolescents with clinically overt hepatic, renal, neoplastic, gastrointestinal, dermatological and endocrine and systemic infective disorders, steroid intake or alcoholism were excluded. Demographic, anthropometric and clinical data were ascertained, and a detailed physical examination conducted. The study was approved by the ethics committee of the Institute of Nuclear Medicine and Allied Sciences and all subjects gave written informed consent.

Pubertal staging was carried out by trained professionals of the same sex based on Tanner criteria. Testicular volume was determined by comparative palpation with Prader orchidometer (Pharmacia and Upjohn, Uppsala, Sweden). Based on testicular volume, subjects were divided into four stages. Stage 1 (prepubertal) included subjects with testicular volume < 4 ml, Stage 2 (early puberty) - volume ≥ 4 but ≤ 8 ml, Stage 3 - volume > 8 ml but ≤ 10 ml, Stage 4 - volume > 10 ml but ≤ 15 and Stage 5 (fully mature) - testicular volume > 15 ml. A testicular volume of 4 ml or greater was considered as the onset of puberty. If there was a discrepancy in the testicular volumes of two sides, the larger one was taken as the final volume.

Fasting blood samples were drawn for the estimation of serum 25-hydroxy Vitamin D (25(OH) D), intact parathyroid hormone (iPTH), total and ionized calcium, inorganic phosphorus, and alkaline phosphatase (ALP). The normal range for different biochemical parameters are as follows: Serum total calcium - 2.2–2.55 mmol/L, ionized calcium 1.12–1.32 mmol/L, inorganic phosphorus 0.9–1.5 mmol/L, and ALP < 240 U/L. The serum concentrations of 25(OH) D (reference range: 22.5–94 nmol/L) and PTH (reference range: 10–65 ng/L) were measured by RIA (Diasorin, Stillwater, MN) and electrochemiluminescence assay (Roche Diagnostics, GmdH-Manheim, Germany), respectively.

BMC and regional distribution, FM and LM were measured using the Prodigy Oracle (GE Lunar Corp., Madison, WI) according to standard protocol. Quality control procedures were carried out in accordance with the manufacturer’s recommendations. Instrument variation was determined regularly using a phantom supplied by the manufacturer and mean coefficient of variation was < 0.5%. For *in vivo* measurements, mean coefficients of variation for all sites were < 1%. BMC index was calculated by total bone weight in kg divided by square of height in meters. Total and regional BMC were adjusted for height, FM and LM by calculating BMC/Ht, BMC/fat, and BMC/lean ratio.

Statistical analysis was carried out using SPSS version 20.0 (Chicago, IL, USA). Data were presented as mean ± standard deviation or number (%) unless specified. Independent two variables (gender) were tested by Student’s *t*-test. A one-way analysis of variance was used to compare the significance level between two groups within each parameter. Pearson’s correlation coefficient was calculated to assess the strength of the relationship between total BMC and its distribution and various anthropometric, biochemical, and densitometric parameters. Multiple regression analysis was done to ascertain association between total and regional BMC as dependable variable and LM or FM, age, SMS, serum calcium, phosphates, serum alkaline phosphatase (SAP), 25(OH) D, and iPTH levels as independent variables.

**Results**

Basic characteristics of 1403 children and adolescents (B - 826; G - 577) ranging from 5 to 18 years and a mean age of 13.2 ± 2.7 years (B - 13.0 ± 2.7; G - 13.4 ± 2.8 years) are as shown in Table 1. Boys were younger, taller and heavier than girls, but their BMI was lower than that of girls. Boys had higher serum 25(OH) D, calcium, phosphates, and ALP levels [Table 1].
BMC at all sites except trunk was higher in boys when compared with girls. When BMC was adjusted for height (BMC index, total BMC/HT ratio), there was no difference between boys and girls. Similarly, total BMC adjusted for weight was also similar between the genders. BMC/FM was higher while BMC/LM was lower in boys than girls, probably reflecting the higher LM in boys compared to girls [Table 1].

Total and regional BMC were higher in more advanced stages of pubertal maturation, and the difference between early and late puberty persisted even after adjustment for age, except the comparison between pubertal Stages 4 and 5 in girls. Similarly, BMC index only increased significantly between pubertal Stage 3 and 4 in girls and Stage 4 and 5 in boys after controlling for age [Table 2]. The percentage increase in total BMC from pubertal Stage 1–5 was comparable between genders (B: 125% vs. G: 134%). A similar pattern of increase in BMC was observed at other regions [Supplementary Table 1]. Girls accumulated more BMC per unit of LM during pubertal maturation when compared to boys. However, BMC accumulation per unit of fat remained constant among girls during pubertal progression as compared to boys.

Total and regional BMC were found to be positively correlated with age, height, BMI, total LM and FM, and 25(OH) D levels and negatively correlated with iPTH, ALP, calcium, and phosphorus in the study population and both genders independently [Table 3]. Importantly, correlation of BMC with height was stronger than that with BMI, and LM stronger than FM [Figure 1]. On multiple regression analysis, with adjustment for age, height, serum calcium, phosphates, ALP, 25(OH) D, iPTH, and SMS, the BMC was positively correlated with LM and FM at all sites. The relationship was stronger for total LM except at trunk in girls, where it was stronger for total FM [Table 4].

### Discussion

In the present study, we report higher total and regional BMC at all ages in boys when compared to girls except at trunk. Similar observations have been reported among UK,[10,13] Polish,[14] Lebanese,[23] and Thai children and adolescents.[7] However, there was no significant difference in BMC index, which takes into account differences in height, between genders except in the prepubertal age group. This suggests that BMC is comparable in both genders, when adjusted for height. This was further supported by comparable BMC/HT ratio and increment in BMC during puberty in both genders. In the present study, the difference in total BMC between genders became significant only after the age of 11 years. A similar observation was made in another Indian study[8] and in healthy Thai children and adolescents[7].
Table 2: BMC according to pubertal staging after adjusting for age

| Pubertal staging* | 1 (boys=103; girls=39) | 2 (boys=194; girls=49) | 3 (boys=183; girls=80) | 4 (boys=148; girls=117) | 5 (boys=198; girls=292) |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Total BMC (kg)    |                        |                        |                        |                        |                        |
| Boys              | 1.69±0.056 (1.583–1.805) | 1.61±0.032 (1.554–1.678) | 1.78±0.027 (1.735–1.843) | 1.97±0.033 (1.906–2.037) | 2.18±0.039 (2.041–2.195) |
|                  | 0.134                  | <0.0001                | <0.0001                | <0.0001                | 0.001                  |
| Girls             | 1.36±0.080 (1.207–1.520) | 1.41±0.060 (1.302–1.536) | 1.59±0.041 (1.516–1.678) | 1.80±0.030 (1.742–1.858) | 1.86±0.028 (1.808–1.918) |
|                  | 0.443                  | 0.003                  | <0.0001                | <0.0001                | 0.137                  |
| Arm BMC (kg)      |                        |                        |                        |                        |                        |
| Boys              | 0.19±0.008 (0.174–0.205) | 0.18±0.004 (0.172–0.189) | 0.20±0.004 (0.200–0.215) | 0.24±0.005 (0.234–0.252) | 0.26±0.006 (0.258–0.280) |
|                  | <0.0001                | <0.0001                | <0.0001                | <0.0001                | <0.0001                |
| Girls             | 0.14±0.009 (0.122–0.159) | 0.14±0.007 (0.135–0.162) | 0.16±0.005 (0.159–0.177) | 0.19±0.003 (0.191–0.204) | 0.20±0.003 (0.199–0.212) |
|                  | 0.323                  | 0.004                  | <0.0001                | <0.0001                | 0.092                  |
| Leg BMC (kg)      |                        |                        |                        |                        |                        |
| Boys              | 0.63±0.024 (0.591–0.687) | 0.63±0.014 (0.607–0.660) | 0.71±0.012 (0.698–0.744) | 0.79±0.014 (0.763–0.819) | 0.83±0.017 (0.801–0.868) |
|                  | <0.0001                | <0.0001                | <0.0001                | <0.0001                | 0.021                  |
| Girls             | 0.44±0.032 (0.381–0.506) | 0.49±0.024 (0.452–0.545) | 0.60±0.016 (0.572–0.636) | 0.67±0.012 (0.648–0.694) | 0.67±0.011 (0.652–0.697) |
|                  | 0.056                  | 0.004                  | 0.001                  | 0.841                  |                        |
| Trunk BMC (kg)    |                        |                        |                        |                        |                        |
| Boys              | 0.51±0.026 (0.497–0.599) | 0.47±0.013 (0.443–0.500) | 0.54±0.015 (0.491–0.541) | 0.58±0.015 (0.553–0.613) | 0.62±0.018 (0.588–0.658) |
|                  | 0.304                  | 0.020                  | 0.001                  | 0.042                  |                        |
| Girls             | 0.43±0.038 (0.358–0.506) | 0.41±0.028 (0.363–0.473) | 0.47±0.019 (0.437–0.513) | 0.57±0.014 (0.552–0.607) | 0.59±0.013 (0.571–0.624) |
|                  | 0.678                  | 0.044                  | <0.0001                | 0.358                  |                        |
| BMC index (kg/m²) |                        |                        |                        |                        |                        |
| Boys              | 0.72±0.017 (0.693–0.759) | 0.69±0.009 (0.675–0.712) | 0.72±0.008 (0.705–0.737) | 0.74±0.010 (0.723–0.762) | 0.78±0.012 (0.762–0.807) |
|                  | 0.037                  | 0.024                  | 0.101                  | 0.001                  |                        |
| Girls             | 0.67±0.027 (0.619–0.726) | 0.67±0.020 (0.638–0.718) | 0.69±0.014 (0.670–0.725) | 0.75±0.010 (0.736–0.775) | 0.77±0.010 (0.758–0.796) |
|                  | 0.326                  | 0.349                  | 0.001                  | 0.133                  |                        |

Values are expressed as mean±SE. SE: Standard error, BMC: Bone mineral content
study from India (B: 125% vs. 184%; G: 134% vs. 177%), but was similar to that reported in young Asian and Caucasian Americans (B: 119%; G: 140%). Studies among Caucasians, Polish, and adolescents from Thailand reported higher bone mass accrual among boys compared to girls. Total and regional BMC increases with age, which may also contribute to the increment in BMC observed during the evolution of puberty. After adjusting for age, there was no difference in total and regional BMC between pubertal Stages 1 and 2, suggesting that this is age related, whereas, the contribution of puberty to increase

Table 3: Correlation of BMC with anthropometric, hormonal, and densitometric (bone and total body composition) parameters

| Parameters       | Total BMC | Trunk BMC | Leg BMC | Arm BMC |
|------------------|-----------|-----------|---------|---------|
| Boys             |           |           |         |         |
| Age (years)      | 0.793 (<0.0001) | 0.707 (<0.0001) | 0.797 (<0.0001) | 0.804 (<0.0001) |
| Height (cm)      | 0.872 (<0.0001) | 0.771 (<0.0001) | 0.900 (<0.0001) | 0.876 (<0.0001) |
| BMI (kg/m²)      | 0.620 (<0.0001) | 0.634 (<0.0001) | 0.609 (<0.0001) | 0.513 (<0.0001) |
| SMS              | 0.767 (<0.0001) | 0.672 (<0.0001) | 0.767 (<0.0001) | 0.795 (<0.0001) |
| Serum 25(OH)D (ng/ml) | 0.114 (0.001) | 0.099 (0.005) | 0.119 (0.001) | 0.121 (0.001) |
| Serum PTH (pg/ml) | -0.183 (<0.0001) | -0.167 (<0.0001) | -0.172 (<0.0001) | -0.173 (<0.0001) |
| Calcium (mg/dl)  | -0.053 (0.131) | -0.033 (0.352) | -0.059 (0.092) | -0.068 (0.050) |
| Phosphorus (mg/dl) | -0.487 (<0.0001) | -0.427 (<0.0001) | -0.480 (<0.0001) | -0.500 (<0.0001) |
| ALP              | -0.460 (<0.0001) | -0.415 (<0.0001) | -0.449 (<0.0001) | -0.481 (<0.0001) |
| Total LM (kg)    | 0.937 (<0.0001) | 0.845 (<0.0001) | 0.944 (<0.0001) | 0.942 (<0.0001) |
| Total FM (kg)    | 0.550 (<0.0001) | 0.580 (<0.0001) | 0.541 (<0.0001) | 0.411 (<0.0001) |

| Girls            |           |           |         |         |
| Age (years)      | 0.760 (<0.0001) | 0.685 (<0.0001) | 0.721 (<0.0001) | 0.775 (<0.0001) |
| Height (cm)      | 0.832 (<0.0001) | 0.745 (<0.0001) | 0.853 (<0.0001) | 0.832 (<0.0001) |
| BMI (kg/m²)      | 0.673 (<0.0001) | 0.675 (<0.0001) | 0.673 (<0.0001) | 0.545 (<0.0001) |
| SMS              | 0.749 (<0.0001) | 0.676 (<0.0001) | 0.720 (<0.0001) | 0.769 (<0.0001) |
| Serum 25(OH)D (ng/ml) | 0.210 (<0.0001) | 0.174 (<0.0001) | 0.222 (<0.0001) | 0.215 (<0.0001) |
| Serum PTH (pg/ml) | -0.215 (<0.0001) | -0.186 (<0.0001) | -0.195 (<0.0001) | -0.211 (<0.0001) |
| Calcium (mg/dl)  | -0.283 (<0.0001) | -0.289 (<0.0001) | -0.250 (<0.0001) | -0.281 (<0.0001) |
| Phosphorus (mg/dl) | -0.499 (<0.0001) | -0.459 (<0.0001) | -0.452 (<0.0001) | -0.494 (<0.0001) |
| ALP              | -0.562 (<0.0001) | -0.520 (<0.0001) | -0.499 (<0.0001) | -0.591 (<0.0001) |
| Total LM (kg)    | 0.883 (<0.0001) | 0.801 (<0.0001) | 0.911 (<0.0001) | 0.867 (<0.0001) |
| Total FM (kg)    | 0.761 (<0.0001) | 0.773 (<0.0001) | 0.757 (<0.0001) | 0.607 (<0.0001) |

Results are expressed as r value and (P value). 25(OH)D: 25 hydroxy Vitamin D, BMC: Bone mineral content, FM: Fat mass, LM: Lean mass, PTH: Parathormone, ALP: Alkaline phosphatase, SMS: Sexual maturity score

Table 4: Correlation of BMC with lean and fat mass after adjusting for age, height, SMS, serum calcium, phosphates, ALP, 25(OH)D, and iPTH

| Parameters       | Total BMC | Trunk BMC | Leg BMC | Arm BMC | BMC Index |
|------------------|-----------|-----------|---------|---------|-----------|
| Boys             |           |           |         |         |           |
| Total lean       | r²=0.880  | r²=0.715  | r²=0.894 | r²=0.891 | r²=0.619  |
|                  | Beta=0.892 | Beta=0.839 | Beta=0.903 | Beta=0.870 | Beta=0.845 |
|                  | <0.0001   | <0.0001   | <0.0001  | <0.0001  | <0.0001   |
| Total fat        | r²=0.776  | r²=0.676  | r²=0.772 | r²=0.728 | r²=0.667  |
|                  | Beta=0.361 | Beta=0.420 | Beta=0.348 | Beta=0.207 | Beta=0.523 |
|                  | <0.0001   | <0.0001   | <0.0001  | <0.0001  | <0.0001   |
| Girls            |           |           |         |         |           |
| Total lean       | r²=0.827  | r²=0.682  | r²=0.846 | r²=0.815 | r²=0.642  |
|                  | Beta=0.693 | Beta=0.638 | Beta=0.804 | Beta=0.615 | Beta=0.589 |
|                  | <0.0001   | <0.0001   | <0.0001  | <0.0001  | <0.0001   |
| Total fat        | r²=0.781  | r²=0.721  | r²=0.741 | r²=0.689 | r²=0.709  |
|                  | Beta=0.490 | Beta=0.562 | Beta=0.516 | Beta=0.269 | Beta=0.568 |
|                  | <0.0001   | <0.0001   | <0.0001  | <0.0001  | <0.0001   |

Results are expressed as r², beta-coefficient and P value. BMC: Bone mineral content, 25(OH)D: 25 hydroxy Vitamin D, ALP: Alkaline phosphatase, iPTH: Intact parathyroid hormone, SMS: Sexual maturity score

adolescents.[1] However, a study from Poland reported no difference in total BMC till the age of 16 years between genders.[3] Since BMC index is adjusted for height and does differ between genders, it can become a useful tool for assessing musculoskeletal health in children and adolescents.

As reported earlier,[8,28] we also found that puberty is associated with an increase in total and regional BMC. The overall increase in total BMC during pubertal progression in present study was lower than that reported in another
in BMC predominantly begins from pubertal Stage 3. A similar observation was made by Ashby et al., who reported no difference between genders in total BMC till pubertal Stage 3.[13] This may be due to the fact that bone accrual follows the peak height velocity.

Our results showed that total and regional BMC was positively related to total LM and FM, which persisted after adjusting for anthropometric and biochemical parameters. Previous studies have also reported a positive association between BMC and LM after controlling for various factors.[5,9,11,17,18,20,21] As reported previously,[13] BMC had a higher Pearson’s correlation coefficient for LM in boys when compared to girls. The relation between BMC and FM has been inconsistent, with reports showing a positive[3,5,9‑11,17,18,20‑22] negative correlation[18,31] and absent correlation.[23] Similar to earlier literature,[3,13,32] we also report that the relation between LM and total and regional BMC was stronger than FM in both genders, except trunk BMC in girls. Other studies, including a longitudinal birth cohort study, showed a stronger correlation between total BMC and BMC in girls as compared to boys.[6,9,23,25,33] On the contrary, a study from Italy found that the association between BMC and FM in boys and girls was comparable.[3] This heterogeneity in observations can be due to differential sensitivity of trabecular and cortical bone to mechanical loading and response to adipokines.[34] It has been reported that FM is a stronger stimulus for the accrual of cortical bone mass in girls with a greater tendency to stimulate periosteal growth and suppress endosteal expansion.[25]

The main limitation was the cross-sectional design of our study, which makes it difficult to assess the sequential changes in BMC with the progression of puberty. Correlation between various factors may differ between cross-sectional and longitudinal studies.[9,18,22] In the present study, there is no information available on genetics, plasma hormones, nutritional status, physical activity, or growth and development in our subjects, which have been shown to have an impact on BMC.[1,2,35]

**Conclusion**

Boys had higher BMC than girls, but height adjusted BMC was comparable in both genders. We demonstrated that LM was more strongly associated with BMC than FM.

**References**

1. Zemel B. Bone mineral accretion and its relationship to growth, sexual maturation and body composition during childhood and adolescence. World Rev Nutr Diet 2013;106:39‑45.
2. Looomba‑Albrecht LA, Styne DM. Effect of puberty on body composition. Curr Opin Endocrinol Diabetes Obes 2009;16:10‑5.
3. Pietrobelli A, Faith MS, Wang J, Brambilla P, Chiumello G, Heymsfield SB. Association of lean tissue and fat mass with bone mineral content in children and adolescents. Obes Res 2002;10:56‑60.
4. Bhudhikanok GS, Wang MC, Eckert K, Matkin C, Marcus R, Bachrach LK. Differences in bone mineral in young Asian and Caucasian Americans may reflect differences in bone size. J Bone Miner Res 1996;11:1545‑56.
5. Burrows M, Baxter‑Jones A, Mirwald R, Macdonald H, McKay H. Bone mineral accrual across growth in a mixed‑ethnic group of children: are Asian children disadvantaged from an early age? Calcif Tissue Int 2009;84:366‑78.
6. Zhu K, Briffa K, Smith A, Mountain J, Briggs AM, Lyse S, et al. Gender differences in the relationships between lean body mass, fat mass and peak bone mass in young adults. Osteoporos Int 2014;25:1563‑70.
7. Nakavachara P, Pooliam J, Weerakulwattana L, Kattisakthavee P, Chaichanwattanakul K, Manorompatarsarn R, et al. A normal reference of bone mineral density (BMD) measured by dual energy X‑ray absorptiometry in healthy Thai children and adolescents aged 5‑18 years: a new reference for Southeast Asian Populations. PLoS One 2014;9:e97218.
8. Khadilkar AV, Sanwalka NJ, Chiplonkar SA, Khadilkar VV, Mughal MZ. Normative data and percentile curves for Dual Energy X‑ray Absorptiometry in healthy Indian girls and boys aged 5‑17 years. Bone 2011;48:810‑9.
9. Ferretti JL, Capozza RF, Cointry GR, García SL, Plotkin H, Alvarez Figuereira ML, et al. Gender‑related differences in the relationship between densitometric values of whole‑body bone mineral content and lean body mass in humans between 2 and 87 years of age. Bone 1998;22:683‑90.
10. Crabtree NJ, Kibirige MS, Fordham JN, Banks LM, Muntoni F, Chinn D, et al. The relationship between lean body mass and bone mineral content in paediatric health and disease. Bone 2004;35:965‑72.
11. Goulding A, Taylor RW, Grant AM, Jones S, Taylor BJ, Williams SM. Relationships of appendicular LMI and total body LMI to bone mass and physical activity levels in a birth cohort of New Zealand five‑year olds. Bone 2009;45:455‑9.
12. Kriemler S, Zahner L, Puder JJ, Braun‑Fahrländer C, Schindler C, Farpour‑Lambert NJ, et al. Weight‑bearing bones are more sensitive to physical exercise in boys than in girls during pre‑ and early puberty: a cross‑sectional study. Osteoporos Int 2008;19:1749‑58.
13. Ashby RL, Adams JE, Roberts SA, Mughal MZ, Ward KA. The muscle‑bone unit of peripheral and central skeletal sites in children and young adults. Osteoporos Int 2011;22:121‑32.
14. Boot AM, Bouquet J, de Ridder MA, Krenning EP, de Muinck Keizer‑Schràma SM. Determinants of body composition measured by dual‑energy X‑ray absorptiometry in Dutch children and adolescents. Am J Clin Nutr 1997;66:232‑8.
15. Kirchengast S. Gender differences in body composition from childhood to old age: An evolutionary point of view. J Life Sci 2010;2:1‑10.
16. Ho‑Pham LT, Nguyen UD, Nguyen TV. Association between lean mass, fat mass, and bone mineral density: a meta‑analysis. J Clin Endocrinol Metab 2014;99:30‑8.
17. Manzoni P, Brambilla P, Pietrobelli A, Beccaria L, Bianchessi A, Mora S, et al. Influence of body composition on bone mineral content in children and adolescents. Am J Clin Nutr 1996;64:603‑7.
18. Wey HE, Binkley TL, Beare TM, Wey CL, Specker BL. Cross‑sectional versus longitudinal associations of lean and fat mass with pQCT bone outcomes in children. J Clin Endocrinol Metab 2011;96:106‑14.
19. Rocher E, Chappard C, Jaffre C, Benhamou CL, Courteix D. Bone mineral density in prepubertal obese and control children: relation to body weight, lean mass, and fat mass. J Bone Miner Metab 2008;26:73‑8.
20. Dorsey KB, Thornton JC, Heymsfield SB, Gallagher D. Greater lean tissue and skeletal muscle mass are associated with higher bone mineral content in children. Nutr Metab (Lond) 2010;7:41.

21. Baptista F, Barrigas C, Vieira F, Santa-Clara H, Homens PM, Fragoso I, et al. The role of lean body mass and physical activity in bone health in children. J Bone Miner Metab 2012;30:100-8.

22. Rauch F, Bailey DA, Baxter-Jones A, Mirwald R, Faulkner R. The ‘muscle-bone unit’ during the pubertal growth spurt. Bone 2004;34:771-5.

23. El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffré C. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. Eur J Appl Physiol 2009;105:759-64.

24. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 2000;27:203-7.

25. Sayers A, Tobias JH. Fat mass exerts a greater effect on cortical bone mass in girls than boys. J Clin Endocrinol Metab 2010;95:699-706.

26. Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy X-ray absorptiometry. Pediatr Radiol 2009;39:647-56.

27. Shivaprasad C, Marwaha RK, Tandon N, Kanwar R, Mani K, Narang A, et al. Correlation between bone mineral density measured by peripheral and central dual energy X-ray absorptiometry in healthy Indian children and adolescents aged 10-18 years. J Pediatr Endocrinol Metab 2013;26:695-702.

28. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, et al. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr 2005;82:477-82.

29. Tanner JM. Growth at Adolescence: With a General Consideration of the Effects of Hereditary and Environmental Factors upon Growth and Maturation from Birth to Maturity. Oxford: Blackwell Scientific Publications; 1969.

30. Pudowski P, Matusik H, Olszaniecza M, Lebiedowski M, Lorenc RS. Reference values for the indicators of skeletal and muscular status of healthy Polish children. J Clin Densitom 2005;8:164-77.

31. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 2000;27:203-7.

32. Courteix D, Lespessailles E, Loiseau-Peres S, Obert P, Ferry B, Benhamou CL. Lean tissue mass is a better predictor of bone mineral content and density than body weight in prepubertal girls. Rev Rheum 1998;65:328-36.

33. Qin MW, Yu W, Xu L, Tian JP, Xing XP, Meng XW, et al. Bone mineral and body composition analysis of whole body assessed by dual X-ray absorptiometry. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2003;25:66-9.

34. El Hage R, El Hage Z, Jacob C, Moussa E, Theunynck D, Baddoura R. Bone mineral content and density in overweight and control adolescent boys. J Clin Densitom 2011;14:122-8.

35. Ferretti JL, Cointry GR, Capozza RF, Frost HM. Bone mass, bone strength, muscle-bone interactions, osteopenias and osteoporoses. Mech Ageing Dev 2003;124:269-79.
## Supplementary Table 1: BMC according to pubertal staging

| Pubertal staging* | 1 (boys=103; girls=39) | 2 (boys=194; girls=49) | 3 (boys=183; girls=80) | 4 (boys=148; girls=117) | 5 (boys=198; girls=292) |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| **Total BMC (kg)**|                        |                        |                        |                        |                        |
| Boys              | 1.114±0.286 (1.058–1.170) | 1.385±0.294 (1.344–1.427) | 1.792±0.429 (1.729–1.854) | 2.153±0.465 (2.077–2.229) | 2.507±0.488 (2.438–2.575) |
| Percentage increase | 24.3 | 29.4 | 20.1 | 16.4 | 14.3 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Girls             | 0.869±0.240 (0.791–0.947) | 1.108±0.194 (1.052–1.163) | 1.431±0.325 (1.359–1.503) | 1.780±0.355 (1.715–1.845) | 2.035±0.488 (1.994–2.076) |
| Percentage increase | 27.5 | 29.2 | 24.4 | 14.3 | 13.9 |
| $P$ *             | 0.005 | 0.001 | <0.0001 | <0.0001 | <0.0001 |
| **Arm BMC (kg)**  |                        |                        |                        |                        |                        |
| Boys              | 0.111±0.033 (0.105–0.117) | 0.150±0.040 (0.144–0.155) | 0.208±0.060 (0.199–0.277) | 0.267±0.086 (0.256–0.278) | 0.322±0.069 (0.312–0.331) |
| Percentage increase | 35.1 | 38.7 | 28.4 | 20.6 | 15.9 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Girls             | 0.082±0.033 (0.072–0.094) | 0.112±0.027 (0.105–0.120) | 0.149±0.038 (0.141–0.157) | 0.195±0.042 (0.187–0.203) | 0.226±0.040 (0.221–0.230) |
| Percentage increase | 34.9 | 33.0 | 30.9 | 15.9 | 13.9 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Leg BMC (kg)**  |                        |                        |                        |                        |                        |
| Boys              | 0.389±0.146 (0.361–0.418) | 0.534±0.143 (0.514–0.554) | 0.723±0.190 (0.695–0.750) | 0.869±0.183 (0.840–0.899) | 1.002±0.201 (0.973–1.030) |
| Percentage increase | 32.5 | 35.4 | 20.2 | 15.3 | 10.2 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Girls             | 0.279±0.102 (0.246–0.312) | 0.395±0.085 (0.371–0.420) | 0.549±0.140 (0.518–0.580) | 0.664±0.143 (0.638–0.690) | 0.732±0.134 (0.716–0.747) |
| Percentage increase | 37.3 | 39.0 | 20.9 | 10.2 | 0.21 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Trunk BMC (kg)**|                        |                        |                        |                        |                        |
| Boys              | 0.320±0.241 (0.273–0.367) | 0.381±0.123 (0.363–0.398) | 0.517±0.163 (0.493–0.540) | 0.654±0.200 (0.622–0.687) | 0.776±0.197 (0.748–0.803) |
| Percentage increase | 19.1 | 35.7 | 26.5 | 18.7 | 15.7 |
| $P$ *             | 0.007 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Girls             | 0.246±0.090 (0.217–0.275) | 0.301±0.074 (0.280–0.322) | 0.413±0.134 (0.383–0.443) | 0.572±0.182 (0.538–0.605) | 0.662±0.164 (0.643–0.681) |
| Percentage increase | 22.4 | 37.2 | 38.5 | 15.7 | 0.20 |
| $P$ *             | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **BMC index (kg/m²)**|                        |                        |                        |                        |                        |
| Boys              | 0.611±0.081 (0.595–0.627) | 0.648±0.086 (0.635–0.660) | 0.722±0.118 (0.705–0.739) | 0.778±0.123 (0.758–0.798) | 0.861±0.144 (0.841–0.881) |
| Percentage increase | 6.1 | 11.4 | 7.8 | 10.7 | 10.7 |
| $P$ *             | 0.010 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Girls             | 0.536±0.078 (0.511–0.562) | 0.592±0.063 (0.574–0.610) | 0.652±0.108 (0.628–0.676) | 0.750±0.119 (0.728–0.772) | 0.825±0.120 (0.811–0.838) |
| Percentage increase | 10.5 | 15.1 | 10.0 | 10.0 | 10.0 |
| $P$ *             | 0.021 | 0.004 | <0.0001 | <0.0001 | <0.0001 |
| $P$ **            | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

*P value trend <0.0001 for all; *Percentage increase of mean lean mass between successive pubertal stage; **P value between successive pubertal staging within each gender; ***P value between gender at each pubertal staging. BMC: Bone mineral content