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ORIGINAL ARTICLE

Determinants of bone mineral content and bone area in Indian preschool children

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Abstract The objective of this study was to examine the lifestyle factors that influence total body bone mineral content (TB BMC) and total body bone area (TB BA) in Indian preschool children. TB BMC and TB BA were measured by dual-energy X-ray absorptiometry (Lunar DPX PRO) in 71 apparently healthy children aged 2–3 years. A fasting blood sample was analyzed for serum concentrations of ionized calcium (iCa), intact parathyroid hormone (iPTH), phosphorus (iP) and 25-hydroxyvitamin D_3 (25 OHD). Dietary intake of energy, protein, calcium and phosphorus was estimated from a 3-day diet recall. The daily physical activity and sunlight exposure were recorded by a questionnaire. The study children were shorter than their age-gender matched WHO counterparts with a mean height for age Z score of -1.3 ± 1.5. The mean dietary intake of calcium was 46% of the Indian recommended dietary intakes (RDI). Seventy-three percent of children had low iCa concentrations, and 57% were deficient in vitamin D. Generalized linear model analysis revealed that height, lean body mass, weight, activity, sunlight exposure in minutes and dietary intakes of calcium, zinc and iron were the significantly influencing factors ($p < 0.05$) of TB BMC and TB BA. In conclusion, attaining optimal height for age, achieving the goals of overall nutrition with adequate calcium, iron and zinc intakes as well as adequate physical activity and sunlight exposure play an important role in achieving better TB BMC and TB BA in preschool children.

Keywords Bone mass · Preschool children · Calcium · Sunlight

Introduction

Bone mass increases normally throughout childhood, reaching peak levels by late adolescence or early adulthood [1]. Bone density at a later age is determined by peak bone mass (PBM) attained during formative years and the rate of bone loss in adulthood. Achievement of peak bone mass during the period of growth is an important determinant of risk of osteoporosis and consequent fractures in later life. Hence, it is important to identify the factors that influence bone mass accrual in children in order to develop preventive strategies for achieving improved bone health. The majority of studies have been conducted primarily in older children (≥7 years of age) [2, 3]. Studies reporting bone mineral content (BMC) in young children below 5 years of age are scarce [4–7], especially in Asian Indians [8, 9]. Considering the ethnic differences in bone mineral content of blacks, whites and Asians [10], it is essential to know the bone mineral status of young Indian children and explore the factors that influence bone mineral density (BMD) in childhood, with the goal of achieving optimal peak bone mass.

The factors that determine PBM can be categorized as genetic or non-modifiable and those that can be modified (i.e., nutrition, physical activity, sunlight exposure). Long-term habitual dietary calcium intake and physical activity are suggested to have a positive influence on BMC in 5-year-old children [11]. Similarly, Janz et al. [12] have reported that moderate activity at the age of 5 years predicts the bone mineral content at the age of 8 and 11 years with adjustments for growth and other possible

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confounders. Furthermore, dietary calcium intake has been found to modify the bone response to activity in young children [13]. Studies in adolescents have demonstrated positive effects of calcium supplementation on total body BMC (TB BMC) [14]. Dietary protein in the presence of optimal calcium and vitamin D supply has also been shown to exert a positive effect on bone health in adolescents [15, 16]. Thus, results of these studies indicate that adequate calcium and protein intake, and physical activity are associated with good bone health. Although there are several studies determining the predictors of bone mineral content in childhood, preschool children have been sparsely studied.

In view of the higher prevalence of malnutrition in developing countries, including India, and the lower bioavailability of minerals due to predominantly vegetarian diets, Indian children may be exposed to a greater risk of low bone mineral status. In Indian children and adolescents, low calcium intakes have been reported by several researchers [17–19]. Association of sunlight exposure and vitamin D status has been reported in other countries [22]. Being a tropical country, ample sunlight exposure is possible; however, limited data are available regarding vitamin D status and sunlight exposure in children and adolescents in India [20, 21]. Therefore, the aim of this study was to examine lifestyle factors as determinants of total body bone mineral content and bone area in Indian preschool children.

Materials and methods

Sample selection

This cross-sectional study was approved by the Ethics Committee of the Hirabai Cowasji Jehangir Medical Research Institute, Pune, India. We studied apparently healthy pre-school children attending our community paediatric clinic, which caters to children from all socioeconomic classes, including a population similar to the one we have published a study about previously [20]. In all, 120 parents of apparently healthy children between 2 and 3 years of age were approached for the study from March 2009 to May 2009.

The selection criteria were such that the children were apparently healthy and were not taking any calcium supplement. Consent was obtained from the children before the study was performed. The exclusion criteria were: (1) history of fracture or prolonged immobilization within the past 12 months and (2) major systemic disease.

A clinical examination was performed at Jehangir Hospital by a pediatrician for evaluating health status. In all, 71 children (boys: 36; girls: 35) meeting the selection criteria were enrolled in the study.

Outcome measures

Anthropometry

Standing height was measured to the nearest millimeter using a portable stadiometer (Leicester Height Meter, Child Growth Foundation, UK), and weight was measured using an electronic scale to the nearest 100 g.

The height and weight Z scores were estimated from the contemporary World Health Organization (WHO) growth data [23].

Dietary food intake and physical activity

A 3-day dietary recall with 2 weekdays and a Sunday was obtained from the mother of each child. Nutritive value tables for raw Indian foods [24] and nutritive values of cooked foods [25] were used to estimate daily dietary intake of calcium, inorganic phosphorus, protein, energy and other micronutrients. Structured questionnaires were administered to the mothers to collect information about the children's daily physical activity and sunlight exposure [20, 26]. Activities like television watching, sitting in the classroom and sleeping in the afternoon were classified as inactivity. Walking, playing around with other children, running and cycling were considered as moderate activities [27]. Daily minutes of each activity were computed for each child. The questionnaires were tested for reliability on a pilot sample of 15 children for physical activity (intraclass correlation coefficient = 0.98, $p < 0.001$) and sunlight exposure (intraclass correlation coefficient = 0.98, $p < 0.001$). The time of the day and duration of sunlight exposure and clothing pattern of children were obtained and expressed as minutes of sunlight exposure per day.

Biochemical measurements (reference range given in parentheses)

A fasting blood sample of 5 ml was collected between 7 am and 9 am in a vacutainer by a trained pediatric nurse. Colorimetric assay was used to measure total calcium (tCa). Ionized calcium (iCa: 1.12–1.23 mmol/l) [28] was measured using an ion selective auto analyzer (AVL, ISE, Graz, Austria). Serum concentration of inorganic phosphorus (iP: 1.25–2.10 mmol/L) [28] was measured using a Semi Auto Analyzer (Biotech, USA). Serum intact parathyroid hormone (iPTH) was measured using the enzyme immunoassay technique (BioSource Europe S.A). The in-house reference range for the iPTH assay was 1.1–6.4 pmol/l, which was established in 100 15- to 45-year-old healthy volunteers from Pune [29]. The sensitivity was 0.22 pmol/l, and interassay variation was 10%. Serum concentration of 25-hydroxyvitamin D_3 (25 OHD) was measured using radioimmunoassay (DiaSorin, Stillwater, MN). The sensitivity of the assay was 3.75 nmol/l, and the interassay variation was <5%.

Bone densitometry

The GE-Lunar DPX PRO (GE Healthcare, Waukesha, WI) Pencil Beam DXA scanner (software encore 2005 version 9.30.044) was used to measure total body (TB) bone mineral content [BMC (g)], bone area [BA (cm^2)], lean body mass [LBM (g)] and fat mass [FM (g)]. The manufacturer's appointed service engineer was requested to review the calibration data and provide a scanner maintenance check to ensure the system's performance before the first subject was scanned, and to confirm that no instrumentation drift occurred. The machine calibration was performed daily using the phantom provided by the manufacturer. All scans and scan analysis were performed by the same operator. The precision of repeat measurements in children for the DPX PRO was estimated to be 0.98% for TB BMC, 1.13% for TB BA, 0.74% for TB LBM and 1.1% for TB FM from two repeat measurements on 31 children. No sedation was used, and the children were kept quiet during scans by their mothers telling them stories.

Statistical methods

All the statistical analyses were performed using SPSS software (version 16.0. 2007, SPSS Inc, Chicago, IL). Differences in means were tested using Student's *t* test. Correlations were computed between the anthropometric, dietary and biochemical parameters and TB BMC, TB BA and TB LBM. Non-normal variables were normalized using suitable transformations or transformed as categorical variables, and a generalized linear model [30] was fitted to TB BMC as well as to TB BA to investigate the significant determinants among independent variables, i.e., height, weight, TB LBM, dietary intake of nutrients, physical activity and sunlight exposure.

Results

Anthropometric and biochemical parameters of 71 preschool children (boys: 36; girls: 35) of mean age 2.8 ± 0.6 years are summarized in Table 1. Since there were no significant gender differences in any of the characteristics of the study children, data are presented as a total cohort. When anthropometric data were compared with the WHO growth standards, it was observed that the mean weight for age *Z* score of the study group was within reference range, with 70% of children having normal *Z* scores [31]. The mean height for age *Z* score was low, with 66% children having normal *Z* scores. The mean weight for height and BMI *Z* score were within the reference range. With respect to weight for height *Z* scores, 52% of children were normal.

Table 1 Anthropometric and biochemical characteristics of the study children

Parameters	Mean ± SD ($n = 71$)
Age (years)	2.8 ± 0.6
Height (cm)	88.6 ± 6.2
Height for age *Z* score	−1.3 ± 1.5
Weight (kg)	11.6 ± 1.8
Weight for age *Z* score	1.17 ± 0.9
Weight for height *Z* score	−0.8 ± 1.2
BMI *Z* score	−0.67 ± 1.2
Hb (g/l)	101.7 ± 14.22
iCa (mmol/l)	1.02 ± 0.20
25 OHD (nmol/l)	29.86 ± 31.53
iPTH (pmol/l)	9.94 ± 5.43
iP (mmol/l)	1.70 ± 0.22

Hb hemoglobin, reference range: 90–140 g/l; *iCa* ionized calcium, reference range: 1.12–1.23 mmol/l; *25 OHD* 25-hydroxy vitamin D_3, reference range: >25 nmol/l [22]; *iPTH* intact parathyroid hormone, in house range: 1.1–6.4 pmol/l; *iP* inorganic phosphorus, reference range: 1.25–2.10 mmol/l

Source: Pesce [28]

The mean serum iCa concentration of all the children was below the lower limit of the reference range, with 73% of children having low iCa concentrations (Table 1). The mean serum iPTH concentration was higher than the upper limit of the reference range, with 30% of the children having normal iPTH concentrations. Though the mean serum 25 OHD concentration was higher than the suggested cutoff of 25 nmol/l for vitamin D deficiency, 57% of children were deficient in vitamin D [22]. The mean serum iP concentration was within reference range. Based upon the normal range of hemoglobin, 18% of children had mild anemia.

Daily energy and protein intakes of the study group were low, with 70% of children consuming less than the Indian recommended dietary intake (RDI) (Table 2) [32]. Intakes of other micronutrients, i.e., zinc, iron and copper, were also less than half the RDI. Inadequate dietary calcium intake (46% of the RDI) was noted in 98% of children, and phosphorus intakes were below the RDI. The dietary calcium-to-phosphorus ratio was 0.64, which is lower than the recommended ratio of 1:1 [32]. The dietary intake of calcium (327 ± 242 mg/day) was found to be significantly greater in normal children than the calcium intake (214 ± 117 mg/day) in malnourished children ($p < 0.05$). However, there was no difference in the intake of energy and other nutrients of the two groups ($p > 0.1$).

Table 2 Dietary intake, physical activity and sunlight exposure of children

Parameters	Mean ± SD
Nutrient intakes/day	
Energy (kcal/day)	935 ± 244
Protein (g/day)	18.6 ± 7.0
Calcium (mg/day)	276 ± 203
Phosphorus (mg/day)	412 ± 85
Calcium-to-phosphorus ratio	0.6 ± 0.25
Zinc (mg/day)	1.5 ± 0.9
Copper (mg/day)	1.1 ± 0.9
Iron (mg/day)	3.4 ± 1.7
Moderate physical activity per day	% children
15–30 min	75.8
30–60 min	12.1
>60 min	12.1
Sunlight exposure per day	% children
15–30 min	48.3
30–60 min	27.6
>60 min	24.1

The majority of the children had less than 30 min of daily moderate physical activity, with around one-fourth of the children having more than 30 min of daily moderate activity. Among these, the percentage of children with moderate physical activity of more than 30 min per day was higher (24%) in the normal group than in the malnourished group (15%).

Children's sunlight exposure pattern was similar because of their playgroup schedules, and almost half of the children had less than 30 min of sunlight exposure per day (Table 2). However, 33% of normal children had more than 60 min sunlight exposure compared to 14% of the malnourished children.

In the absence of normative data or Z scores from the Lunar DXA machine, it was not possible to evaluate the adequacy of the TB BMC of these children. Since height has a significant influence on bone area and in turn on bone mass, the TB BMC of the group was assessed with respect to their height for age Z scores. Using the WHO definition of child malnutrition, study children were classified into four nutritional grades. Children with Z scores above -1 SD were classified as normal. The children with Z scores between -1 SD and -2 SD were classified as mildly malnourished, those with scores between -2 SD and -3 SD were classified as having moderate malnutrition, and children with Z scores <-3 SD were classified under severe malnutrition [31, 33, 34]. TB BMC was assessed with respect to their nutritional status in terms of height for age Z scores. There was a decreasing trend in TB BMC and TB BA with the reduction in nutritional status (Fig. 1); however, the difference was marginally significant only between normal children versus those with severe malnutrition ($p < 0.1$). Further comparison of the bone parameters of normal children with all children of grade I to III malnutrition according to their height for age Z scores revealed that the TB BMC, TB BA and TB BMD of normal children were significantly higher than those of malnourished children (Table 3) ($p < 0.05$). However, there were no significant differences in TB LBM and percent body fat of the two groups.

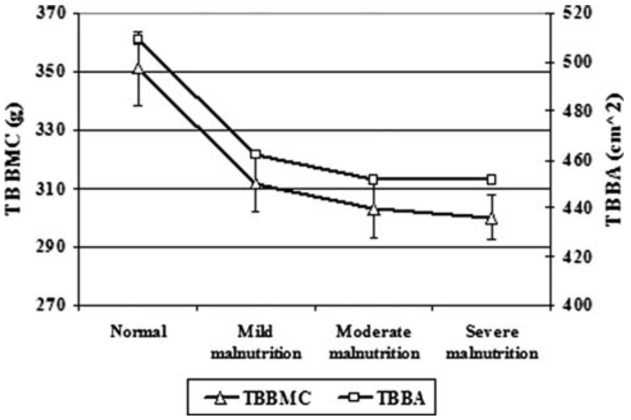

Fig. 1 Reduction in TB BMC and TB BA with respect to malnutrition using grades of height for age Z score

To further assess the relationship of anthropometric parameters with bone status, correlations were calculated. There was a high correlation between height and TB BA (Table 4) ($p < 0.01$). Figure 2 illustrates the graph of TB BA versus height along with the 95% confidence interval for individual predicted values. TB BA was also significantly correlated with weight and TB LBM ($p < 0.01$) (Table 4). Similarly, correlation of TB BMC with height, weight and TB LBM was positive and statistically significant ($p < 0.01$).

Figure 3 illustrates the change in TB BMC versus TB BA showing a close band of the 95% confidence interval for individual predicted values. Pearson's correlation coefficient between TB BA and TB BMC was high and significant ($r = 0.98$, $p < 0.01$).

Table 4 also describes the correlation coefficients of the biochemical parameters with bone parameters in children as a cohort and also grouped according to their nutritional status. TB BMC was significantly positively correlated with iCa and 25 OHD and negatively with iPTH concentrations ($p < 0.05$) (Table 4). Similarly, TB BA was also significantly positively correlated with iCa, 25 OHD and negatively with iPTH concentrations ($p < 0.05$). However, the concentrations of iP and ALP showed no significant association with either TB BMC or TB BA. When the associations were examined separately for normal nutritional status children and children with malnutrition grade I

Table 3 Bone measurements of the children

Variables	Mean ± SD		
	Normal children	Malnourished children	All
TB BMC (g)	340.29 ± 93.80	295.35 ± 48.09*	322.4 ± 80.59
TB BA (cm^2)	493.64 ± 105.61	446.53 ± 59.42*	475.3 ± 92.09
TB BMD (g/cm^2)	0.68 ± 0.05	0.66 ± 0.03*	0.67 ± 0.04
TB LBM (g)	9210 ± 1456	8752 ± 1083	9009 ± 1348
Body fat %	15.62 ± 4.54	14.16 ± 3.97	15.0 ± 4.30

TB BMC total body bone mineral content, *TB BA* total body bone area, *TB BMD* total body bone mineral density, *TB LBM* total body lean body mass

* $p < 0.05$

Table 4 Pearson's correlation coefficients of anthropometric measurements, dietary parameters and biochemical parameters with TB BMC and TB BA

Parameters	Normal children		Malnourished children		All	
	TB BMC	TB BA	TB BMC	TB BA	TB BMC	TB BA
Anthropometric measurements						
Height	0.84**	0.86**	0.78**	0.85**	0.78**	0.82**
Weight	0.81**	0.83**	0.57**	0.61**	0.71**	0.75**
TB LBM	0.83**	0.87**	0.67**	0.72**	0.79**	0.84**
Dietary parameters						
Protein	0.62**	0.65**	0.44*	0.57**	0.55**	0.58*
Calcium	0.58**	0.60**	0.48*	0.49*	0.58**	0.59*
Zinc	0.39**	0.40**	0.53**	0.60*	0.51**	0.54*
Iron	–	–	–	–	0.27*	–
Copper	–	–	–	–	0.40**	0.42*
Biochemical parameters						
iCa	0.45*	0.45*	0.61*	0.55*	0.30*	0.31*
25 OHD	0.50*	0.50*	0.41*	0.44*	0.36*	0.35*
iPTH	−0.48*	−0.37*	−0.44*	−0.52*	−0.34*	−0.34*

* $p < 0.05$, ** $p < 0.01$, "–" value of correlation coefficient was traces

to III, similar significant positive correlations were obtained with iCa and 25 OHD, whereas negative correlation was found with iPTH.

Association of lifestyle factors with bone parameters

The relationship of dietary intakes with bone parameters was examined in the total cohort as well as in normal and malnourished children separately (Table 4). In the total study group, the mean TB BMC (397.8 ± 85.70 g) and TB BA (565.0 ± 87.7 cm^2) were significantly higher in children with calcium intakes above the RDI than the mean TB BMC (305.8 ± 65.85 g) and TB BA (455.2 ± 74.65 cm^2) of children with calcium intakes below RDI; ($p < 0.01$). The intake of calcium showed a significant ($p < 0.01$) positive correlation with TB BMC and TB BA (Table 4). Also, the intake of protein ($p < 0.01$), zinc ($p < 0.01$), iron ($p < 0.05$) and copper ($p < 0.05$) were positively correlated with TB BMC after adjusting for energy. Similarly, significant positive correlations of protein ($p < 0.01$), zinc

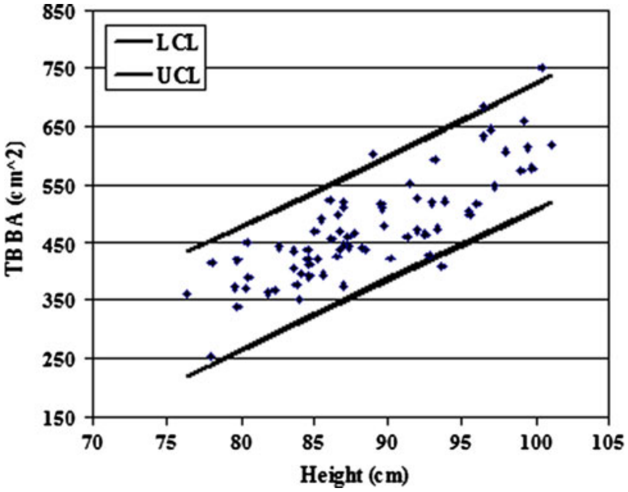

Fig. 2 Relationship between TB BA and height with 95% CIs for individual predicted values. *LCL* lower 95% confidence limit, *UCL* upper 95% confidence limit

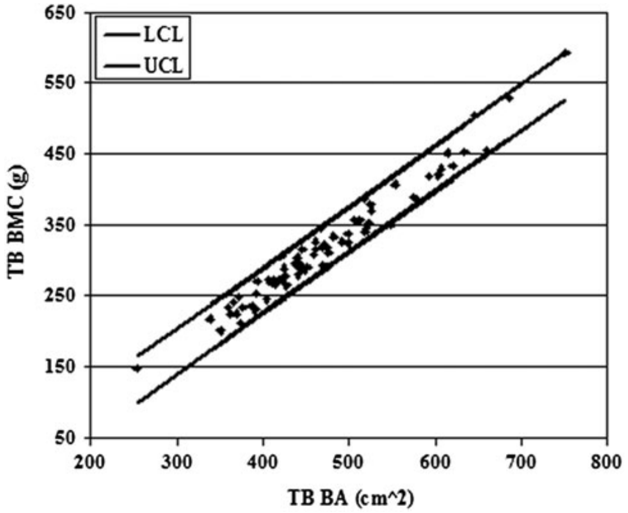

Fig. 3 Relationship between TB BMC and TB BA with 95% CIs for individual predicted values. *LCL* lower 95% confidence limit, *UCL* upper 95% confidence limit

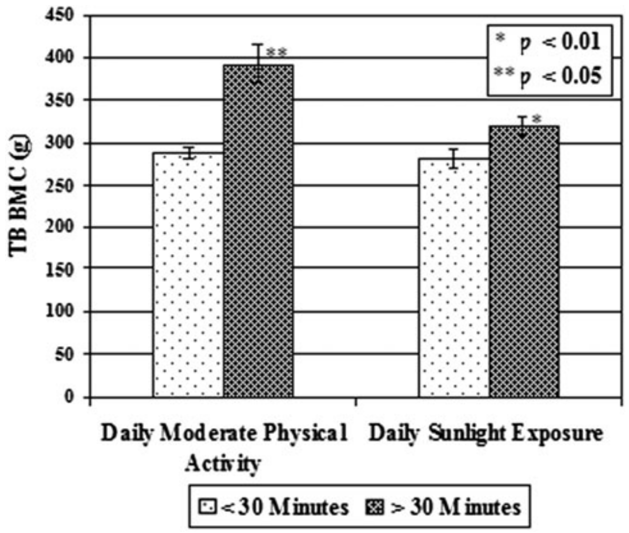

Fig. 4 Difference in TB BMC with respect to the daily sunlight exposure and daily moderate physical activity of <30 min and >30 min

($p < 0.01$) and copper ($p < 0.05$) after adjusting for energy were observed with TB BA.

In children with normal nutritional status, intake of calcium, protein and zinc showed a significant positive correlation with TB BMC and TB BA after adjusting for energy.

In malnourished children (from grade I to III), intake of calcium was positively correlated with TB BMC and TB BA after adjusting for energy. The other nutrients showing positive correlation with TB BMC and TB BA were protein and zinc after adjusting for energy (Table 4). Thus, in both groups similar correlations were obtained.

In addition to nutrient intakes, other lifestyle factors were sunlight exposure and physical activity. Sunlight exposure was significantly correlated with 25 OHD concentration ($r = 0.35$, $p < 0.05$), but not with TB BMC or TB BA. The mean TB BMC and TB BA were significantly greater in children having more than 30 min of daily sunlight exposure than those with less exposure ($p < 0.01$, $p < 0.01$, respectively) (Fig. 4). In children with daily moderate physical activity of more than 30 min, a significantly greater mean TB BMC and TB BA were observed than in those with less physical activity ($p < 0.05$, $p < 0.05$, respectively) (Fig. 4).

Thus, bivariate analysis showed significant associations between DXA bone measurements and lifestyle factors. When these variables and potential confounders (weight, height) were analyzed simultaneously with generalized linear model techniques, the final model for TB BMC revealed that height, TB LBM, weight, activity, sunlight exposure in minutes and dietary intakes of calcium, zinc and iron were the significant factors ($p < 0.05$). The model for TB BA exhibited similar influencing factors as those for TB BMC ($p < 0.05$) (Table 5). When similar generalized linear model analysis was performed in children with normal nutritional status, the significant influencing factors were similar to those of the entire cohort for TB BMC and TB BA, i.e., with height, TB LBM, weight, activity and dietary intakes of calcium, zinc, iron ($p < 0.05$) and sunlight exposure in minutes being marginally significant ($p < 0.1$).

Table 5 Association of various factors with bone parameters using generalised linear model

Independent variable	$\beta \pm$ SE	
	TB BMC	TB BA
Weight	19.61 ± 0.30*	17.26 ± 0.30*
Height	5.66 ± 0.05*	6.68 ± 0.05*
TB LBM	0.01 ± 0.0002*	0.012 ± 0.0002*
Activity	11.72 ± 0.65*	7.06 ± 0.65*
Sunlight exposure	7.05 ± 0.30*	10.31 ± 0.30*
Calcium intake	0.23 ± 0.004*	0.16 ± 0.004*
Zinc intake	1.66 ± 0.54*	5.7 ± 0.54*
Iron intake	1.57 ± 0.30*	0.30 ± 0.02*

$\beta \pm SE$ regression coefficient ± SE, obtained from the model

* $p < 0.05$

Discussion

Preschool children in the present study were apparently healthy, yet half of them were below the WHO reference standard with respect to height for age *Z* scores. Bone mineral content of the preschool children was found to decrease with the grade of malnutrition. In the absence of normative data for the Lunar DPX PRO, our data provide estimates of TB BMC and TB BA of Indian preschool children with varying degrees of malnutrition. The reduction in TB BMC along the grades of malnutrition with respect to weight reported by Chaturvedi et al. was among malnourished and sick children (2–14 years) versus normal children [5–9], and their bone measurements were performed using single photon absorptiometry. Our data showed a reduction in TB BMC and TB BA with increasing grades of malnutrition among apparently healthy children.

Our findings suggest that height, weight, lean body mass, activity, sunlight exposure and dietary calcium, zinc and iron were the predictors of TB BMC and TB BA ($p < 0.01$, $p < 0.01$, respectively). Significantly greater TB BMC and TB BA were noted in children with more than 30 min of daily physical activity. In 2001, Specker et al. also suggested height, weight, activity and dietary calcium as the predictors of TB BA and TB BMC in 3- to

5-year-old children [35]. In line with our results, other studies have reported height, weight, activity and average dietary calcium intake as predictors of TB BMC in children less than 5 years of age [11, 36]. Similarly, in 4- to 20-year-old children, height and calcium intake has been shown to positively influence TB BA [37–39].

Studies reporting the effect of zinc, iron and sunlight exposure in children are scanty. Zinc is necessary for bone matrix synthesis and plays a role in normal growth and bone mineralization [40, 41]. A positive significant correlation of dietary zinc with TB BMC was reported by Bounds in 8-year-old children [42]. Our study reports a significant association of zinc, iron and TB BMC and TB BA in 2- to 4-year-old children. Studies are lacking reporting the effects of iron on bone mass in children. Heaney et al. [40] stated that iron may also play an important role in bone formation, acting as a cofactor with enzymes involved in collagen synthesis. Jones et al. [43] observed an association of sunlight exposure and BMD in 8-year-old children. Our findings of a significant association of sunlight exposure and 25 OHD concentrations and the significantly greater TB BMC and TB BA in 2- to 4-year-old children with daily sunlight exposure of more than 30 min are in line with Jones et al.'s results [43], suggesting a role of sunlight exposure in TB BMC and TB BA.

The associations of iCa, 25 OHD and iPTH concentrations with the TB BMC and TB BA in 2- to 4-year-old children have been very scarcely reported. Our findings of significant positive associations of TB BMC and TB BA with iCa and 25 OHD concentrations and a negative association with iPTH against the background of low serum iCa concentrations support the need for assessing the effect of calcium supplementation along with vitamin D repletion on their TB BMC and TB BA.

Although the sample size in this study was small, in view of the scarcity of data in this age group, our results underline the importance of attaining optimal height for age, achieving the goals of overall nutrition with adequate dietary calcium, iron and zinc intakes, physical activity and sunlight exposure in preschool children for achievement of peak bone mass. Further validation of the results with a larger sample size is warranted.

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