Chapter 1

Importance of calcium nutrition in growth and development: a review of current status and need for research

Adequate nutrition is essential for normal growth and development of a child. Apart from the major nutrients; i.e. energy and protein, vitamins and minerals are necessary for various body functions and attaining growth potential. Growth is largely assessed in terms of height which is the result of bone development. Studies have shown that calcium, phosphorus, proteins, vitamin D, magnesium, zinc, copper, iron, fluoride, and vitamins D, A, C, and K are some of the most essential nutrients required for adequate bones (Tucker et al 2003, Ahmadieh and Arabi 2011, Heaney 2005). Of these, calcium is the most vital nutrient for adequate bone growth and mineralization.

Calcium is an important mineral in human metabolism and it ranks fifth in the elementary composition of the human body after oxygen, carbon, hydrogen and nitrogen. The new born infant has about 24 gm of calcium as a store which rises to 1300 g at maturity, requiring an average daily positive calcium balance of 180mg during first 20 years of growth (FAO & WHO 2001). Ninety nine percent of the body's calcium supply is stored in the bones and teeth where it supports their structure and function. The remaining 1 % of calcium is required for other critical metabolic functions such as vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion. Serum calcium is very tightly regulated and in absence of adequate calcium intakes, the body uses bone tissue as a source of calcium, to maintain constant concentrations of calcium in blood, muscle, and intercellular fluids (NAP 2010).Calcium is involved in many biological functions, i.e. regulation of the cardiovascular and nervous systems, helps to metabolize iron, formation of bones and teeth, blood clotting, muscle contraction, blood pressure regulation and nerve transmission.

Calcium adequacy in the diet is directly related to the dairy consumption (Weaver et al 2005). In developing countries like India, calcium intake is mainly from plant
foods than dairy products (NNMB 2006). The low bioavailability of calcium from plant foods due to the presence of calcium absorption inhibitors such as oxalates and phytates remains to be a major concern in reaching children’s optimal calcium intakes (Miller 2001, NAP 2010). Without adequate dairy products, calcium requirement can only practically be met by consuming fortified foods or supplements (Weaver 2006, Miller 2001).

Skeletal growth is the major determinant of the dietary requirement for calcium during the first 20 years of life. A high prevalence of low calcium intake during childhood and adolescence is vastly reported. (Salamoun et al. 2005; Rozen et al. 2001; Ahmed 1998; Puri et al. 2008; Ekbote et al. 2010). Since the amount of bone accumulated during growth is dependent to some extent on the amount of calcium in the diet, calcium deficiency during skeletal formation decreases the peak bone mass achieved in turn increasing fracture risk in the young years and later in life (Heaney 2000). Thus, achieving a high peak bone mass in early life predicts a relatively higher bone mass, and hence greater protection from osteoporosis and fracture, late in life.

Along with nutrition, growth hormone plays a major role in linear growth and bone mineralisation. In growth hormone deficiency, children’s statural growth is severely affected. Inadequate dietary calcium intakes in addition to growth hormone deficiency may lead to reduced bone mineralization and in turn put them at risk for osteoporosis and fracture.

Considering the risk of calcium deficiency in children and its importance in growth, there is a need to examine available evidence of calcium status in both healthy and GH deficient children. A review of existing knowledge about, role of calcium and other micronutrients in growth and metabolism is presented below which will lead to the need for present research.
1. Role of Calcium in Human Body:

Calcium, the most abundant mineral in the body, makes up about 1.5 to 2% of the body weight and 39% of total body minerals. It is most commonly associated with the formation and metabolism of bone. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodeling.

1.1 Calcium Homeostasis:

The 1% of total calcium circulates in plasma in three fractions. The most important is the ionized fraction, which constitutes about 50% of the total and is maintained at a concentration of between 1.12 and 1.23 mmol/L. This ionized fraction determines optimal neuromuscular function and is itself maintained by the various endocrine factors responsible for its stability. Most of the remainder, approximately 40%, circulates bound to albumin, and conditions associated with hypoalbuminemia may reduce the total circulating concentration without affecting the ionized calcium. The remainder of the total calcium circulates complexed to other molecules such as citrate and sulfate.

Calcium ion concentration in extracellular fluid (ECF [Ca\(^{2+}\)]) is the central, controlled quantity in the operation of the calcium economy. ECF [Ca\(^{2+}\)] is sustained by three independent control loops, involving bone resorption, renal clearance, and intestinal absorption. Figure 1.1 illustrates the normal calcium homeostasis over 24 hours in a healthy individual consuming 1 g of calcium per day (Allgrove J 2005).
Figure 1.1: Normal calcium homeostasis (In All)

This system functions optimally when dietary calcium intakes are at or above currently recommended values, i.e. both ECF [Ca^{2+}] and bone mass are protected. At lower calcium intakes, ECF [Ca^{2+}] is sustained, but decreased calcium intake or altered calcium demands reduce bone mass. Parathyroid hormone (PTH) acts on all three effector systems to protect against hypocalcaemia by stimulating calcium removal from bone, improving calcium absorption from food and regulating loss of calcium through the kidneys. Figure 1.2 depicts the 3-arm control loop regulating ECF [Ca^{2+}] showing specifically the response to a drop in [Ca^{2+}]

(Mundy 1999)
1.1.1 Vitamin D and PTH as calcium regulators:

Further, the vitamin D metabolite, 1,25 dihydroxyvitamin D(1, 25 [OH]$_2$D$_3$) and PTH are the major hormonal regulators of calcium homeostasis. The response of these 2 hormones to a low calcium diet is shown in figure 1.3. Here, low calcium is sensed at the level of parathyroid gland through a calcium sensing receptor. The calcium sensing receptor relays a signal that leads to the increased production and release of PTH into the circulation. Once released, PTH has several important functions. First, it promotes bone resorption by stimulating osteoclastic activity. Second, it stimulates renal calcium reabsorption in the proximal renal tubule and also suppresses renal phosphate reabsorption. Finally, PTH is a strong stimulator of the renal enzyme 25 hydroxyvitamin D (25[OH]D)-1 $\alpha$ hydroxylase, that catalyzes the conversion of 25(OH)D to 1, 25(OH)$_2$D$_3$, the hormonally active form of vitamin D.
The 1, 25(OH)$_2$D$_3$ stimulates bone resorption, renal calcium reabsorption in the distal convoluted tubule, and active calcium absorption in the proximal small intestine.

**Figure 1.3:** The responses of PTH and 1, 25 [OH]$_2$D$_3$ controlling whole body calcium homeostasis during habitual low dietary calcium intake

Thus, during low habitual dietary calcium intakes, through the PTH and 1, 25 (OH)$_2$D$_3$ system the ionized calcium concentrations are controlled at the expense of bone mineral content. This further results in low bone mass in turn affecting normal bone development (Fleet JC 2006).

### 1.2 Growth Hormone:

Growth Hormone influences calcium homeostasis in children. Growth hormone and its physiological mediator, insulin-like growth factor (IGF)-1, have a major role in linear bone growth and accrual of bone mass during childhood and adolescence. In addition, growth hormone can also promote intestinal calcium absorption. This is indirectly due to the activation of the renal 1α hydroxylase and elevation of serum 1, 25(OH)$_2$D$_3$ levels. The vitamin D-dependent mechanism by which the growth
hormone-IGF-1 axis may regulate intestinal calcium absorption is not clear at this time.

Human growth hormone (GH) is produced as a single chain, 191 amino acid protein, that is synthesized, stored, and secreted by somatotropes in the anterior pituitary and released in pulses. The alternating secretion of growth hormone–releasing hormone (GHRH), which stimulates GH release, and somatostatin, which inhibits GH release, accounts for the rhythmic secretion of GH. Physiologic effects of GH occur through both direct and indirect (via IGF peptides) mechanisms. In general, linear growth-promoting effects of GH appear to depend upon production of IGF-1 and perhaps other IGF peptides (Parker & Felner 2007). Apart from its actions on linear growth, GH is anabolic, lipolytic, and diabetogenic. It also increases calcium absorption (Mehta & Dattani 2005)

**Figure 1.4: Schematic presentation of the human GH regulation**

Circulating IGF-1 is synthesized primarily in the liver and formed locally in mesodermal and ectodermal cells, particularly in the growth plate of children, where its effect is exerted by paracrine or autocrine mechanisms. Circulating levels of IGF-
1 are related to blood levels of GH and to nutritional status. The biologic effects of GH include increases in linear growth, bone thickness, soft tissue growth, protein synthesis, fatty acid release from adipose tissue, insulin resistance, and blood glucose levels (Parker & Felner 2007). Figure 1.5 presents the multiple sites of GH action.
Figure 1.5: Multiple sites of GH action

(Carrel & Allen 2000).
Normal GH secretion and action during childhood and adolescence promotes growth of lean tissue and limits the formation of fat in the abdominal visceral depot. GH increases net muscle protein synthesis primarily by enhancing amino acid transport, and hence the availability of amino acids for protein synthesis. GH-enhanced lipolytic activity in adipose tissue, combined with reduction of triglyceride accumulation via inhibition of the lipoprotein lipase activity are important mechanisms by which GH reduces adipose tissue (Carrel & Allen 2000). A negative relationship exists between body mass index, a marker of body fatness, and GH secretion in normal prepubertal children as well as in obese children (Patel & Clayton 2005).

The major role of GH during growth and development is to promote longitudinal bone growth. Two hypotheses have been generated to explain the mode of action of GH in generating a growth response. The somatomedin hypothesis proposes that GH mediates its effects on its target tissues via stimulation of hepatic IGF-I production, which in turn acts as a classical endocrine hormone (Fig. 1.6a). The alternative hypothesis, the “dual effector theory”, is based on the premise that growth is a result of the differentiation of precursor cells, followed by clonal expansion. The dual effector theory proposes that GH acts directly to stimulate differentiation of prechondrocytes into early chondrocytes (Chondrocyte: A connective tissue cell that occupies a lacuna within cartilage matrix. Also called cartilage cell.) that in turn stimulates production of IGF-1. IGF-1 then stimulates clonal expansion of and maturation of chondrocytes (Fig. 1.6b) (Patel & Clayton 2005, Ohlsson C et al, 1998)
GH plays a key role not only in longitudinal bone growth, but in the accretion of bone mass during childhood and adolescence through the regulation of both bone formation and bone resorption. GH increases bone formation in two ways: via a direct interaction with GHRs on osteoblasts and via an induction of endocrine and autocrine/paracrine IGF-1 (Ohlsson C et al 1998).

Thus, in addition to linear growth, GH serves many metabolic functions in human body. In children with growth hormone deficiency, with height, bone, adipose and muscle metabolism is also affected (Photograph 1.1).

1.3. Growth Hormone Deficiency:

1.3.1. Causes of Growth Hormone Deficiency:

GH deficiency may be congenital (e.g. associated with septooptic dysplasia) or acquired (e.g. secondary to cranial irradiation or, rarely, a tumor in the area of the hypothalamus or pituitary). A congenital deficiency may arise through deletion of the GH gene, in which case no GH molecule can be produced, or by mutations within the gene, often at exon–intron splice sites, that result in an abnormal dysfunctional GH molecule.
Isolated GH deficiency presents in early childhood without a family history. It is usually associated with pituitary hypoplasia secondary to a deficiency in the secretion of GH-releasing hormone from the hypothalamus. The etiology of this form of GH deficiency is unclear. There may be a history of birth trauma or prematurity, and abnormalities in pituitary development genes may contribute. Both congenital and acquired forms of GHD may be isolated (IGHD) or involve multiple pituitary hormones (MPHD). Deficiency of other hormones besides GH can develop later.

The reported incidence of GH deficiency is 1 in 3480 to 1 in 4,000 live births in USA and UK, with the majority of cases being idiopathic. Familial cases account for 5–30% of all the cases (Desai & Colaco 2008, Patel & Clayton 2005, Mehta & Dattani 2005).

1.3.2. Diagnosis of Growth Hormone Deficiency:

The diagnostic tests for growth hormone deficiency are indicated when,
- Other physiologic and systemic causes of short stature are ruled out,
- The bone age (bone age: surrogate for skeletal maturation) and height age (height age: by projecting given subject’s height on to the corresponding median height of the reference population) are assessed in relation to the chronologic age and found lower.
- A subnormal growth velocity is documented (< 3-4 cm/year) precisely over a period of at least 6 months (preferably over a year)

GH secretion is pulsatile and serum concentrations are low during many hours of the day. Currently, the diagnosis of GHD is based on eliciting a GH peak in response to physiological conditions such as sleep and exercise or provocation by pharmacologic stimulation test. The pharmacologic stimulation agents include insulin, clonidine, L-dopa, arginine, glucagon, propranolol and GHRH (Growth hormone releasing hormone). A variety of combinations of these tests has been used and, in some centers, two provocative stimuli are administered sequentially or in combination. The GH response set-point is 10 ng/ml (Desai & Colaco 2008, Shalet 1998) for the
stimulation agents except for l-dopa and GHRH. For l-dopa and GHRH, the set-point is 6 and 7-10 ng/ml respectively (Desai & Colaco 2008).

1.3.3. Growth Hormone Deficiency Treatment:

GH deficiency is treated with daily injections of recombinant GH. In children, body size dosing is used based on body weight (BW) (Mg/Kg) or body surface area (BSA) (Mg/m²). The dose varies by country. The suggested dose for Indian children is 7.5 – 10 mg/m²/week or 0.23-0.30 mg/Kg/week (Desai & Colaco 2008).

1.3.4. GH Deficient Child:

If GH deficiency is an isolated pituitary hormone problem, birthweight is characteristically normal, and the early postnatal course is uneventful. A diminished growth velocity becomes obvious towards the end of the first year as the hormone-dependent childhood phase of growth fails to take over from the nutrition-dependent infant phase. However, despite the congenital nature of this disorder, GH deficiency may not present until later childhood. The lack of sufficient GH causes a characteristic appearance with truncal obesity, immature cherubic facies, and central crowding of the facial features from maxillary hypoplasia (Photograph 1.1) (Patel & Clayton 2005). These children have a typical ‘doll- like’ appearance (Photograph 1.2). The weight of a GHD child is equal to or more than expected for his height with skinfold thickness in the upper centiles. However, low BMI and low skinfold thickness are seen in some children of Asian origin and observed in GHD of congenital or familial nature (Desai & Colaco 2008).

In children with growth hormone deficiency, puberty may occur spontaneously but may be slightly delayed (Patel & Clayton 2005). As many children fail to reach their target height on current GH regimens at the time of entering puberty, strategies are being investigated to maximise the pubertal growth spurt. However, no official consensus has been reached about the GH dosage during puberty (Desai & Colaco 2008).
Thus, in the absence of growth hormone, there is a marked decrease in lean body mass accompanied by increased adipose tissue. Growth hormone deficiency leads to diminished muscle mass and muscle strength. Researchers have shown that GHD children exhibit reduced bone mass (Boot et al 1997, Hogler et al 2005). However, it is also suggested that the bone mass in GHD children may be adequate for their muscle mass (Hogler et al 2010). Moreover, ethnic differences in attainment of muscle mass have been reported in children. Also, in addition to GH therapy, optimization of nutrition is essential (Desai & Colaco 2008).

1.3.5. Growth Hormone and Zinc:

The role of zinc in growth is well known (Prasad 2009). It is reported that zinc deficiency decreases serum IGF1 concentrations, increases GH resistance, and impairs the action of IGF1 on skeletal growth in rats. Also, zinc supplementation significantly increases plasma IGF1. Zinc deficiency reduces IGF1 production and can lower cell responsiveness, thus affecting membrane signaling systems and intracellular second messengers that coordinate cell proliferation in response to IGF1. Consequently, zinc status may explain the response to GH treatment in children with GH deficiency (Alves et al 2012). Thus, zinc plays an important role in GHD children in their response to GH therapy. Moreover, zinc has also shown to have an effect on the bone metabolism. The role of zinc in bone metabolism is further discussed in the review.
Photograph 1.1: A growth hormone deficient child showing central crowding of the facial features

Photograph 1.2: A 5 year old growth hormone deficient child
1.4 Role of calcium, vitamin D and Zinc in bone status:

1.4.1. Bone:

Bones play many roles in the body. They provide structure, protect organs, anchor muscles, and store calcium. Bone is composed of 50 to 70% mineral, 20 to 40% organic matrix, 5 to 10% water, and <3% lipids. The mineral content of bone is mostly a form of calcium phosphate in the form of hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, with small amounts of carbonate, magnesium, and acid phosphate, with missing hydroxyl groups that are normally present. Bone undergoes longitudinal and radial growth, modeling, and remodeling during life. Longitudinal and radial growth during growth and development occurs during childhood and adolescence (Clarke et al 2008).

1.4.2. Calcium:

Calcium serves two major functions for bone. First, calcium is the bulk cation out of which bone mineral is constructed. As such it must be absorbed in sufficient quantity from ingested foods to build a skeleton during growth and to maintain skeletal mass in maturity (the latter by offsetting obligatory losses from the body). Second, calcium serves as an indirect regulator of skeletal remodelling. Adequate dietary calcium has long been recognized to play an important role in building peak bone mass as a strategy to decrease incidence of fracture later in life. More recently, it has become apparent that even childhood fractures are also related to low bone mass, and that childhood bone mass in turn is influenced by diet and physical activity (Heaney et al 2005).

Remodeling of bone serves two, closely linked purposes: 1) the repair of fatigue damage and the reshaping of bone to accommodate growth and altered usage; and2) a source and sink for calcium in the protection of extracellular fluid (ECF) $[\text{Ca}^{2+}]$. In both, small packets of bone are resorbed by osteoclasts, and the released bone mineral either recycled or used to offset excretory losses.
The first role of remodeling is generally divided into two types:

i) **“Remodeling”** the replacement of damaged structures. Bony resorption and formation occur at the same skeletal site, though separated in time (resorption by osteoclasts first, followed by formation by osteoblasts). (Figure 1.7)

**Figure 1.7: Remodeling of bone**

![Remodeling of bone](Office of the Surgeon General (US); 2004)

ii) **“Modeling”**, i.e., the reshaping of bone. Formation and resorption occur on different surfaces (e.g., periosteal, endosteal), but simultaneously.(Figure 1.8)

**Figure 1.8: Modeling of bone**

![Modeling of bone](Office of the Surgeon General (US); 2004)

During growth both processes are active, while after growth, when adult skeletal shape is approximately stable, true remodeling predominates. Both types share a common feature: bone mineralization in the formation phase of remodeling takes calcium and phosphorus out of the circulating blood, creating a mineral deficit in the
ECF which constitutes the principal systemic basis for stimulating parathyroid hormone (PTH) secretion. PTH in turn is the principal determinant of the quantity of bone resorption occurring throughout the skeleton. In this sense, bone mineralization “pulls” bone resorption.

During periods of fasting or low calcium intake, PTH secretions rises, and with it bone resorption (and, thereby, total remodeling). From a homeostatic perspective, such remodelling provides the calcium needed to maintain ECF [Ca$^{2+}$]. However, structurally, homeostatic remodeling contributes only weakness, since bone at sites being remodeled is reduced in mass and hence in strength. Figure 1.9 illustrates the strength reduction which makes the point that a resorption cavity in the side of a load-bearing bone trabecular produces local weakness out of proportion to the modest reduction in mass.

**Figure 1.9: Diagramatic illustration of strength reduction due to homeostasis remodeling caused by low calcium intake**

Over the short term, this loss in strength is trivial, but if inadequate calcium intake is continuous, then remodeling remains high and fragility increases. The numbers of these compromised trabeculae accumulate and ultimately bone mass declines as well. It is important to note that the increase in fragility precedes appreciable loss of mass, and is due to compromised structures Thus, calcium augmentation increases bone strength by both, increasing the bone mass and reducing the remodeling (Heaney et al 2005).
1.4.3. Vitamin D:

Vitamin D plays an indirect role in stimulating mineralization of unmineralized bone matrix (Figure 1.10). After absorption or skin production of vitamin D, the liver synthesizes 25-hydroxyvitamin D and the kidneys subsequently produce biologically active 1, 25-dihydroxyvitamin D (1,25-(OH)₂D) (Clarke et al 2008). 1,25(OH)₂D₃ is the major controlling hormone of intestinal calcium absorption. As the body’s demand for calcium increases from a diet deficient in calcium, from growth, pregnancy or lactation, the synthesis of 1,25(OH)₂D₃ is increased resulting in the stimulation of intestinal calcium absorption (Christakos et al 2012). The principal function of vitamin D in calcium homeostasis is to increase calcium absorption from the intestine (Christakos et al 2011).

Figure 1.10: Vitamin D metabolism

Source: http://medicaljournalonline.blogspot.in/2012/04/vitamin-d-deficiency-symptoms-and.html
1.4.4. Zinc:

Zinc (Zn) plays an important role in growth (Seo et al 2010, Kawakubo et al 2011). The presence of a large amount of Zn in bone tissue suggests that this ion also plays an important role in the development of the skeletal system (Nishi 1996). Zinc interacts with important hormones involved in bone growth such as somatomedin-c, osteocalcin, testosterone, thyroid hormones, and insulin. It is intimately linked to bone metabolism, thus, zinc acts positively on growth and development. Zinc is considered an essential component of the calcified matrix (Salgueiro et al 2002). It has a potent stimulatory effect on osteoblastic bone formation through collagen synthesis and an inhibitory effect on osteoclastic bone resorption (Seo et al 2010, Kawakubo et al 2011). Zinc enhances the anabolic IGF-I effects in osteoblastic cells (Matsui et al 1995). Zinc depletion attenuates growth and decreases circulating IGF-I (Ninh et al 1995). It also enhances vitamin D effects on bone metabolism through the stimulation of DNA synthesis in bone cells (Salgueiro et al 2002).

1.5. Assessment of Bone status:

Bone mass measurement also known as Bone mineral density test has long been one of the primary tools used to evaluate bone health and predict fracture risk. Bone mineral density test measures the amount of mineral matter (bone mineral content) per square centimeter of bone (bone area). In children, bone assessment helps to understand the growth status.

Various non-invasive methods such as single photon absorptiometry (SPA), dual photon absorptiometry (DPA), single X-ray absorptiometry (SXA), dual-energy X-ray absorptiometry (DXA), quantitative computerized tomography (QCT), quantitative ultrasound (QUS), radiographic absorptiometry (RA) and magnetic resonance imaging (MRI) have been developed and used over the years to analyze bone mineral density. Of these, DXA is the gold standard used to assess BMD in children and adolescents.
1.5.1. Dual Energy X-Ray Absorptiometry (DXA):

Dual-energy X-ray absorptiometry (DXA) is the most commonly used bone densitometry technique for children throughout the world and preferred over other techniques because of its speed, precision, safety, low cost and easy availability (Gordon et al 2008). A DXA scan measures areal BMD (g/cm$^3$) defined as the integral mass of bone mineral per unit project area (Blake & Fogelman 1997). The first commercial DXA scanner was introduced in 1987.

1.5.2. Principle of DXA and Measurement Site:

Dual energy X-ray Absorptionmetry determines the amount of mineral in a given region by the differential absorption of x rays of two different energies. Using the same differential absorption, it can also take account of the depth and composition of adjacent soft tissue and generate measurements of fat and lean mass. Currently, DXA machines are manufactured by 3 companies (GE Lunar, Hologic, and Norland). In DXA technique, machines are manufactured either with fan beam or pencil beam of x-rays. Pencil beam machines acquire data using a small angle beam of x-rays that moves across the part being scanned in a rectilinear fashion and thus the exposure to radiation dose is less than the fan beam. Hence, the use of pencil beam DXA is preferred in children due to less exposure to the radiation. The DXA machine used for the present study was a GE Lunar Pencil beam machine (Fewtrell et al 2003).

DXA measurements can be obtained of the total body as well as regions such as the lumbar spine, hip, and distal radius (Fewtrell et al 2003). In children, the spine and total body less head are the most accurate and reproducible skeletal sites for DXA measurements (Bianchi et al 2010). The DXA measurement for total body less head has been carried out for all children in this study.
1.5.3. Interpretation of DXA Measurements in Children:

DXA machines measure bone mineral content \([\text{BMC (g)}]\) and bone area \([\text{BA (m}^2\text{)}]\), then calculate bone mineral density (BMD) as \(\frac{\text{BMC}}{\text{BA}} \text{ (g/m}^2\text{)}\). During growth, body size and maturation are major determinants of bone mineral content (BMC) and bone mineral density (BMD). As there is longitudinal and cross-sectional growth of skeleton, BMC increases. The two-dimensional measurement does not include the depth of bone; therefore, smaller bones of comparable volumetric BMD (measured in grams per cubic centimeter) appear to have lower areal BMD (Zemel et al 2010). Also, since many children with chronic disease such as growth hormone deficiency are small for their age, their BMD measurement will frequently appear to be low as well.

In research, DXA measurements are often performed to examine the effects of dietary interventions and also in comparison between 2 groups of children. The variability in the DXA measurements in children increases with age due to their growth and there is no precise point of reference to compare children’s DXA measurements. Hence, the BMC measurements are assessed as Z-scores in comparison to a reference data which takes into consideration gender, age, and race/ethnicity. A low BMC Z-score is defined as less than -2 after adjusting for age, gender and body size. (Zemel et al 2010, Fewtrell et al 2003). Also, it is necessary to consider other corrections such as bone size, height, lean body mass, bone age, height age, and pubertal stage, either alone or in various combinations (Bianchiet al 2010).

DXA measurements can be repeated after minimum 6 months to detect actual changes in the skeletal. In case of children receiving any therapy or with disease, repetition of DXA measurements after a longer interval may be appropriate. Also, a child may act as his own control with serial scans to monitor progress. Table 1.1 illustrates the various types of Z-scores calculated to assess the total body bone health in children.
### Table 1.1: Types of Z-scores used to assess total body bone health in children

<table>
<thead>
<tr>
<th>Type of Z-scores</th>
<th>Particulars</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC for Age</td>
<td>Adequacy of Total Body Bone Mineral Content for Age</td>
<td>Molgaard C et al 1997</td>
</tr>
<tr>
<td>BA for Age</td>
<td>Adequacy of Total Body Bone Area for Age</td>
<td>Molgaard C et al 1997</td>
</tr>
<tr>
<td>BA for Height</td>
<td>Adequacy of Total Body Bone Area for Height</td>
<td>Molgaard C et al 1997</td>
</tr>
<tr>
<td>BMC for BA</td>
<td>Adequacy of Total Body Bone Mineral Content for Bone Area</td>
<td>Molgaard C et al 1997</td>
</tr>
<tr>
<td>LBM for Height</td>
<td>Adequacy of Total Lean Body Mass for Height</td>
<td>Crabtree N et al 2004</td>
</tr>
<tr>
<td>BMC for LBM</td>
<td>Adequacy of Total Body Bone Mineral Content for Total Lean Body Mass</td>
<td>Crabtree N et al 2004</td>
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Thus, in the present study, various adjustments are carried out to assess the DXA measured total body BMC in growth hormone deficient children. Other Z-scores used are BMC for height age and BMC for bone age (refer chapter 6). To calculate the Z-scores of BMC with various adjustments, Indian reference data was used (Khadilkar et al 2011). However, in supplementation studies, children have acted as their own controls.

### 1.6. Nutritional Influences on Linear Growth:

Growth can be defined as a process of increase in size by accretion of tissue. Child’s growth generally refers to skeletal or linear growth as increase in their height. Though majority of the height potential is genetically determined, nutrition and other life-style factors can help to maximize increase in height. The gain in bone length contributes to 98% gain in height (Pipes et al 1988, Ress & Mahan 1988). Thus proper bone development especially during pubertal age is important and may be optimized through adequate nutrition.
With the progressive elimination of energy-protein deficits in the diets of children, micronutrient deficiencies emerge as the major bottleneck to growth (Maiya & Karunakara 2012). An inadequate dietary supply of nutrients from whatever cause may result in negative effects on growth and development (Prentice et al 2006). Inadequate intakes and/or poor absorption of the bone-forming minerals, especially calcium and zinc, may also contribute to linear growth retardation (Prentice & Bates, 1994). Moreover, calcium can enhance both the longitudinal and the cross-sectional growth of the bones. Calcium has an effect on bone modelling which is associated with an increase in statural growth (Bonjour et al 1997). Furthermore, the effects of diet on children’s bones may depend on their stage of maturity. For example, the skeleton appears to be more responsive to Ca, protein or exercise before the onset of puberty (Bonjour et al, 2003, Davies et al. 2005). Abundant studies can be found describing calcium status, the effect of calcium supplementation on bone health, peak bone mass in adolescent age group. However, studies in calcium status and its impact on growth during early childhood are scarce (Abrams & Hawthorne 2006).

Although, nutritional status data of GHD children is scarce, many studies have reported that daily calcium and zinc intakes of majority of children and adolescents in India are much below the requirements. Also, low biochemical vitamin D levels are reported in Indian children (Wayse et al 2004, Marwaha et al 2005, Ekbote et al 2010). Very few international studies have also reported the nutritional status in GHD children (Antoniazzi et al 2004). Figure 1.11 shows the conceptual model of the effect of growth hormone deficiency in addition to dietary calcium and zinc deficiency on calcium status in children.
Thus, there is a lacuna in research in Indian GHD children with respect to the studies of their bone status and nutritional status. Hence it is essential to study GHD children’s nutritional status and bone health status and also the effect of GH therapy on their bone mass.

Although the role of calcium in bone metabolism is well known and dietary calcium deficiency in children from all parts of India is common, studies examining the effect of calcium on bone status of GHD children are scarce. Moreover, GHD children on GH therapy exhibit a significantly higher growth rate, in turn higher rate of longitudinal bone growth, which may increase their requirement of calcium. Since calcium requirement is driven by skeletal growth rate. Zinc plays an important role in GH treatment response as well as in bone metabolism. Dietary zinc deficiency is also reported in Indian children (Khadilkar et al 2012, Tupe et al 2010). Hence it is essential to study the effect of calcium, zinc and vitamin D supplementation on bone status in children with GHD in addition to GH therapy.
1.7. Dietary Calcium Status:

To evaluate dietary calcium sufficiency, daily food intakes are assessed using various methods which include: (i) 24-hour Dietary Recall, (ii) Food Record and (iii) Food Frequency Questionnaires (FFQ). Amongst these, 24 hour recall and food record are reported to be more accurate than FFQ (McPherson et al 2000). Adequate dietary calcium is a prerequisite for maximizing peak bone mass during the first 3 decades of life and for minimizing subsequent bone loss. Dietary requirements for calcium are determined by the needs for bone development and maintenance, which vary throughout the life stage, with greater needs during the periods of rapid growth in childhood and adolescence (Flynn et al 2003) Inadequate dietary calcium in early life impairs bone development.

1.7.1. Calcium Requirements in Children and Adolescents:

Dietary standards, regardless of the name they go by – Recommended Dietary Allowances, Recommended Nutrient Intakes, Recommended Daily Amounts of Nutrients, or Safe Intakes of Nutrients – are the average daily amounts of essential nutrients estimated, on the basis of available scientific knowledge, to be sufficiently high to meet the physiological needs of practically all healthy persons in a group with specified characteristics (ICMR 2009). The Recommended Dietary Allowance (RDA) is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97 to 98 percent) individuals in a life stage and gender group (NAP 1997).

The amount of each nutrient needed for an individual depends upon his/her age, body weight and physiological status. The dietary allowances for a population also take into consideration individual variation within the group, quality of the diet, effect of cooking and processing and bio-availability of the nutrient from diet. Many countries have published recommended calcium intakes since 1988. A large variations in these recommendations is noted due to differences in the conceptual basis (e.g. avoidance of deficiency vs prevention of chronic disease), the endpoints being used (calcium balance vs. bone mineral density) and also due to variations of
calcium requirements from culture to culture for dietary, genetic, body size, lifestyle and geographical reasons. Figure 1.12 illustrates the range of recommendations made by various countries for various age groups.

**Figure 1.12: Range of calcium recommendations from various countries.**

![Graph showing the range of calcium recommendations from various countries](image)

(Adapted from Looker AC, 2006)


Recently, the recommended dietary allowance for Indians was revised with addition of nutrients like zinc for which earlier there was no recommendation. Also the intake of calcium has been revised especially for children.

The recommendations for calcium requirements are based on 3 major approaches: 1) Calcium balance studies, 2) A factorial model using calcium accretion based on bone mineral accretion data 3) clinical trials investigating the response of change in calcium balance or BMC/BMD or fracture rate varying on calcium intake. Using these 3 approaches, an ICMR expert committee has defined the minimum amount of calcium needed to accrue enough BMC for good bone health during childhood and adolescence (ICMR 2009).
The first Indian recommended dietary allowances (RDA) for calcium were published in 1989; these have been recently revised by the expert group of Indian Council of Medical Research (ICMR 2009). The revised recommended allowances are higher than the earlier; however, they are still lower than the FAO/WHO recommendations (Table 1.2).

**Table 1.2: Recommendations for dietary calcium (mg/day) during growing years**

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>FAO/WHO, 2004</th>
<th>ICMR, 1989</th>
<th>ICMR, 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 3 years</td>
<td>500</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>4 – 6 years</td>
<td>600</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>7 – 9 years</td>
<td>700</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>10 – 18 years</td>
<td>1300</td>
<td>600</td>
<td>800</td>
</tr>
</tbody>
</table>

(Adapted from ICMR 2009)

1.7.2 Calcium Intake in Children and Adolescents:

The risk of inadequate intakes is likely to be much higher than the risk of excessive intakes. Dietary calcium deficiency is observed in children and adolescents from across the world. Most researchers have assessed children’s calcium intake by 24 hour diet recall on 2 or 3 non-consecutive days. The adequacy of calcium intakes is expressed in comparison to the recommended intakes or adequate intakes.

*International Studies:*

When world regions for their calcium intakes were compared, it was seen that, calcium intakes generally appeared highest in Scandinavian countries, lowest in Asian countries, and intermediate in western European, Oceanic (Australia/New Zealand), and North American countries (Looker 2006).

Various researchers have reported deficient calcium intakes in children from United States from various rounds of NHANES (National Health and Nutrition Examination Study).
Survey, United states) data on dietary intakes. Calcium intakes assessed from the 1999 to 2004 NHANES round showed greater % of inadequacy in children more than 9 years of age as judged by their “Adequate Intake” (Inadequate intakes in 77 % children from 9 to 18 years, as opposed to 22 % in 4-8 year and 4 % in 2-3 year old children) (Nicklas et al 2009). From NHANES 2005-2006, in 4,032, 1 to 18 year old children, 32 %, 83 % and 58 % children between 4 - 8, 9 - 13 and 14 - 18 years of age had inadequate calcium intakes respectively (Moshfeh et al 2009).

Forty six % of 1-3 year old children from Greater New Haven, New England, were consuming calcium less than the RDA (recommended dietary allowance) as assessed by 24 hour recalls (Carpenter et al 2012) out of 750 enrolled children. Calcium intakes assessed by 24 hour diet recalls were also reported in 643 children (9 to 13 years of age) from Poland, with 82 % of boys and 92% of girls having inadequate intakes. The mean calcium intakes of these children were 19 % and 25 % of the RDA in girls and boys respectively (Rusińska et al 2011). Researchers from Japan and China have also reported deficient calcium intakes in children. Mean daily calcium intakes were less than the RDA (432 mg/day) in Japanese preschoolers (3 to 5 years) as assessed by 3 day 24 hour diet recalls (Shibata et al 2008). Prevalence of dietary calcium deficiency was found in > 97 % of 7 to 17 year old Chinese children (Du WW et al 2010)

From 12 to 16 year old Israeli girls (n = 2000), 51 % of Jewish and 48 % of Arab girls were consuming inadequate amounts of calcium (Rozen GS et al 2001). Two to 4 year old children from rural Bangladesh and 6 to 17 year old children from Saudi Arabia have also had inadequate calcium intakes < 50 % and < 60 % of RDA respectively (Arsenault et al 2012, Al-Musharaf et al 2012).

These reports thus show that calcium intakes of children from developed and developing countries worldwide are less than desired.
Indian Studies:

Similar to children from other countries, dietary deficiency of calcium is common in Indian children. The National Nutrition Monitoring Bureau (NNMB, 2006) of India analysed the dietary intakes in rural areas each in 9 states of India from the year 2004-2005 using 24 hour diet recall. Calcium intake was less than the RDA in children from rural villages in all states in India. The mean dietary calcium intake was 245±246 mg/d in 1 – 3 year old children and 272± 238mg/day in 4 to 6 years old. In 7-9 year old children, it was 291±243 mg/d. Calcium intake was 330±285 mg/d and 307±259 mg/d in 10 – 12 year boys and girls respectively (NNMB 2006). Thus, the calcium intake was less than 50% of the RDA in all children.

In a cross-sectional study from Pune, Western Maharashtra carried out in preschoolers to examine the lifestyle factors influencing their total body bone mineral content and bone area, calcium intake was studied by a 3 day, 24 hour diet recall method. The average calcium intake was 276 ± 203 mg/day. Inadequate calcium intake (< 50% of the RDA) was noted in 98% of the children (Ekbote et al 2010). Similarly, calcium intake as low as 28% of the RDA in adolescent girls from an urban slum from Pune, Western Maharashtra was noted as assessed by 3 day 24 hour recall method (Khadilkar et al 2012).

Studies from other parts of India have also reported deficient calcium intakes. In 4 to 14 year old children from Mysore, Karnataka, South India, inadequate calcium intakes were reported (Kulsum et al 2008). In 2 to 4 year old children from rural Punjab, meagre calcium intakes (62 % of the RDA) were reported (Kaur et al 2007). In 4 to 5 year old children from urban Delhi, mean calcium intake of 440 mg/day (73 % of RDA) was reported (Aggrawal 2002). Seven to 9 year old children from urban as well as rural parts of Ludhiana, Punjab were studied for their calcium intake by 24 hour diet recall method by Mehta et al (2013). Low calcium intakes with a mean of 375±79 mg/day (63 % of RDA) in urban children and 400±128 mg/day (67 % of RDA) in rural children were noted.
Ahmed (2012) has reported calcium intakes to be 58 % of RDA in boys and 49 % of RDA in girls of 3 – 6 years of age from Assam. An intake of 324±634 mg/day of calcium (54 % of RDA) was noted in 10 to 19 year old girls from an urban slum from Hyderabad and Secunderabad, Andhra Pradesh (Saibaba et al 2002). Calcium intake analysed using 5- 7 day diet recall was found to be less than the RDA in children (11 to 13 years) living in urban (293±6mg/d) as well as the rural (277±6 mg/d) areas of Tirupati, Andhra Pradesh, South India (Harinarayan et al. 2008).

Thus, reports from urban and rural areas of various parts of India suggest deficient calcium intakes in children from all age-groups.

1.8. Sources of Calcium in the Diet:

Milk and milk products such as curd, cheese and cheddar cheese are the richest sources of calcium. Dietary calcium adequacy is directly related to the adequacy of milk and milk products intake. Early humans are thought to have consumed a diet rich in calcium from a wide range of plant sources. With cultivation of plants, a few staple cereal crops became the major source of energy for humans. Cereal grains are the part of the plant which accumulates the least amount of calcium. Since the agricultural revolution, the main source of calcium in the diet of most populations is dairy products. Dairy products provide nearly ¾th of the calcium requirement in western diets. However, insufficient milk intakes are also reported in children from developed countries. In a National Australian children’s survey, milk and milk products intake was measured by 24 hour diet recalls in 4487 Australian children of 2-18 years of age. It was found that children above 4 years of age did not achieve the recommended milk intake of 2 servings /day (Baird 2012). National nutrition surveys of England and Japan have also shown low milk intakes in 11 to 18 and 6-11 year old children (Bates et al 2009, Koga et al 2012) An average milk intake of around 350 ml/day was noted in 2 to 18 year old German children (Alexy et al 2002).

However, inadequate milk intakes are more pronounced in children from various developing countries. Arsenault et al (2012) assessed dietary intakes in 480, 2-4 year old children by 24 hour diet recall for 2 days. They found that only 41 % of children
consumed at least 10 g of milk/milk products on either of the 2 days. In 4 to 12 year old Bangladeshi children (from an urban slum) milk intake less than once a week was noted (Turin 2007). An intake of less than 3 servings of milk and milk products was noted in Pakistani children of 6 month to 8 years of age (Khan et al 2013). Also, the National Nutrition Survey of Pakistan reports inadequate milk intake (average 162 ml/day) in 1-2 year old children (Bhutta 2011). The average milk consumption was found to 26 g/day in 7 to 17 year old Chinese children when assessed by 3-day 24 hour recalls which was very low as compared to their required intake of 300 ml/day (Du WW et al 2010).

Indian Council of Medical Research (ICMR) has recommended a minimum dietary milk intake of 500 ml/day to meet the dietary calcium requirements in Indian children and adolescents (ICMR 2003). Although, milk production in India has increased in past decade or so, the per capita availability still remains at 140 ml/day (Narasinga Rao 2007). According to the NNMB report (2006), dietary milk intake was 29% and 25% of the RDA for milk in rural Indian children aged 1-3 years and 4- 6 years respectively. On the other hand, mean milk intake was reported to be merely 62 - 54 ml in children aged 7 – 12 years in rural India.

Adolescent girls from Pune were reported to consume 45±8 g/d of milk (Tupe 2009). Similarly, Sanwalka et al have reported low milk intakes (45 % and 30 % of Milk RDA in boys and girls respectively) in 7-19 year old children from urban Pune. In 2-6 year old children from Mysore, the adequacy of milk intake ranged from 5 to 17%.

Thus, intake of milk and milk products is reported to be grossly inadequate in Indian children from all agegroups. Whereas, plant foods such as cereals, pulses and green leafy vegetables contribute to the 36 to 50 % of the total calcium intake of Indian children (Tupe et al 2009, Sanwalka et al 2010).

Table 1.3 gives a list of Indian foods rich in calcium. Many plant foods may contain more amount of calcium per 100 grams as compared to milk and milk products. However, the use of these foods as major sources of dietary calcium is restricted due to poor calcium bioavailability (Weaver & Heaney 2006). The bioavailability of calcium from milk and milk products is reported to be 30 % as compared to around
10% from plant foods Weaver & Heaney 2006). Moreover, the amount of calcium from 1 serving of milk can be higher than from the plant foods.

Table 1.3: Indian foods rich in calcium content

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Food Item</th>
<th>Range of Calcium Content/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals and Legumes</td>
<td>Bengal Gram (Whole), Finger Millet Horse Gram (Whole), Rajmah and Soyabean</td>
<td>200 – 340 mg</td>
</tr>
<tr>
<td>Green Leafy Vegetables</td>
<td>Amaranth, Cauliflower Greens, Colocasia Leaves, Curry Leaves, Fenugreek Leaves, Fetid Cassia, Knol-Khol Leaves</td>
<td>400 – 800 mg</td>
</tr>
<tr>
<td></td>
<td>Cumin Seeds, Gingelly Seeds</td>
<td>1080 – 1450 mg</td>
</tr>
<tr>
<td>Fish</td>
<td>Bacha, Katla, Mrigal, Pran and Rohu</td>
<td>320 – 650 mg</td>
</tr>
<tr>
<td>Milk and Milk Products</td>
<td>Buffalo’s Milk, Cow’s Milk, Curds (cow’s milk)</td>
<td>120 – 210 mg</td>
</tr>
<tr>
<td></td>
<td>Cheese, Khoa, Skimmed Milk Powder and Whole Milk Powder</td>
<td>790 – 1370 mg</td>
</tr>
</tbody>
</table>

(ICMR 2010)

1.9. Absorption of Calcium:

Intestinal calcium absorption proceeds by two mechanisms, (1) an active transcellular process that takes place in the duodenum and (2) a passive paracellular process throughout the small intestine.

(1) The transcellular process is saturable, has limited capacity, takes place largely in the duodenum and proximal jejunum and it is controlled through the action of 1,25 dihydroxyvitamin D₃. This mechanism is important when calcium intakes are low and body requirements are not being met. Transcellular movement can be described as involving three sequential steps: entry, intracellular diffusion, and extrusion. Entry across the brush border into the cell is down an electrochemical gradient via calcium channels that are not voltage gated. Intracellular diffusion of the calcium ion, i.e.,
movement from brush border to the basolateral pole of the duodenal cell, is the rate-limiting step. In the absence of the vitamin-D dependent, cytosolic calcium-binding protein (calbindin, Mr. 9 kDa), self-diffusion of the calcium ion in the intestinal cell is only 1/70 of the measured transport rate. The transcellular calcium transport varies directly with the cellular content of calbindin (Bronner et al. 1987, 1998).

(2) The paracellular process is non-saturable, concentration dependant, and takes place all along the small intestine. When large amount of calcium is ingested in a single meal, much of the calcium absorption occurs by this passive route. Since the active transcellular process plays only a minor role in the distal jejunum and the ileum, the paracellular process is predominant there. The reason for this is that high calcium intake leads to the down regulation of active transport (Bronner et al. 1987, 1998).

Figure 1.13 illustrates the intestinal calcium absorption.
Figure 1.13: Schematic representation of calcium absorption in intestine

The epithelial Ca$^{2+}$ transport occurs by paracellular and transcellular pathways. The paracellular Ca$^{2+}$ transport is carried out through tight junctions (TJ) by an electrochemical gradient (long arrow between cells). The transcellular Ca$^{2+}$ transport consists of three steps: (1) apical entry of Ca$^{2+}$ through epithelial Ca$^{2+}$ channels TRPV5 and TRPV6 (the last one is the most abundant in intestine); (2) cytosolic diffusion bound to calbindins, and (3) extrusion across the basolateral membranes by the plasma membrane Ca$^{2+}$-ATPase (PMCA 1b) and the Na$^{+}$/Ca$^{2+}$ exchanger (NCX1). Calcitriol (1,25(OH)$_{2}$D$_{3}$) stimulates the individual steps of transcellular Ca$^{2+}$ transport. Calcitriol molecules bind to their nuclear receptors (VDR), and the complex1,25(OH) 2 D 3 -VDR interacts with specific DNA sequences inducing transcription and increasing the expression levels of TRPV 5/6, calbindins and the extrusion systems (Pérez AV 2008).

1.9.1. Factors Affecting Calcium Absorption:

Nutrients, for which metabolism is usual and appropriate and the route of administration is nearly always oral, the notion of bioavailability generally designates simply the quantity or fraction of the ingested dose that is absorbed.

Absorption of dietary calcium depends upon the total calcium content of the meal and the presence of food constituents that enhance or inhibit calcium absorption.
Calcium bioavailability of plant foods depend on their content of Phytates and oxalates which are the known inhibitors of calcium absorption (Weaver et al 1999). Phytic acid is the storage form of phosphorus in seeds. The negative charges of the phosphate groups bind divalent cations such as calcium and negatively effect its gastrointestinal absorption (Cámara-Martos et al 2002). A 3-fold increase in phytic acid reduces calcium absorption by 25 %. High-phytate bran cereals can physically absorb great quantities of calcium and reduce its absorbability (Food sources, supplements and bioavailability). Higher fractional absorption of calcium was reported from tortillas prepared from maize with reduced phytate content than maize with typical phytate content (Hambidge 2005).

In Indian diets, the cereals, pulses, nuts and oilseeds are rich sources of phosphorus. About 40-60 % of cereal phosphorus is present as phytin (Narasinga Rao et al 2007). Phytic acid reaches 3 to 6% of the weight of the grain (Febles et al 2002). Indian diets both in rural and urban areas are predominantly based on cereals Narasinga Rao et al (2007) and Harinarayan et al (2007) have reported high phytate consumption through Indian diets although in adults.

Oxalic acid is the most inhibitor of calcium absorption. Oxalic acid, a dicarboxylic acid or its salts (oxalates) are widely distributed in plant foods and can be found in green leafy vegetables and pulses. It forms an extremely insoluble salt with calcium and reduces calcium absorption considerably. Spinach is the least bioavailable of the calcium sources, due to its high oxalic acid content (Heaney et al 1988). However, other green leafy vegetables with low oxalate content show better calcium bioavailability. Gupta et al also have shown reduced bioavailability of calcium in green leafy vegetables with high oxalate content from Mysore, India (Gupta 2006). However, soyabean has high bioavailability despite its high oxalate content, the reason for which is still unknown to the researchers (Weaver et al 1998).

Thus, the presence of these inhibitors in plant foods considerably affects the calcium absorption in diets of Indian children. Hence suggesting strategies to improve the calcium content as well as its bioavailability from plant foods are necessary in Indian children’s diets.
1.9.2. Assessment of Calcium Absorption:

Calcium absorption from a number of sources has been estimated from areas under the curve (AUC) of profiles of total or ionized calcium or its regulator i.e. parathyroid hormone or vitamin D metabolites after an overnight fast (Clinically Studying calcium metabolism). This approach is based on the fact that the serum concentrations of calcium rise into the circulation as calcium enters the circulatory system during its absorption.

Although, the extracellular calcium concentration is tightly regulated, calcium absorption produces small increase in the concentration. This increase is measurable and can be captured by the measurement of area under the curve. The degree of increase in calcium concentrations reflects the amount of calcium absorbed (Heaney et al 2003). Moreover, this method is better suited when 2 or more sources are to be compared (Heaney et al 2001). This method has been successfully used to determine responses in large doses i.e a 500 mg calcium supplement vs placebo. Moreover, when measuring the absorption from a particular source, it is advisable to compare it with a reference source such as calcium carbonate (Clinically studying calcium metabolism).

Thus, the pharmacokinetic method by assessing the AUC can be used successfully to measure the calcium absorption and especially when comparing 2 or more sources of calcium.

Researchers have analysed the changes in serum ionized calcium and parathyroid hormone after the ingestion of the calcium sources and their changes in the area under the curve for various time periods ranging from 4 hours postprandial to 24 hours (4 hours, Talbot et al 1999, Martini & Wood 2002, 4.5 hours Hanzlik et al 2005; 6 hours Heller et al 2000, 24 hours Heaney et al 2003). Thus postprandial response of ionized calcium and parathyroid hormone up to 5 hours may provide sufficient information for comparison of absorption of calcium from 2 or more sources.
1.10. Strategies to Improve Calcium Intake:

Strategies to improve calcium intakes include (1) Dietary Modification and Diversification (2) Use of calcium fortified foods and (3) The use of Calcium supplements.

1.10.1. Dietary Modification and Diversification:

Dietary modification and diversification includes changes in food selection patterns (use of foods naturally rich in calcium) and traditional household methods for preparing and processing indigenous foods.

Use of foods naturally rich in calcium:
Incorporation of indigenous foods which are naturally rich in calcium in day to day diets can be encouraged. Also to enable the use of such foods, modifications in their usual menus can be suggested (Gibson et al 1998).

Use of food processing methods:
Use of certain food processing methods has shown to improve the calcium absorption and or bioavailability from plant foods. Table 1.4 shows the effect of various food processing methods on the bioavailability of calcium from plant foods:
Table 1.4: Effect of food processing methods on the calcium absorption / bioavailability from plant foods

<table>
<thead>
<tr>
<th>Processing Method</th>
<th>Technical Influence</th>
<th>Effect on Calcium Absorption/Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal Processing</td>
<td>May degrade Phytate</td>
<td>May increase Calcium bioavailability</td>
</tr>
<tr>
<td>Germination and Malting</td>
<td>Increases phytase (phytate degrading enzyme) activity via de novo synthesis or activation of endogenous phytase, hence induces hydrolysis of phytate</td>
<td>Increases Ca absorption</td>
</tr>
<tr>
<td>Fermentation</td>
<td>Induces hydrolysis of phytate by microbial phytase</td>
<td>Increases Ca bioavailability</td>
</tr>
</tbody>
</table>

(Gibson 2006)

The above mentioned food processing methods increase calcium absorption or bioavailability principally through the degradation of phytate content. Fermentation of sorghum flour for over 72 hours to prepare Injera (a traditional Ethiopian bread) has been shown to decrease phytate content from 317.6±13.5 mg/100g in sorghum flour to 286.7±4.3 mg/100 g in Injera (Mohammed et al 2010). A significant decrease of around 18 to 21 % in the phytic acid in various legumes and millets has been reported (Ghavidel et al 2007). Malt pre-treatment of sorghum flour has shown to increase calcium extractability to 102 – 103% which is a result of reduction in the phytin content (Idris et al 2005, 2007).

The dietary modifications and diversifications are culturally acceptable, equitable, economically feasible and sustainable. Moreover, they are community based and hence can enhance community and human development (Gibson et al 1998).

However, for successful implementation of these strategies in any community, the knowledge of their dietary pattern and knowledge of available local foods is essential. Thus, a study of existing dietary patterns of children is necessary.
Dietary Patterns:

Dietary diversification involves assessing of dietary patterns before suggesting any new strategies (Tontisirin et al 2002). It is essential to consider the existing dietary patterns, the socio-economic, cultural factors and biologic & physical environment in which the population lives and then indicating what aspects should be modified (FAO 2001).

Cereal based diet patterns were reported by Tupe & Chiplonkar (2009) in Indian adolescent girls. They found that girls were consuming diets based either on pearl-millet, rice, sorghum or wheat. In 2 to 6 year old Indian children, Kaur et al (2007) found that the consumption of cereals was more (> 80 % of the recommended intake) than all other food groups. The adequacy of intakes of cereals, pulses, roots-tubers and other vegetables were better than intakes of green leafy vegetables, milk and milk products in 4-14 year old urban children from Mysore (Kulsum et al 2008). A higher adequacy of cereal intake as compared to pulses, vegetables and milk and milk products in 3 – 6 year old children from Assam (India) was reported (Ahmed 2012). In 7 to 19 year old children from Pune, India, 51 % of total intake was of cereals (Sanwalka et al 2010). Thus, various studies have described Indian children’s dietary intakes in terms of food groups and majority of children’s diets are based on cereal intakes. However, studies reporting dietary patterns with special reference to their calcium intakes are scarce. Examining Indian children’s diet patterns would help to develop suitable recipes rich in calcium content.

1.10.2. Food Fortification:

Fortification of a habitual food has a potential for better compliance over supplementation when the micronutrient intake of a population needs to be improved (Weaver & Heaney 2006). Food fortification has the dual advantage of being able to deliver nutrients to large segments of the population without requiring radical changes in food consumption patterns. Fortification of a staple food affects everyone, including the poor, pregnant women, young children and populations that can never be completely covered by social services. In addition, fortification reaches secondary
at-risk groups, such as the elderly and those who have an unbalanced diet. Food fortification is usually socially acceptable, requires no change in food habits, does not alter the characteristics of the food, can be introduced quickly, can produce nutritional benefits for the target population quickly, is safe, and can be a cost-effective way of reaching large target populations that are at risk of micronutrient deficiency (Allen et al 2006).

Calcium Salts for Food Fortification:

Various calcium salts can be used as food fortificants such as calcium carbonate, tricalcium phosphate, dicalcium phosphate, calcium citrate, calcium citrate malate, calcium lactate, calcium gluconate, calcium glubionate etc. The amount of the total calcium salt required to fortify a food depends on the proportion of calcium in the salt. Salts with lower concentrations will have to be added in larger amounts, a factor that may affect the final choice of fortificant. All of these salts are either white or colourless. Most are bland although the citrate has a tart flavour, and high concentrations of the lactate can be unpleasant. The cost of calcium carbonate is very low, usually less than that of the food vehicle. The % calcium content of various salts ranges from 6.5 % in Calcium Glubionate to 40 % in Calcium carbonate (Weaver & Heaney 2006). Thus calcium carbonate has higher percentage of calcium content than most of the other calcium salts. The use of calcium carbonate as a fortificant has shown positive effects on bone mineral content or density (Weaver 1998). Calcium carbonate is being used for wheat flour fortification in United Kingdom (Allen et al 2006). It is also often used in cereal based food products such as food bars and breakfast cereals (Rafferty et al 2007). Thus, calcium carbonate can be used for fortification in cereal based diets.

Most pure salts have similar calcium absorption, but the food matrix can affect absorption substantially so they must be tested (Weaver & Heaney 2006). The absorption of calcium from calcium carbonate as pure salt was higher than with meal or as a fortificant through food (Rafferty et al 2007). Similar results are reported with the use of other calcium salts (Heaney et al 2005, 2005). Moreover, few
fortified foods have been tested for their calcium absorption, even fewer have been tested for their benefits on bone (Weaver & Heaney 2006).

Hence, it is necessary to test the calcium absorption from fortified food and further test its effectiveness on bone mineral content in children.

Calcium fortification of milk and milk products such as cheese, yoghurt, cottage cheese, beverages like soy milk, fruit juice has been suggested (Allen et al 2006). Most calcium food fortification studies in children have used milk and milk products or milk extracts (Bonjour et al 1997, Cadogen et al 1997, Lambert et al 2008). Bonjour et al (1997) used milk extracts to fortify several food products and supplemented them to pre-pubertal girls for a year. They showed greater bone mass accrual (greater by 3.5 to 5 %) in supplemented girls than the non supplemented group. Moreover, greater benefit was also seen in girls with spontaneous low calcium intakes than in girls with spontaneous high calcium intakes. Such studies examining the effect of fortification on bone mineral content in Indian children are lacking.

Most studies were conducted in Caucasian children, around the pubertal years. Studies examining the effect of supplementation of calcium in toddlers are scarce (Greer & Krebs 2006). Examining the effect of calcium supplementation on toddlers bone mass is of importance due to the high growth rate during toddler years. Developing dietary practices which may be associated with adequate calcium intake during later years of life can also be achieved during the toddler years (Greer & Krebs 2006). Considering the ethnic differences in bone mineral accrual (Khadilkar et al 2011) and a gap of knowledge in preschool and toddler years it is necessary to study the effect of calcium fortification in Indian toddlers.
1.10.3. Calcium Supplementation:

Supplementation is the term used to describe the provision of relatively large doses of micronutrients, usually in the form of chemical compounds as pills, capsules or syrups. It has the advantage of being capable of supplying an optimal amount of a specific nutrient is often the fastest way to control deficiency in individuals have been identified as being deficient (Allen et al 2006). Although a number of calcium supplementation trials have been conducted, most of them are studies in healthy Caucasian populations with adequate dietary intake of calcium (Johnston CC et al 1992, Lloyd et al 1993, Dibba et al 2000, Moyer-Mileur et al 2003, Rozen GS 2003, Cameron et al, 2004, Courteix et al, 2005, Matkovic et al, 2005, Molgaard et al, 2004). The dosage of supplementation in these studies ranged from 300-1200mg/d. Also, it has been established that calcium intake above the threshold does not provide any additional benefit (Matkovic & Heaney 1992). Thus, based on the Indian RDA, 500mg/d of calcium supplement in addition to the dietary calcium may be sufficient to show positive results.

To observe the effect of supplementation on bone mass accrual requires longer time period. Most of the calcium supplementation trials have shown positive effect on bone mass accrual in children and adolescents with 1 year of supplementation with a few studies giving supplementation for 8.5 months (Iuliano-Burns et al 2003). This indicates that 12 months may be a sufficient period to see any effect of supplementation on bone mass accrual.

However, reliance on the use of supplements for improving a population’s calcium intake has restricted effectiveness due to the issues concerning long time adherence (Weaver & Heaney 2006). Nevertheless the strategy of supplementation to increase calcium intakes can be appropriately used in growth hormone deficient children since they are at greater risk of impaired bone mineralization. Moreover, considering the increased growth rate of these children when on growth hormone therapy, supplementation appears to be more appropriate measure than only diet diversification or fortification. Studies, examining the nutrition intake of GHD children and effect of calcium supplementation are scarce. Only one study so far by
Zamboni et al (2006) has shown a positive effect of calcium supplementation in addition to GH therapy in Italian prepubertal GHD children. In view of the abundance of the dietary calcium deficiency in Indian children and ethnicity being a major determinant of bone mass, it is essential to study the effect of calcium supplementation in addition to GH therapy in Indian prepubertal GHD children.

Figure 1.14 shows the conceptual model. The above review of available literature suggests that Indian children and adolescents diets majorly comprise of cereals and pulses with meagre intakes of milk. Socio-economic status and dietary patterns of children also affect their total and bioavailable calcium intakes. This leads to dietary deficiency of calcium an also the bioavailability of such plant food calcium is very low. Dietary inadequacy of calcium may lead to impairment of bone mineralization in children and adolescents. In case of growth hormone deficient children, calcium and zinc deficiency may further impair their height gain and bone mineralization. Hence, apparently healthy children as well as growth hormone deficient children may be at increased health risk and the risk of developing osteoporosis in later life.

**Figure 1.14: Conceptual model**

- Socio-economic Status ➔ Dietary Pattern ➔ Cereal-Based Diet
  - Low Total and Absorbable Dietary Calcium ➔ Impaired Bone Mineralization in Childhood
  - Growth Hormone Deficiency ➔ Impaired Height Gain and Impaired Bone Mineralization in GH Deficient Children
  - Zinc Deficiency ➔ Increased Health Risk, Increased risk of developing Osteoporosis in later life
To summarize, the available evidence suggests that:

- Deficiency of calcium in children and adolescent’s diets hampers the bone mineralization.
- Indian children’s diets are deficient in calcium and also the bioavailability of the dietary calcium is low.
- The main source of calcium is cereals, pulses and vegetables in Indian children’s diet and milk consumption is meagre.
- Bioavailability of the plant food calcium is low than that of milk and milk products.
- There is a need to study children’s dietary pattern and accordingly suggest strategies to increase calcium intake.
- Strategies such as dietary diversification and modification, calcium fortification and calcium supplementation may be used to increase the calcium in children and adolescent’s diet.
- Fortification of foods from existing diet pattern has great potential to enhance calcium intakes of a population.
- Absorption of calcium from the fortified food and its benefits on bone need to be examined.
- The calcium status and effect of calcium supplementation on calcium status in toddlers has been scarcely studied and it is essential to study due to their high growth rate.
- Growth hormone plays an important role in linear growth as well as bone mineralization in children. However, information on nutritional status and bone health status in Indian growth hormone deficient children is lacking.
- Zinc also plays an important role in the action of growth hormone in growth hormone treated children and bone mineralization. Thus there is a need to study the effect of growth hormone therapy, zinc, calcium and vitamin D supplementation in growth hormone deficient children.
Therefore aim and objectives of the present study are:

**Aim:** To assess diet patterns for calcium intakes in children and to study the effect of calcium supplementation on bone mass in toddlers; to study the effect of calcium and zinc supplementation on bone mass of growth hormone deficient children on growth hormone therapy.

**Objectives:**

1) To assess diet patterns of children and adolescents for their milk-equivalent calcium density and suggest strategies to improve total and bioavailable calcium intake.
2) To evaluate Ca absorption of one of the alternatively developed diet/meal in children.
3) To study the effect of Ca supplementation on Ca status in hypocalcaemic toddlers.
4) To assess effect of GH alone and GH with Ca and Zn supplementation on bone mass in GHD children.

The entire research work is presented in the form of 7 chapters covering study design, methodology, results of each objective and summary & conclusions. Major part of the results have been published in international, peer-reviewed journal as indicated under various chapters.