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Bone Mineral Density and Nutritional Status of Healthy Sri Lankan PreSchool Children

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ABSTRACT

Background: We wished to assess bone mineral density (BMD) values and factors that contribute to bone mineral accrual among preschool children in Sri Lanka. Currently, this information is not available.
Objectives: To measure BMDs of spine and hip, using a central-type DXA, in a representative sample of preschool children in Southern Sri Lanka and to study their anthropometry and micronutrient status in order to study the associations of such measurements with bone mineral status.
Patients and Methods: We measured BMD of the spine and hip using dual-energy absorptiometry in 105 preschool children (52 boys) aged 3–5 years in Southern Sri Lanka. We also studied their anthropometric characteristics and micronutrient status (iron, zinc, calcium, ceruloplasmin, free thyroxine, and vitamins A and D).
Results: Although spine BMD showed no sex difference (mean BMD 0.451 g/cm^2 and 0.447 g/cm^2 in boys and girls, respectively; $p = 0.70$), proximal femur BMD values were significantly higher ($p = 0.02$) among boys (0.594 g/cm^2) than among girls (0.557 g/cm^2). Boys had significantly higher bone area in the spine, compared to girls (mean 25.58 vs. 24.05 cm^2; $p = 0.02$). After controlling for other independent variables studied (anthropometry and biochemistry), weight and serum calcium accounted for 26% of the BMD variation ($R^2 = 0.26$). One unit change in body weight (1 kg) or serum calcium level (1 mmol/L) was associated with a change in spine BMD of 0.051 g/cm^2 or 0.016 g/cm^2, respectively.
Conclusions: In addition to higher BMD in the proximal femur boys have broader bones, particularly in the spine. Among different indices of body measurements and multiple nutritional factors, body weight and serum calcium appear to be the main determinants of BMD accrual.

Implication for health policy/practice/research/medical education:
Study on determinants of childhood bone mineral accrual would identify the major determinant of peak bone mass in later life.

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1. Background

Optimizing peak bone mass accrual during childhood is one of the strategies of preventing age-related osteoporosis (1). Two dietary nutrients that have been most associated with building optimal bone mass are vitamin D and calcium. Childhood and adolescence are particularly relevant with regards bone mass because a major portion of peak bone mass is accrued during this period

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and the process is potentially modifiable (2). Dual-energy x-ray absorptiometry (DXA) is an accepted technique for the estimation of bone mineral status in children. Bone mineral density (BMD) and bone mineral content (BMC) estimated by DXA can be interpreted only if age- and sex-specific normal reference values are known. Normal BMD reference values are expressed as age- and sex-specific means and standard deviations, allowing clinicians to calculate the deviation of a given BMD value from the normal age- and sex-specific mean and express it as a function of standard deviation (Z-score). Areal BMD estimation is influenced by body size; therefore, any two subjects with the same BMD could have different areal BMD values if their body measurements are different. Attempts have been made to adjust BMD measurements in children to take into account the influence of body size (3, 4). In Sri Lanka, normal BMD values among Preschool children are not available. Restricted availability of DXA and the low priority given to metabolic bone diseases in current medical practice may have contributed to this. Furthermore, the main contributory factors of bone accrual during adolescence are poorly documented in Sri Lanka. Since BMD as well as factors that contribute to BMD accrual vary according to ethnicity and geography, ethnically and geographically specific information is required when bone health promotion programs are designed for local populations.

2. Objectives

We measured BMD_s of the spine and hip using a central-type DXA in a representative sample of Preschool children in Southern Sri Lanka and studied their anthropometric characteristics and micronutrient status in order to determine the association of these measurements with bone mineral status.

3. Patients and Methods

Healthy children (n = 105, 52 boys) aged between 3 and 5 years, who were free of infectious diseases and diarrhea within the preceding month, were selected from the University Field Training area of the Faculty of Medicine, Galle, Sri Lanka. The Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, granted approval for the study. An announcement regarding the study was displayed in public and private places several weeks prior to screening. Parents were invited to a meeting in which the purpose of the study, possible risks, and benefits were explained in detail. They were given an opportunity to ask questions regarding the study. Informed written consent was obtained from parents of each study subject. Subjects who had a history of medical conditions, such as endocrine or chronic diseases affecting major organs, and chronic diseases of an inflammatory nature lasting more than one month were not included in the study. Subjects who consumed medications, including vitamin or mineral preparations, for more than one month were also excluded. Height, weight, and medical history were obtained and a physical examination was conducted in order to assess eligibility for the study. Height and weight were measured using a portable stadiometer and a beam balance, respectively. A 5-mL sample of venous blood was drawn from each child and an aliquot of 0.20 μL was taken for hemoglobin assessment. The remaining portion of the blood sample was delivered to the laboratory where it was centrifuged at 5000 rpm for 10 min, and serum was separated and stored in acid-washed polystyrene tubes at -80°C until analysis for ferritin, folate, zinc, calcium, ceruloplasmin, vitamin A, and vitamin D (25 (OH) vitamin D was done).

3.1. Measurement of BMD and BMC

BMD, BMC, and bone area of the total spine from L1 to L4 in the posteroanterior projection and of the left proximal femur were measured using a dual-energy X-ray absorptiometer (Hologic Discovery, Bedford, MA, USA). The in vitro precision error of the machine was <0.6%, 0.9%, and 1.5%, respectively, for the spine scans (L1–L4) at slow, medium, and fast speeds, whereas the error was 1.2% and 1.5–2% for the femur scans at slow and medium speeds, respectively. The in vivo precision error of the machine has been reported earlier (5). The scans were acquired using the appropriate scan mode for the patient's weight. Bone mineral measurements were performed with the same machine and by the same operator throughout the study period.

3.2. Laboratory Analysis

Hemoglobin (Hb) concentration was measured spectrophotometrically by using the cyanmethemoglobin method at the Nutrition Research Laboratory, Faculty of Medicine. Serum concentrations of ferritin, ceruloplasmin, folate, and vitamin D were measured by using immunoradiometric assays (IRMAs) at the Radioimmunoassay Laboratory of the Nuclear Medicine Unit; the reagents were provided by the North East Thames Regional Immunoassay (NETRIA) Center, London. Serum calcium level was determined by enzyme-linked immunosorbent assay (ELISA) performed at the Nuclear Medicine Unit; the test kits were provided by Bioassays System, USA. Serum zinc level was determined by flame atomic absorption spectrophotometry at the Industrial Technology Institute, Colombo. The CV% and the details of biochemical analysis have been reported earlier (6). Anemia was defined as a Hb level < 11.0 g/dL, and depleted iron store was defined as a serum ferritin (SF) concentration < 15.0 μg/L (7). Iron deficiency anemia (IDA) was defined as the combination of a Hb level < 11.0 g/dL and an SF concentration < 15.0 μg/L. The following cut-off values were used to define the respective micronutrient deficiency states: vitamin A < 0.7 μmol/L, vitamin D < 35.0 nmol/L, calcium < 1.20 mmol/L, zinc < 9.945 μmol/L, ceruloplasmin < 240 mg/dL, and serum free T4 < 12.0 pmol/L (8).

3.3. Statistical Analysis

BMD values were converted to Z-scores by using the reference data provided by the manufacturer, based on the following formula: score = (patient's BMD – mean BMD of the reference population)/age- and sex-adjusted SD of the mean BMD of the reference population.

Statistical analysis was carried out using open-source statistical software (9). The prevalence of malnutrition was assessed on the basis of weight-for-age, height-for-age, and weight-for-height by using the Center for Disease Control's (CDC) 1978 reference standards. The relationship between bone mineral accrual and nutritional and anthropometric determinants was assessed by linear regression analysis. Because the distributions of serum levels of ferritin, free T4, folate, ceruloplasmin, vitamin D, and vitamin A were skewed, they were log transformed in all calculations. For presentation, these variables were transformed back to the original scale. Differences between groups (male and female) in anthropometric indices, DXA measurements, and biochemical measurements were tested using an independent sample *t*-test. Multiple regression analysis was performed to ascertain the effect of anthropometric and biochemical parameters on DXA measurements such as BMD, BMC, and bone area. Descriptive statistics are expressed as the mean (SD). Two-tailed p-values less than 0.05 were considered statistically significant.

4. Results

One hundred five children (52 boys) were recruited for the study. There was no difference in age distribution (p = 0.17) in these subjects (mean age of boys, 51.0 months [SD, 7.2 months]; mean age of girls, 48.8 months [SD, 7.5 months]). Boys had a mean weight of 14.56 (2.2) kg, and a mean height of 102.78 (5.8) cm, whereas the girls had a mean weight of 14.00 (1.7) kg, and a mean height of 101.65 (5.2) cm. The weight and height were not significantly different in boys and girls (p = 0.20 for weight, and p = 0.35 for height) (*Table 1)*. Body mass index (BMI) did not show any significant difference either (13.73 kg/m^2 in males and 13.52 kg/m^2 in females; p = 0.35). Eighteen percent (n = 19) of children were underweight (weight-for-age Z-score [WAZ] < -2.0). Twenty-one percent (n = 11) of boys and 15% (n = 8) girls were underweight. Only 2 children (1 boy and 1 girl) were stunted (height-for-age Z-score [HAZ] < -2.0) and 14 children (13%) were malnourished (weight-for-height Z-score [WHZ] < -2.0). There were no significant sex differences in any of the measurements studied, namely, hemoglobin, serum levels of ferritin, folate, zinc, calcium, ceruloplasmin, free T4, vitamin A, and vitamin D. When a cut-off value of Hb level < 11.5 g/dL was used to define anemia, 50% of subjects (n = 53) were found to be anemic (26 boys and 27 girls). The serum zinc and calcium levels of the anemic children were significantly lower than those of non-anemic children. Serum zinc levels among anemic and non-anemic children were 2.63 (0.9) and 3.20 (1.1) μmol/L, respectively (p = 0.01), and serum calcium values

Table 1. Baseline Anthropometric and Biochemical Characteristics of the Study Subjects

	Male, Mean ± SD (n = 52)	Female, Mean ± SD (n = 53)
Weight, kg	14.5 ± 2.2	14.0 ± 1.7
Height, cm	102.8 ± 5.8	101.6 ± 5.2
BMI [a], kg/m^2	13.7 ± 1.1	13.5 ± 0.9
HAZ [a]	-0.5 ± 1.0	-0.3 ± 0.8
WAZ [a]	-1.4 ± 0.9	-1.2 ± 0.9
WHZ [a]	-1.4 ± 0.9	-1.3 ± 0.7
Hemoglobin, g/dL	11.2 ± 1.1	11.1 ± 0.9
Calcium, mmol/L	1.9 ± 0.4	1.9 ± 0.5
Zinc, μmol/L	8.7 ± 3.1	9.0 ± 3.1
Free T4, pmol/L	15.7 ± 1.5	14.7 ± 1.3
Ferritin, μg/L	34.8 ± 1.7	37.5 ± 1.7
Ceruloplasmin, mg/dL	55.3 ± 1.5	55.7 ± 1.6
Folate, mmol/L	8.1 ± 1.8	8.1 ± 1.8
Vitamin D, nmol/L	64.6 ± 1.6	59.3 ± 1.7
Vitamin A, μmol/L	0.7 ± 1.5	0.9 ± 1.8

[a] Abbreviations: BMI, body mass index; HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.

Table 2. DXA Measurements of BMD (g/cm^2), BMC (g), and Bone Area (cm^2) of the Study Subjects

Region of Interest	Male, Mean ± SD (n = 52)	Female, Mean ± SD (n = 53)
Total spine		
BMD [a]	0.4 ± 0.0	0.4 ± 0.0
BMC [a]	11.6 ± 1.8	10.8 ± 1.9
Area [b]	25.5 ± 2.8	24.0 ± 2.8
Total femur		
BMD [c]	0.6 ± 0.0	0.5 ± 0.0
BMC	8.3 ± 2.0	8.2 ± 1.9
Area	14.1 ± 1.8	14.2 ± 2.6
Neck of femur		
BMD	0.4 ± 0.0	0.4 ± 0.0
BMC	1.2 ± 0.3	1.1 ± 0.2
Area	2.6 ± 0.7	2.7 ± 0.4
Trochanter		
BMD [d]	0.6 ± 0.08	0.5 ± 0.0
BMC	1.5 ± 0.4	1.5 ± 0.5
Area	2.5 ± 0.5	2.6 ± 0.6
Inter-trochanter		
BMD [e]	0.6 ± 0.07	0.6 ± 0.0
BMC	5.6 ± 1.3	5.6 ± 1.4
Area	8.9 ± 1.3	9.2 ± 1.3
Total spine Z-score	-0.6 ± 0.7	-0.5 ± 0.9

[a] Abbreviations: BMD, bone mineral density; BMC, bone mineral content
[b] Males had significantly higher, bone area (p = 0.02) of the total spine
[c] Males had significantly higher BMD (p = 0.02) in the total femur
[d] Males had significantly higher BMD (p = 0.05) in the trochanter
[e] Males had significantly higher BMD (p = 0.04) in the inter-trochanter

Table 3. Correlation Coefficients (r) of Age and Anthropometry With DXA Measurements of the Total Spine

	BMD [a, b]	BMC [a, b]	Bone Area	Total Spine Z-Score
Age, mo	0.25 (0.05)	0.37 (< 0.001)	0.39 (< 0.001)	-0.2 (0.08)
Weight, kg	0.50 (< 0.001)	0.68 (< 0.001)	0.56 (< 0.001)	0.25 (0.02)
Height, cm	0.47 (< 0.001)	0.72 (< 0.001)	0.65 (< 0.001)	0.14 (0.22)
BMI [a]	0.22 (0.05)	0.21 (0.05)	0.09 (0.41)	0.27 (0.01)
WAZ [a]	0.47 (< 0.001)	0.53 (< 0.001)	0.35 (< 0.001)	0.45 (< 0.001)
WHZ [a]	0.34 (0.002)	0.33 (< 0.001)	0.17 (0.14)	0.36 (< 0.001)

Results presented as r (p-value).
[a] Abbreviations: BMD, bone mineral density; BMC, bone mineral content; BMI, body mass index; ; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.

were 1.12 (0.3) and 1.24 (0.2) mmol/L, respectively ($p = 0.04$). There were 16 children (15%) with low serum vitamin D levels (<35.0 nmol/L), of which 6 were boys and 10 were girls. There were no significant differences in any of the other biochemical parameters measured between children with low and normal serum vitamin D levels. There were only 4 subjects (2 from each sex) with serum calcium levels below 1.20 mmol/L. However, it was revealed that 56% of children (n = 59) were zinc deficient (zinc level < 9.945 μmol/L), of which 26 were boys and 33 were girls. Serum ferritin and ceruloplasmin levels in zinc-deficient children were significantly lower than in children with no zinc deficiency (data not shown). The DXA measurements of the children are presented in (*Table 2*). There was no significant sex difference in mean spine BMD (0.451 g/cm^2 in boys and 0.447 g/cm^2 in girls; $p = 0.70$) and BMC (11.60 g in boys and 10.84 g in girls; $p = 0.08$). However, bone area was significantly higher in boys than in girls (25.58 vs. 24.05 cm^2; $p = 0.02$). Further, we observed significant differences in BMD values of the femur, trochanter, and the inter-trochanteric area, with boys having higher values than girls (*Table 2*). There was no significant difference in total spine Z-score ($p = 0.54$) between boys and girls. There were no significant correlations between DXA measurements and hemoglobin or other serum estimations. However, the total spine BMD,BMC, bone area, and Z-scores showed significant correlations with most of the anthropometric measurements (*Table 3*). The BMD, BMC and bone area were showed positive correlation with age. Multiple regression analysis with stepwise elimination (forward and backward) was used to assess the effect of selected explanatory variables on the DXA measurements among the study subjects. After controlling for the other independent variables included (anthropometric parameters: weight, height, body mass, WAZ, HAZ, and WHZ; biochemical measurements: Hb, folate, ferritin, free T4, calcium, zinc, ceruloplasmin, vitamin A, and vitamin D; and age), weight and serum calcium status were found to have statistically significant correlations with the BMD measurements. Furthermore, serum calcium and weight accounted for 26% of the BMD variation ($r^2 = 0.26$). One unit change in body weight (1 kg) or serum calcium (1 mmol/L) was associated with a change in spine BMD of 0.051 g/cm^2 or 0.016 g/cm^2, respectively. In contrast, height, WAZ scores, vitamin D deficiency status, and zinc deficiency of the children had a statistically significant positive correlation with BMC level. These variables together explained 59% of BMC variation. Only height and sex of the individual had significant correlations with bone area. These two variables together explained 45% of the variation in bone area. Further, spine BMC, BMD,

Table 4. DXA Measurements of Total Spine, Anthropometric Measurements, and Serum Parameters in Tertiles of Serum Vitamin D Levels

	Serum vitamin D		
	Lower Third (n = 31)	Middle Third (n = 31)	Upper Third (n = 43)
BMD [a], g/cm^2	0.445 (0.046)	0.449 (0.051)	0.457 (0.060)
BMC [a], g	10.88 (1.9)	11.26 (1.9)	11.71 (2.1)
Bone area, cm^2	24.20 (3.0)	25.04 (3.0)	25.51 (2.5)
Z-score	-0.62	-0.61	-0.50
Weight, kg	13.84 (1.5)	14.51 (2.1)	14.79 (2.4)
Height, cm	101.48 (5.8)	101.97 (4.7)	103.71 (5.5)
Hemoglobin, g/L	110.52 (10.2)	110.71 (11.5)	113.24 (8.5)
Serum calcium, mmol/L	1.96 (0.4)	1.79 (0.5) [b]	2.06 (0.4)
Serum zinc, μmol/L	8.35 (2.8)	9.44 (3.7)	8.91 (2.8)
Serum vitamin A, μmol/L	1.06 (0.6)	0.83 (0.4)	1.04 (0.5)

[a] Abbreviations: BMD, bone mineral density; BMC, bone mineral content.
[b] serum calcium levels in the middle third children were significantly lower ($p = 0.05$)

anthropometric indices, and other serum measurements were analyzed in tertiles of serum vitamin D distribution in the sample (*Table 4*). While biochemical indices showed no significant differences in the tertiles of vitamin D levels, BMD, BMC, bone area, height, and weight showed a uniform pattern across vitamin D tertiles. Compared to the children in the upper tertile, children with the lower third of vitamin D values were shorter, lighter, and had low spine BMD, BMC, and bone area. Furthermore, there was an upward trend in all variables across the 3 categories of vitamin D levels. The BMD of the neck of the femur among children in the upper third (0.453 g/cm^2) was significantly higher than that of the children in the lower tertile (0.426 g/cm^2; $p = 0.01$, data not shown). Similarly, total spine BMC (1.34 g) and total spine bone area (3.00 cm^2) in children in the upper tertile were significantly higher than those in the children in the lower tertile (BMC, 11.71 g; $p = 0.04$, and bone area, 25.51 cm^2; $p = 0.001$).

5. Discussion

Our study reports data of the total spine and hip BMD, BMC and bone area in a representative sample of Preschool children aged 3–5 years. There were significant differences in the BMD values of the proximal femur, with boys acquiring higher values than their female counterparts. Further, boys had higher bone area in the total spine. This difference is probably a reflection of the sex variation in the velocity of mineral gain in different age groups. However, this increase in bone mass measures not only reflects a true increase in BMD but also an increase of bone volume that occurs with growth. The lumbar spine BMD values that we found in our sample are higher than those of Finnish children (10), but comparable to those of Spanish (11) and Dutch children (12). This finding suggests that there may be geographical differences in BMD. We could not trace any Asian study to compare our results. Studies examining the prevalence of vitamin D deficiency among children are sparse. A comparison of our vitamin D data with other studies may not be entirely appropriate given the fact that different studies have been conducted in different seasons and using different assays. Nonetheless, severe hypovitaminosis D (< 12.5 nmol/L) as defined in the Lips classification (13), was not seen in our study sample, whereas it was seen in 8.6% of Indian children (14), 23.5% of Finnish children (15), and 45.2% of Chinese girls (16) during winter. In the Chinese study, vitamin D deficiency prevalence dropped to 6.7% when the same cohort was evaluated during summer. Current evidence suggests that increased serum vitamin D levels have only a little influence on skeletal calcium accrual in children in whom calcium intake and vitamin D status are not optimal but not overtly deficient (2). We observed that the children in the lower tertile of serum vitamin D levels had lower BMD and BMC measurement than the children in the upper tertile did. Viljakainen *et al*. (17) demonstrated that improving the vitamin D status of adolescent Finnish girls via vitamin D supplementation for 12 months significantly increased bone mineral augmentation of the femur and lumbar spine, and that serum vitamin D concentrations of >50 nmol/L at baseline may be optimal. Lehtonen-Veromaa *et al*. (18) reported that none of the girls in their study who had baseline serum vitamin D concentrations of >50 nmol/L lost BMD at the lumbar spine. There is no ideal measurement of body zinc status (19, 20). Serum zinc is not an ideal indicator of individual zinc status, because serum zinc levels decrease during sepsis and inflammation, and this measure lacks sensitivity to identify deficiency (20). However, we found normal serum ferritin and ceruloplasmin levels among children with zinc deficiency, indicating that ongoing sepsis or inflammation is an unlikely explanation for the low zinc levels observed. Therefore, the association between zinc deficiency and BMC observed in the regression analysis would indicate a role for zinc in bone metabolism and normal growth (21). Our data show a significant association of age and anthropometric measurments, and DXA measurements (BMD, BMC and bone area). The model incorporating weight and serum calcium explains approximately 26% of BMD variation. Height, and WAZ explain 59% of BMC variation. Several studies have shown an association of BMD with age, height, and weight. In an Indian study (22) age, weight, and height together accounted for 50% of BMD variation, whereas Pettifor & Moodley (23) found that these measures explained 57% of BMD variation. The relative individual contribution of each of these variables cannot be entirely determined because of the interdependence of each of these factors. A study among children aged 4–20 years by Boot et al. (12) revealed that age, sex, genetic-ethnic factors, hormonal status, calcium intake, physical activity, and weight are determinants of BMD. However, the major determinant of BMD during childhood was weight (and pubertal development in girls), which was similar to our findings. There are several limitations in our study. The participants of the current study were primarily from the University Field Training area and may not be representative of the entire country. They responded to open invitations; hence, a selection bias may have occurred. Although we intended to include a larger sample initially, sample size had to be restricted because of practical reasons. It is uncertain whether the results of the current study can be generalized to older children. Therefore, further studies in older age groups are needed.

In conclusion, the results shown here support the idea that anthropometric variables (weight and height) and nutritional factors are important determinants of BMD, BMC and bone area in children. Although vitamin D deficiency was seen among healthy schoolchildren, its clinical significance was not very clear. Compared to children with high vitamin D levels, those with low vitamin D levels were lighter, shorter, and had lower BMD, BMC. This

observation calls for further research, given the diverse roles of vitamin D in many physiological functions of the body.

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