ABSTRACT

**Background:** Adequate calcium intake during childhood and adolescence is necessary for the attainment of peak bone mass, which may be important in reducing the risk of fractures and osteoporosis later in life. Dietary calcium deficiency is omnipresent in Indian children. Especially intake of milk and milk products which are the richest and highly bioavailable sources of calcium is meager, while cereal, pulses, green leafy vegetables which are high in phytin and oxalates (that bind with calcium and decrease its absorption) form a major source of calcium in the diets of Indian children and adolescents. Also, vitamin D deficiency in Indian children is reported, which further reduces intestinal calcium absorption. Thus, dietary calcium deficiency in addition to vitamin D deficiency in childhood impairs the bone mineralization. Hence, strategies to increase the total and bioavailable calcium from plant food need to be devised and investigated for their effect on bone mineralization.

Growth hormone (GH) is essential for linear bone growth and accrual of bone mass during childhood and adolescence. Growth hormone can also promote intestinal calcium absorption. Untreated growth hormone deficiency during childhood and adolescence often leads to reduced peak bone mass in addition to severe height deficit. Data on nutritional status and bone health status in Indian growth hormone deficient children is scarce. Moreover, it is reported that zinc deficiency decreases serum Insulin like Growth Factor 1 (IGF1) concentrations, increases GH resistance, and impairs the action of IGF1 on skeletal growth. Calcium and zinc supplementation with growth hormone therapy may increase the growth hormone deficient (GHD) children’s bone mineralization.

**Objectives:** Therefore the specific objectives of the present work were: i) To assess diet patterns of children and adolescents for their milk-equivalent calcium density and suggest strategies to improve total and bioavailable calcium intake. ii) To evaluate Ca absorption of one of the alternatively developed diet/meal in children. iii) To study the effect of Ca supplementation on Ca status in hypocalcaemic
toddlers. iv) To assess effect of GH alone and GH with Ca and Zn supplementation on bone mass in GHD children.

**Materials and study design:** i) A cross-sectional study on 220 children of 2 to 16 years of age from 3 socioeconomic strata of society was conducted to assess their diet patterns with reference to their calcium intake. Children were enrolled from schools catering to different strata of society. According to the diet patterns, recipes rich in calcium and with different food techniques to improve their bioavailability were developed. Calcium fortification was examined as a strategy to improve calcium intakes. ii) An in-vivo bioavailability study of calcium fortified cereal-legume snack (Ragi-Bengal gram Laddoo) was carried out in 8 to 12 year old children. The bioavailability of the fortified snack was tested against a standard calcium supplement. Serum ionized calcium (iCa) and intact parathyroid hormone (iPTH) concentrations were measured at 0, 1, 2, 3, 4 and 5th hour of the administration of the snack. iii) Effect of 1 year supplementation of the calcium fortified snack on bone mineralization was studied in preschool children. Sixty preschool children were randomly allocated to 2 groups; Group A receiving the calcium fortified cereal-legume snack (fortified with 500 mg of calcium) and Group B received unfortified snack for 5 days a week. Also, both groups received 30,000 IU of vitamin D₃ supplementation once in every 3 months. iv) In a cross-sectional study, nutritional status and bone health status was studied in 50 Indian prepubertal GHD children. v) In a 2 year supplementation study, 31 prepubertal GHD children were randomly enrolled in 2 groups, Group A and Group B. Both groups received GH therapy in first year, in the second year, in addition to GH therapy, Group A received calcium (500 mg) and vitamin D₃ (30,000 IU) supplementation, while Group B received zinc supplementation (as per Recommended Dietary Allowances) in addition to calcium and vitamin D.

Structured questionnaire was used for socio-demographic information. Standing height was measured using a wall-mounted stadiometer to the nearest 1 mm. Weight was measured on electronic digital scale to the nearest of 0.1 kg. Dietary intake was assessed by a 24 hour diet recall for 3 days. Bone and body composition parameters were assessed by dual energy x-ray absorptiometry (DXA) scan. Fasting serum iCa, vitamin D₃ (25OHD), iPTH were assessed by standard kit methods. In case of GHD
children, serum GH, Insulin like growth factor 1 (IGF 1) and zinc were assessed by standard methods. The study protocols were approved by Institutional ethics committee. And the power of the study was 0.8 ($p < 0.05$).

**Results and conclusions:** Children’s dietary intake data were classified in the following major food groups, i.e.; Cereals and Millets, Pulses and Legumes, Milk and Milk products, Fruits and salads, Vegetables, meat/ fish products and Fried foods and Sweets., and k-mean cluster analysis was performed, to identify dietary patterns.

In children aged 2 to 9 years, 2 diet patterns viz “Wheat and Milk & Milk Products (WM)” and “Rice and Pulse (RP)” were obtained. The calcium intake as % of recommended dietary allowances was 44% (14% from milk & milk products) in children from RP diet and was significantly lower ($p < 0.05$) than 60 % (38% from milk & milk products) in children from WM diet pattern. In adolescents (10 to 16 years of age), 3 diet patterns were identified i.e. “Mixed Food (MF)”, “Rice and Pulse (RP)”, and “Wheat, Milk & Bakery (WMB)” diet pattern. The % RDA calcium intake was 93% (44% from milk & milk products) in adolescents from WMB pattern and was significantly ($p < 0.05$) greater than in adolescents from RP pattern (35%, 6% from milk & milk products) and MF pattern (40%, 5% from milk & milk products). However, the % RDA calcium intake in adolescents from RP and MF pattern was similar ($p > 0.1$). Twenty plant food based meals were developed considering children’s diet patterns. These recipes were developed using calcium rich foods and using food processing techniques such as germination, fermentation known to improve calcium bioavailability (calcium per serving > 200 mg). The meals were tested by hedonic rating scale. Out of the 20 meals, “Ragi-Bengal gram Laddoo”, “coriander leaves wadi”, “sprout bhel” were well accepted by the panellists. One serving of the suggested meal provides 42% of the calcium RDA in children and 32% in adolescents.

Thus, strategies were devised to improve the Indian children and adolescents dietary intake of calcium through plant food sources.

In the in-vivo study, to examine the absorption of the fortified Ragi-Bengal Gram Laddoo (fortified with 500 mg calcium carbonate), age, weight, and height of the
children in the 3 experimental groups were similar (P > 0.1). In group A (Fortified Ragi-Bengal Gram Laddoo), a peak of 6% above baseline was observed at 1 h in serum ionized calcium, whereas group C (Calcium Carbonate) showed a peak of 5.5% at 4 h and group B (non fortified Ragi-Bangal Gram Laddoo) showed a small increase of 1.8% at 1 h. The change in area under curve of groups A and C were of similar order (4.6 and 5.5, respectively), whereas that of group B was significantly lower (0.82). Serum parathyroid hormone was lowest at 2 h in groups A and B and at 3 h in group C. These results thus indicate that the calcium absorption of the fortified snack was on par with the calcium supplement and can be used as a strategy to increase children’s dietary calcium.

In the 1 year calcium supplementation study through the Fortified Ragi-Bengal Gram Laddoo, children’s anthropometric, dietary, biochemical and bone parameters were similar in calcium-vitamin D (Group A) supplemented and only Vitamin D (Group B) supplemented groups at baseline. A significantly greater % increase in bone mineral content (BMC) (of around 7%) was seen in the Group A (Ca-VitD) than Group B (VitD) (p < 0.05). The % change in BMC was assessed after adjusting for height, bone area (BA) and also dietary calcium and vitamin D status. The % change in BMC in the Group A (Ca-VitD) (35.5%) was significantly (p < 0.01) higher than that in Group B (VitD) (28.1%) even after the adjustments. The results of this study show that one year calcium supplementation using fortified Laddoos as a vehicle along with monthly vitamin D supplementation led to a significantly greater gain in BMC in children having habitually low dietary calcium intake.

In the cross-sectional study of prepubertal growth hormone deficient children, all children were very short (mean height for age Z-score < -3) and they were also underweight. The energy and protein intake was 57% and 84% of the recommended dietary allowances (RDA) for Indian children. The % contribution of fat to the total calories was 28% and was within the recommended range of 25 to 30% (ICMR, 2010). The mean calcium, phosphorus and zinc intakes were 49%, 86% and 40 % of the RDA respectively. To assess children’s bone health, various approaches were used. Applying the Molgaard approach, all children had “short bones” (Height for age Z-score < -2),” 86 % had “narrow bones” (Bone area inadequate for Height), and
72 % had “light bones” (Bone mineral content inadequate for bone area). Further, the adequacy of bone mineral content was assessed in relation to lean body mass (LBM), bone age (surrogate for skeletal maturity), height-age (age matched for height). The mean BMC Z-score remained below – 2 even after adjustments for LBM, bone age, and Height-age, suggesting a deficit of BMC in spite of all adjustments. When adjusted for LBM, 87 % of the children had low LBM for height and 33 % had low BMC for LBM. Thus, the low bone mass was possibly a combination of growth hormone deficiency working through short stature, vitamin D deficiency, secondary hyperparathyroidism and poor nutrition.

In the 2 year supplementation study, at baseline, the GHD children were prepubertal with a mean age of 8.7 ± 2.8 years with 18 boys and 13 girls. Boys and girls from both groups were similar in their anthropometric characteristics. After 1 year of GH therapy, the height-adjusted gain in BMC was of the order of 48 % in Group A and B \( (p > 0.1) \). Likewise, height-adjusted % gain in BA was of the order of 40 % in Group A and B \( (p > 0.1) \). The LBM also showed a similar \( (p > 0.1) \) height adjusted increase in both the groups of the order of 47 %. At end of 2 years, post calcium vitamin D and zinc supplementation, when height-adjusted % gain in BMC, BA, and LBM was examined, a significantly greater \( (p < 0.05) \) gain was found in Group B \( (\text{Ca + Vit D + Zn}) \) than in Group A \( (\text{Ca + Vit D}) \) in these parameters. The gain in BMC was 49 and 51 % in Group A and B, respectively \( (p < 0.001) \), while % gain in BA was 34 and 36 % in Group A and B, respectively \( (p < 0.001) \). Similarly, the height-adjusted % gain in LBM was 30 % in Group B, significantly \( (p < 0.001) \) greater than 28 % in Group A. Thus, the results of this study results suggest that GH therapy in prepubertal GHD Indian children, known to have reduced dietary intakes of calcium and Zn, and vitamin D deficiency, significantly increases their bone mass accrual. Supplementation with calcium and vitamin D along with GH therapy results in a greater gain in BMC. Moreover, supplementation of Zn in addition to the GH, calcium, and vitamin D further enhances bone mineralization.
**Principal conclusions:**

1) Plant food based meals developed using food processes like germination and fermentation have the potential to increase the total and bioavailable dietary calcium.
2) The calcium bioavailability from a calcium fortified cereal-pulse food was on par with standard pharmacologic calcium supplement. Thus, cereal-pulse food product can act as a vehicle for calcium fortification.
3) One year supplementation of the calcium fortified cereal-pulse food in addition to vitamin D improves the bone mineral content in toddlers than only vitamin D supplementation.
4) Indian prepubertal growth hormone deficient children had low bone mass. Inadequate nutritional intake and vitamin D insufficiency in addition to growth hormone deficiency may contribute towards the low bone mass.
5) Growth hormone therapy increases the bone mineralization in prepubertal growth hormone deficient children.
6) Supplementation of calcium and vitamin D along with growth hormone therapy in GHD Indian children has the potential for enhancing bone mass accrual; this effect is further enhanced through the addition of zinc supplement.

**Contribution to Existing Knowledge:**

The plant food based calcium rich meals developed in the present study can be used as food based sustainable approach for improving Indian children’s dietary calcium. This study for the first time demonstrates that the calcium absorption from a calcium fortified cereal-pulse food is on par with a standard pharmacologic calcium supplement, and thus cereal-pulse food can be used as a vehicle for calcium fortification. It has also been shown for the first time that calcium supplementation through cereal-pulse food fortification in addition to vitamin D supplementation improves bone mineralization in toddlers. The nutritional status and bone and body composition status of prepubertal Indian growth hormone deficient children has been studied for the first time. This study demonstrates the positive effect of zinc, calcium and vitamin D supplementation on bone mineralization in prepubertal growth hormone deficient children.