CHAPTER 1

Introduction and Review of Literature

Bones play many roles in the body; providing structure, protecting organs, anchoring muscles and storing calcium. Adequate dietary intake of calcium and weight bearing physical activity builds strong bones, optimizes bone mass, and may reduce the risk of fractures later in life. While it’s particularly important to take steps to build strong and healthy bones during childhood and adolescence, it is equally essential to protect bone health during adulthood too. Thus, bone development and maintaining bone is a lifelong process that begins at birth and continues in childhood and adulthood (National Osteoporosis Foundation, 2009).

Peak bone mass (PBM) is a key determinant of skeletal health throughout life. Peak bone mass refers to the time in an individual’s lifespan when bone mineral density reaches its maximum potential. However, several other factors such as physiological and environmental conditions are also important in bone formation and maintenance of bone health. Moreover, women experience greater bone loss than men. In a country like India, plant-based diets, different environmental conditions and gender bias necessitate identification of bone health determinants particularly in girls to develop strategies for improving bone status.

Throughout life, bone is highly metabolically active through a process called bone turnover (bone modelling and remodelling) which involves breakdown or removal of old bone and formation of new bone (Hadjidakis & Androulakis, 2006). During childhood and adolescence, bone modelling is very rapid and the formation of new bone exceeds the resorption or breakdown resulting in the accumulation of bone mass until the third decade of life when peak bone mass is achieved (Matkovic 1991). After the middle age, micro-architectural deterioration of the bone structure begins with bone mineral resorption (breakdown) exceeding bone formation, resulting in a gradual loss of bone mineral content (BMC) and bone mineral density (BMD) (Figure 1.1).
Figure 1.1: Lifetime changes in bone mass.


At a given age, bone mass results from the amount of bone acquired during growth, i.e. the peak bone mass (Bonjour et al 1991, Theintz et al 1992) minus the age-related bone loss (Marwaha et al, 2010; Rizzoli et al, 2001). The higher the peak bone mass, the more bone one has "in the bank" and less likely one is to develop osteoporosis as age advances. However, low peak bone mass, excessive bone resorption, and inadequate formation of new bone during remodelling leads to increased bone fragility and an increased susceptibility to fractures, a condition known as osteoporosis; i.e. porous bones. The most common bones that are affected are hip bone, spine and wrist.

In case of women, rapid bone loss is observed near menopause which is higher than the age-related bone loss in men (Figure 1.1). Therefore it is essential to target bone health in women by identifying specific risk factors and developing awareness programs. Moreover, failure to attain sufficient peak bone mass in childhood and adolescence and lack of maintenance of peak bone mass for a sufficient period of time during early adulthood increases the risk of osteoporosis. Hence, targeting girls to achieve maximum peak bone mass by supplementation of essential nutrients such as calcium would go a long way in maintaining strong bones throughout life. Thus, the main aim of the present research is to study girls and
women for their bone status and study the effect of calcium supplementation on bone mass in girls. A detailed review of available literature is presented below which elaborates the need for concentrating on bone health in young girls as also in women.

1.1 Basic Bone Structure

Bone is a complex, highly organised and specialised connective tissue, composed chiefly of calcium, phosphorus, and a fibrous substance known as collagen. Every bone, whether big or small, has an outer layer of compact bone. Compact bone is dense tissue made up of osteons arranged in rings (Figure 1.2). The rings have white spots at the centre which allow for blood vessels and nerves to travel through. Inside the compact bone is a lighter spongier layer of bone (Figure 1.2). The spongy layer has a honeycomb appearance and keeps the skeleton from being overly heavy. Bone marrow is located within the spongy layer. Between the layers there are tiny spaces for bone cells and fluid to provide nutrients to the bone.

Figure 1.2: Inner structure of Compact Bone & Spongy (Cancellous bone)

Bone cells may be divided into two broad classifications depending on whether they make bone or resorb it. Osteoblasts make bone, while osteoclasts resorb or take away bone. However, there are actually three
different sub-categories of bone cells related to osteoblasts: 1) the osteoblasts themselves, 2) bone lining cells, and 3) osteocytes. Osteoblasts synthesize and deposit bone matrix. Bone lining cells are basically inactive osteoblasts (in terms of making bone) that cover all available bone surfaces. Osteocytes are osteoblasts that become encased in bone matrix during bone tissue production. Osteoclasts on the other hand, secrete acids and enzymes (collagenase) to break down bone matrix.

**Bone turnover (modelling and remodelling)**

There is a continuous cycle of active bone formation (through the activity of osteocytes and osteoblasts) and bone resorption (involving osteoclasts). The terms osteogenesis and ossification are often used synonymously to indicate the process of bone formation. Figure 1.3 shows the sequence of events in the bone remodelling. Osteoclasts are attracted to a quiescent bone surface (A) and then excavate an erosion cavity (B, C). Mononuclear cells smooth off the erosion cavity (D), which is a subsequent site for the attraction of osteoblasts that synthesise an osteoid matrix (E). Continuous new bone matrix synthesis (F) is followed by calcification (G) of the newly formed bone. When complete, lining cells once more overlie the trabecular surface (H).

**Figure 1.3: Steps in the remodelling sequence of cancellous bone.**

Bones contain more calcium than any other organ. The intercellular matrix of bone contains large amounts of calcium salts, the most important being calcium phosphate. When blood calcium levels decrease below normal, calcium is released from the bones to maintain an adequate supply for metabolic needs. When blood calcium levels are increased, the excess calcium is stored in the bone matrix. The dynamic process of releasing and storing calcium goes on almost continuously. If more calcium is removed from bones than is replaced, bones become weaker and have a greater chance of breaking.

Overall conformation of adult bone is genetically determined. Once maturity is achieved bone ceases to grow, but mechanical stresses of weight bearing, muscle attachment, and applied loads will all result in constant adaptation of the internal structure and external appearance of bone. Bone mass refers to the amount of minerals (mostly calcium and phosphorous) that a specific volume of bone contains. Around the age of 17 yr, 90% of the adult bone mass is established. A person with low bone mass is at high risk for fractures and osteoporosis.

1.2 Osteoporosis: Thin bones

Osteoporosis is defined as a ‘metabolic bone disease characterised by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk’ (Consensus development conference, 1993). An example of osteoporotic and normal bone is shown in Figure 1.4. In osteoporosis the bone mineral density (BMD) is reduced and the amount and variety of protein in bone is altered. There are three main types of osteoporosis, primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is referred to as primary type 1 or postmenopausal osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs after age 75 and is seen in both females and males in a ratio of 2:1. Finally, secondary osteoporosis may arise at any age and affect men and women equally. This form of osteoporosis results from chronic predisposing medical problems or disease, or prolonged use of medications such as glucocorticoids, when the disease is called steroid- or glucocorticoid-induced osteoporosis. The underlying
mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation. Osteoporosis can be caused by three different scenarios: 1) a failure to attain a sufficient peak bone mass during childhood and adolescence, 2) a failure to maintain peak bone mass for a sufficient period of time during early adulthood, 3) accelerated bone loss in later years.

**Figure 1.4: Examples of normal and osteoporotic bone under the microscope**

![Normal Bone and Osteoporotic Bone](image)


### 1.3 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).

Various methods like quantitative ultrasonography (QUS), quantitative computerized tomography (QCT), single photon absorptiometry (SPA), dual-photon absorptiometry (DPA), single energy x-ray absorptiometry (SXA) and dual-energy X-ray absorptiometry (DXA) are used to measure BMD. Most of the methods used
for measuring BMD are fast, non-invasive and painless (Cefalu 2004). When all these techniques are evaluated according to their precision, accuracy, cost-effectiveness and irradiation dose, bone mineral density measured with DXA has been the most commonly used measure to assess bone health status in both paediatric as well as adult populations (Zhu et al, 2001).

**Dual energy X-Ray Absorptiometry (DXA) technique to measure BMD**

DXA has been available since the late 1980s and is used extensively for diagnosis and monitoring of osteoporosis (Mazess & Barden 1989; Compston et al, 1995; Genant et al, 1996). Studies have shown that bone mass as evaluated by DXA predicts 80% to 90% of its strength in vitro (Mazess & Whedon 1983), which translates into a high predictive value for osteoporotic fractures in postmenopausal women (Wasnich 1993; ISCD, 2004). The fundamental principle of DXA is the measurement of the transmission of x-rays through the body at high and low energies. The use of two energies allows discrimination between soft tissue and bone; low-energy photons are attenuated by soft tissue and the high-energy photons by bone & soft tissue. By subtracting the soft tissue from soft tissue & bone, it is possible to quantify the amount of bone within the x-ray scan path. Pixel-by-pixel attenuation values are converted to areal BMD (aBMD; g/cm²) by comparison with a bone mineral phantom. In most clinical and research reports and throughout the thesis, aBMD is designated simply as BMD.

DXA may be applied to the whole body or to the skeletal regions of interest, for example, the spine, the proximal femur, and the radius. The advantages of DXA include rapid scan times, a low ionizing radiation dose [the amount of effective dose of radiation exposure for a whole body DXA is very low (0.02 μSv)] and the availability of reference data for interpretation of results (Sawyer et al, 2007). Also, DXA can measure as little as 2% of bone loss per year (Fawzy et al, 2011) making it a useful tool for monitoring osteoporosis.

DXA machines are manufactured by three major commercial manufacturers: GE Medical Systems Inc. (formerly known as Lunar) Madison, WI; Hologic Inc., Waltham, MA; and Cooper Surgical (formerly known as Norland Medical Systems, Inc.) Trumbull, CT (Brownbill & Illich, 2005). Though all three are known for their
accuracy and precision in quantifying the bone density at all skeletal sites, the BMD as measured on any one machine is not identical to that obtained from a DXA by a different manufacturer as a result of difference in their calibration (Bonnick 1998) and difference in normal population used as reference database (Faulkner et al, 1996). Thus, while comparing bone status of different groups of individuals, it is necessary to have measurements from the same type of machine.

Bone status is assessed by measurement of BMD in absolute terms (mg/cc) and by comparison with a known standard (reference population used by the machine) to give the “T score”, which is the number of standard deviations above or below the mean for a healthy 30 year old adult of the same sex and ethnicity as the patient, or the “Z score”, which is the number of standard deviations above or below the norm for a person of the patient’s age, sex, weight, and ethnic origin. “Osteoporosis” in adults is defined on the basis of T score and “low bone mass” in paediatric population based on Z scores.

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T\text{ score} = \frac{\text{subject’s BMD value} - \text{mean young normal BMD value}}{\text{Young normal BMD standard deviation}}
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According to World Health Organisation (WHO), T score above -1 is considered normal, between -2.5 and -1 it is “osteopenia” (mild reduction in BMD) and less than -2.5 is considered “osteoporosis” (severe reduction in BMD) (Lau, 2001). As per the International society for clinical densitometry (ISCD, 2004), Z score of less than -2 in children can be labelled as “low bone density for chronological age”.

1.4 Prevalence of osteoporosis in adults

Osteoporosis is a global problem occurring in every geographic area and affecting 150 million men and women worldwide. It is a silent disease, reflected only in a low bone density, till a fracture occurs. The highest risk of hip fractures are seen in Norway, Sweden, Iceland, Denmark and the USA with an increasing incidence of hip fractures seen in the developed cities in Asia (Kanis et al, 2002). Worldwide, lifetime risk for osteoporotic fractures is 30-50% in women and 15-30%
in men (Randell et al, 1995). Both men and women reach “peak bone mass” in their mid-thirties, but there are significant differences in pattern of bone loss between men and women. Studies have shown that peak bone mass tends to be higher in men than in women by almost 12-25% as estimated by DXA (Joseph & Melton et al, 2001). Thus at all stages of life, bone mass in females is lower as compared to males (Ebbesen et al, 1999; Nieves et al, 2005). Further, in women, there is a period of bone mass stability between peak level and the onset of menopause, when declining levels of oestrogen significantly affect bone mass. Oestrogen is thought to play a role in maintaining bone mass by slowing the process of bone breakdown or resorption. As with menopause oestrogen levels fall, bone breakdown accelerates. During the first decade after onset of menopause, the annual rate of bone loss in women may be as high as 7%, but after menopause it is between 1-2%. By contrast, the annual rate of bone loss in men is only about 1% after peak bone mass is achieved (Miller 2009). Another gender difference is that men have more cortical bone than women (Kenny & Prestwood, 2000). Consequently, bone diameter increases in men and confers a mechanical advantage in protecting from fractures (Shreyasee & Felson, 2001). Thus, though osteoporosis occurs in both women and men, women have a much higher percentage of bone loss over their lifetime and also bone loss starts at an earlier age, putting them at a higher risk for osteoporosis.

Using the criteria determined by the WHO, it is estimated that 13- 18% of women worldwide aged 50 and over have osteoporosis. Recent estimates suggest a prevalence of osteoporosis to be 42.5% in Indian women above 50 years of age (Marwaha et al, 2011). Further, high prevalence of osteopenia (moderately reduced BMD) in Indian premenopausal women and a high prevalence of osteoporosis has been reported in postmenopausal women (Mittal et al, 2011) indicating that bone loss begins before the onset of menopause. Studies in Indian women aged 30-60 years from low income groups have reported much BMD values at all skeletal sites than those reported from developed countries, with a high prevalence of osteopenia (52%) and osteoporosis (29%) seen in this age group (Shatrugna et al, 2005). Thus, there is a need to focus on bone health of women nearing menopause.
1.5 Bone health status in children and adolescents

One of the best defenses against developing osteoporosis is building enough calcium stores during the growth period. The critical years for building bone mass are from childhood to about age 20 years. Approximately, 40% of adult Peak Bone Mass (PBM) is accrued during Puberty. Since peak bone mass is a major risk factor in the aetiology of osteoporosis, it is important to assess bone health status during years of rapid bone mass deposition i.e. during childhood and adolescence. Studies have reported that Asian girls have lower bone mineral density than their Caucasian counterparts (Boot et al, 1997). In apparently healthy pubertal Chinese girls a high prevalence of low body weight (BMI<18) associated with low bone mass was observed (Du et al, 2003). Barrack et al (2008) have reported low bone mass in female endurance runners with dietary restraints. In children and adolescents, especially those belonging to the lower-socio-economic stratum with low intake of nutrients and short stature, lower bone mineral density has been demonstrated compared to children from upper socio economic stratum (Arabi et al. 2004). Inadequate bone health in children from low income groups has been reported by an Indian study (Khadilkar et al, 2010) where significantly lower bone mass was seen in adolescent girls compared to relatively ‘well off’ age-matched South Asian and white Caucasian girls in the UK. Another study by Marwaha et al (2005) in healthy North Indian children reported lower bone mass in girls from lower socioeconomic strata compared to girls from higher socioeconomic groups. Thus available data suggests the existence of a socioeconomic gradient for bone health and the need to focus on improvement of bone health of girls from underprivileged sections of the society.

In South Asian countries like India, discrimination against the girl child is a well-documented fact with intra-familial distribution of food showing significant male preference (Puri et al, 2008). Poor nutrition and inadequate health care during growing years may lead to achievement of lower peak bone mass during adulthood since of the total adult body bone mineral content (TBBMC), 53% is achieved during the premenarchal period in girls (Lloyd et al, 1992; Bailey 1997). This makes premenarchal years important for peak bone mass attainment.
1.6 Need for attention to bone health in females

The etiologic framework for risk of low bone mass in females can be postulated as shown in Figure 1.5. Gender bias, especially in underprivileged class coupled with poor environmental conditions and less health care facilities leads to nutritional deficiencies. Further, Asian ethnicity adds to the risk of lower bone mass accrual at young age and low peak bone mass attained at young age would increase the risk of low bone mass during adulthood. In the reproductive years, calcium and bone metabolism is substantially altered during periods of pregnancy and lactation with women losing 300 to 400 mg of calcium daily through breast milk. This calcium demand is met by a 5-10% loss of skeletal mineral content during 6 months of exclusive lactation which is usually recovered during the weaning period (Kovacks 2005). However, in case of inadequate post-partum nutrition these losses may not be recovered especially in women with habitually low intakes of calcium (O’Brien et al, 2006; Sowers et al, 2000). Moreover, women experience rapid bone loss during and post menopause as a result of oestrogen deficiency which increases the risk of osteopenia and subsequently osteoporosis. Thus, premenarchal girls and women near menopause need to be investigated for betterment of their bone health.

It can be predicted from epidemiological studies that a 10% increase in peak bone mass would reduce the risk of fragility fractures after menopause by 50% (Daly & Petit, 2007). Therefore, these are the two stages in woman’s life that should be targeted for preventive strategies to achieve optimum bone health: primary prevention aimed at maximizing the PBM in formative years and secondary prevention, in adulthood and after menopause, aimed at reducing bone loss with age. Low peak bone mass or weak bones cannot be easily observed since bone loss is gradual and painless. Often there are no symptoms until the first fracture occurs. Hence, it is imperative to understand the risk factors for low bone mass and appropriately plan corrective measures for bone health in growing years as also in adulthood.
Figure 1.5: Conceptual model for bone health in Indian women throughout life cycle:

Discrimination against girl child -> Poor nutrition, inadequate health care

Socio-economic status, environmental conditions

Asian Indian

Less bone mass accrual in Adolescence

Low peak bone mass at adulthood

Pregnancy & child birth – bone loss

Rapid bone loss after 40 years of age

Osteopenia at postmenopausal age

Osteoporosis and increased risk of fractures at old age
1.7 Determinants of bone health

Many interrelated factors can influence bone health of which, some are modifiable such as lifestyle and some are not e.g.; age, gender or family history. Figure 1.6 illustrates the interplay of various factors influencing peak bone mass. The amount of bone accrued during lifetime by an individual is controlled to a large extent by their genetic makeup (Balock and Eisman, 2004). Though heritability, i.e. the additive effects of genes, accounts for 40-80% of the variance in adult bone mineral density (Lloyd et al, 2002), modifiable or lifestyle factors, such as diet, physical activity, body composition, and general health, are thought to explain anywhere from 20 to 40% of the variability seen in peak bone mass variance (Bonjour et al, 2009).

Figure 1.6: Determinants of peak bone mass.

The black arrows show interdependency of the 4 types of factors
The following section examines the role of these factors in bone health during childhood and adulthood:

**Ethnicity:** Racial difference is one of the most important determinants of peak bone mineral density. A review by Pollitzer & Anderson (1989) concluded that blacks have higher bone density than Caucasians and Asians. Globally, highest prevalence of vertebral and hip fractures has been reported in Caucasians and Asians and lowest among Blacks (Lau, 1996; Ross, 1995; Lau, 2001). It has been reported that Asians are at higher risk of achieving lower peak bone mass and thus developing osteoporosis later in life. Available data indicate that Indians have lower spinal bone density than their North American and European counterparts (Nangia et al, 1998) (Kadam et al, 2009) putting them at higher risk of developing osteoporosis especially after menopause. Further, recent data indicate lower age of peak incidence of osteoporosis seen in Indians compared to Western countries (Shatrugna et al, 2005). In most Western countries, while the peak incidence of osteoporosis occurs at about 70-80 years of age, in India it may afflict those 10-20 years younger (at age 50-60 yr). Therefore, Indian women are at higher risk of developing osteoporosis and also at an earlier age. Keeping in view these facts, there is a need to assess bone status of Indian women and identify measures for improving their bone health.

**Frame size:** Extremely thin individuals or those with a small body frame are at greater risk of osteoporosis because they tend to have less bone mass. Low body mass index (BMI) is an indicator of lower BMD (Ravn et al, 1999). Post menopausal women with below average BMI should be considered as being at increased risk of osteoporosis (Omland et al, 2000). Cross-sectional and prospective studies have shown a positive relationship between height, weight and bone mass in children and adolescents, with body weight appearing to be the most important determinant among modifiable factors in this group (Turner et al. 1992; Moro et al. 1996; Boot et al. 1997; Uusi-Rasi et al. 1997). Although in adult males and females, height is not a determinant of bone mass, a significant positive association of height in children and adolescents has been reported, at least up to 15 years of age (Glastre et al. 1990). Similar to weight-bearing activity, added body weight places mechanical stress on bone, resulting in increased bone modelling and possibly greater bone strength.
**Genetics/family history:** The amount of bone mass accrued during lifetime by an individual is controlled to a large extent by the genetic makeup (Baldock and Eisman, 2004). Research suggests that heredity and genetics play a major role in osteoporosis and fractures (Jouanny et al, 1995). Osteoporosis is a polygenic disorder, determined by the effects of several genes, each with relatively modest effects on bone mass and other determinants of fracture risk (Stewart & Ralston, 2000). Thus, an individual with a parent or sibling who has osteoporosis or a history of fractures has increased risk of osteoporotic fractures.

**Gender:** Before puberty, there are no consistent gender related differences in bone mass at any skeletal site (Bonjour et al. 1991) (Kadam et al, 2009). Once pubertal maturation has been reached, the gender differences in bone mass essentially result from a greater bone size increment in male subjects. In boys, the onset of puberty occurs later than in girls and the period of accelerated bone growth lasts for four as compared to three years in girls (Theintz et al, 1992). These two characteristics probably account to a large extent for the gender difference in mean PBM observed in healthy young adults (Bonjour et al, 1994). As a result, peak bone mass tends to be higher in men than in women by almost 12-25% (Joseph & Melton et al, 2001). In our previous study we found 4% lower bone mass in Indian women compared to men at 30 years of age when the peak bone mass is said to be achieved (Kadam et al, 2009). Thus, women are at higher risk of osteoporosis especially due to the years of rapid bone loss associated with menopause.

**Puberty:** Puberty is associated with increased rate of linear growth as well as bone mass accrual. During adolescence, growth hormone as well as sex hormone levels increase and both have a positive influence on BMD (Slootweg, 1993; Albertsson-Wikland et al. 1994). A number of cross-sectional studies in adolescents have shown a positive correlation between pubertal stage and BMD (Lloyd et al. 1996; Lonzer et al. 1996; Boot et al. 1997; Goksen et al, 2006). Research indicates that puberty has a greater influence on BMD in girls than in boys (Boot et al, 1997, Kun et al, 2001). A study by Rubin et al. (1993) in 299 white girls aged 6–18 years showed that up to 80% variation in axial BMD was explained by weight and pubertal stage, with pubertal stage the strongest single predictor. Further, studies indicate that 90% of the adult reference height and 53% of the adult reference total
body bone mineral content (TBBMC) is achieved during the premenarchal period in girls. Of this, as much as 86% of BMD is gained before 14 years of age with the highest rates of mineralisation seen to occur during pubertal stage II and III (Lehtonen-Veromaa et al, 2002; Saggese et al, 2002). Recently, a study reported that early puberty was associated with greater bone mass while later puberty resulted in lower BMD (Gilsanz et al, 2011). Thus, in growing years, premenarchal age with respect to improvement in bone health needs to be explored as this age may provide a window of opportunity for improvement in bone mass accrual and attainment of higher peak bone mass.

**Hormonal influences:** Influence of growth hormone and sex hormone is evident in adolescence, and, oestrogen has known effects on bone mass in adulthood. Oestrogen can decrease the rate of the bone turnover, and inhibit the osteoclastic resorption of bone by affecting bone cell differentiation and function and also has effects on parathyroid hormone and vitamin D metabolism. Thus, lower levels of oestrogen may adversely affect bone mass (Saggese et al. 1997). Thus, women undergoing menopause experience a higher bone loss compared to women who are premenopausal. Examining the effect of endocrine factors on bone health of pre and postmenopausal women, Ito et al. (1995) found that length of reproductive period had the strongest correlation with BMD, but early menarche was also significantly associated with BMD in both groups of Japanese women. This may be because early menarche is associated with earlier onset and longer duration of puberty (Marti-Henneberg & Vizmanos, 1997) providing the opportunity for more bone mass accumulation. Age at menopause, years since menopause, age at first pregnancy, number of pregnancies, and duration of breast feeding are other reproductive factors affecting bone health in women. A study by Ozdemir et al (2005) also found negative correlation between number of parities, number of pregnancies and lower age at first pregnancy. Hence, pubertal stage and other reproductive factors are also important determinants of bone heath that need to be taken into account when addressing bone health in adolescent girls and women.

Another important hormonal factor in bone metabolism is the thyroid hormone. Thyroid hormones are necessary for normal skeletal growth, maturation, basic metabolism, and bone turnover (Allain et al, 1993). Hyperthyroidism is
accompanied by osteoporosis or osteopenia with increased rates of bone formation as well as bone resorption, with predominance of resorption (Akalin et al, 2002; Udayakumar et al, 2006) leading to incidence of fractures (Van de ven & Erdtsieck, 2008). Thus, these facts must be taken into consideration while assessing bone health in apparently healthy individuals.

1.7.1 Lifestyle/Environmental factors:

The genetic potential for peak bone mass can only be reached when the modifiable factors that contribute to bone acquisition are favourable. Though heredity accounts for majority of variance in adult bone health (as described in the previous section), factors like diet, activity, body composition and general health that are modifiable, can greatly influence attainment of peak bone mass as well as maintaining strong bones in later life. Following section deals with each lifestyle factor separately and examines its importance in bone health.

a) Physical activity

Weight bearing physical activity includes mechanical loading and strain on the skeleton, which stimulates bone remodelling and increases the rate of bone mineral accrual (Frost 2003). Early in life, these exercises are known to promote higher peak bone mass. During midlife, exercise provides many bone-related health benefits, although these effects have not been clearly established. Some exercises such as moderate intensity aerobics and walking have been associated with reduced bone loss (Shea et al, 2004), although few studies have directly evaluated their effect on BMD (NIH, 2001). The prepubertal human skeleton is sensitive to the mechanical stimulation elicited by exercise and there is increasing evidence that regular weight-bearing exercise is an effective strategy for enhancing bone mineral throughout growth. Physical activity or participation in sports needs to start at prepubertal ages and be maintained through pubertal development to obtain the maximal peak bone mass achievable. Moderate exercise has been shown to benefit prepubertal boys and is site specific. Moderate exercise for prepubertal girls may be beneficial, but only if the girls do not have a prior history of loading, and the
benefits are believed to be site specific. High intensity exercise seems to be beneficial for both boys and girls pre-pubertally (Burrows, 2007).

Intervention studies in pre- and peripubertal children confirm the findings from cross-sectional studies that high-impact physical activity (Nurmi-Lawton et al, 2004; French et al, 2000; MacKelvie et al, 2003) and regular physical exercise increases BMD (Linden et al, 2006; Gunter et al, 2008). In adults, the effect of physical activity is smaller and less consistent (Morseth et al, 2011). Thus, factors that favour the acquisition of lean body mass (muscle mass) such as physical activity in addition to optimum weight gain during growing years may promote bone health in childhood and adolescence.

A positive relationship between both BMD and current physical activity and physical activity during adolescence has been shown in young Canadian females (18-35 yr) (Rubin et al, 1999) and middle-aged Italian women (Bidoli et al, 1998). Current exercise has been associated with higher bone density in post menopausal English women (Coupland et al, 1999) and in Norwegian women aged 50-75 yr with fractures (Omland et al, 2000). However, a study in Australian population has shown current physical activity being positively associated with BMD but after adjustment for age, BMI, calcium intake and quadriiceps strength, the relationship was not statistically significant (Nguyen et al, 2000). Findings from intervention studies in premenopausal women indicate that young women who exercise continue to increase bone mass compared to non-exercising controls. In postmenopausal women, systematic reviews indicate that physical activity may slow the rate of bone loss on weight-bearing sites with an effect of approximately 1% per year (Wallace & Cumming 2000; Wolff et al, 1999). Consequently, individuals with a sedentary adolescent lifestyle should be considered at higher risk of osteoporosis. Those who currently have a sedentary lifestyle may also be at higher risk.

b) **Tobacco use and excessive alcohol consumption**

Research suggests that tobacco use contributes to weak bones. Cigarette smoking has been shown to lower bone mass causing decreased bone strength and increased incidence of fractures (Johansson & Mellstrom, 1996). In addition, fracture healing also appears to be impaired in smokers (Cobb et al, 1994; Schmitz
et al, 1995). Similarly, alcohol consumption has also been associated with increased risk of fracture (Gomez Navarro, 2011) possibly because alcohol can interfere with the body's ability to absorb calcium.

c) Health conditions and procedures that affect bone health

Bone mass accrual during years of growth is also affected by various illnesses and diseases with low bone mass reported in health conditions like inflammatory bowel disease, celiac disease (Blazina et al, 2010), Crohn’s disease (Hill et al, 2011), anorexia nervosa (Munoz and Argente 2002), congenital hypothyroidism (Demartini et al. 2007), growth hormone deficiency (de Boer et al. 1994) and neuromuscular disorders (Moynahan et al. 1996; Tasdemir et al. 2001; Henderson et al. 2002; Bianchi et al. 2003). Low bone mass in these conditions is a result of combination of risk factors including malnutrition, vitamin D insufficiency, mal-absorption, deficiency or resistance to sex steroids or growth hormone and increased cytokine production (Borges & Brandao, 2006). Immobilization associated with some of the health conditions is also known to affect bone turnover (Biering-Sorensen et al, 1990). In addition, procedures like stomach surgery (gastrectomy) and weight-loss surgery can also affect the body's ability to absorb calcium. Therefore, in the present study, while selecting individuals, such specific diseases or health conditions were excluded as the reasons for low bone mass would be these health disorders rather than lifestyle factors.

d) Use of certain medications

Long-term use of corticosteroid medications, such as prednisone, cortisone, prednisolone and dexamethasone has been shown to adversely affect bone growth and density in children and adults (Weldon, 2009). Other drugs associated with an increased risk of osteoporosis include long-term use of aromatase inhibitors to treat breast cancer, antidepressant medications such as selective serotonin reuptake inhibitors (SSRIs), cancer treatment drugs like methotrexate, some anti-seizure medications, acid-blocking drugs such as proton pump inhibitors and aluminum-containing antacids. Therefore, use of certain medications, was considered among the exclusion criteria while selection of subjects in the present study.
Diet and Nutrition

Nutrition is one of the important modifiable factors in the development and maintenance of bone mass and the prevention and treatment of osteoporosis (New et al, 2000). About 90% of total adult bone mass is accrued by age 20, and a significant proportion of this (40%) is achieved during puberty alone (Cashman 2002). Thus, gaining an understanding of the role of dietary components in bone metabolism and bone mass in these early life stages is important for developing new strategies to maximize the accretion of bone during growth which may help reduce the risk of osteoporosis in later life.

The strength of the bones depends on size and density; bone density depends on the amount of calcium, phosphorous and other minerals that bones contain. When the bones contain fewer minerals than normal, they are less strong and eventually lose their internal supporting structure. Variations in the dietary intakes of nutrients, especially calcium and vitamin D, impact the integrity of the bone through alterations in nutrient homeostasis in the body. Bone is a storage site for several minerals within the body (Broadus, 1996) and dietary intakes of nutrients are required for synthesis and function of enzymes (Czajka-Narins, 1992), hormones (Seelig et al, 1993; Zofkova & Kancheva, 1995), and bone cells (Czajka-Narins, 1992), all of which are necessary for maintenance of bone metabolism. These dietary factors range from inorganic minerals (e.g., calcium, magnesium, phosphorus, zinc and various trace elements) and vitamins (vitamins A, D, E, K, C, and certain B vitamins), to macronutrients, such as protein and fatty acids (Cashman 2007). Although an abundance of research has focused on the relationship between calcium and BMD, dietary factors important to bone metabolism are not limited to calcium. Several other nutrients affect bone directly or through effects on calcium economy (New et al. 2000, Rubin et al. 1999). However, role of these micronutrients during the critical periods of high bone turnover is not clearly understood and studies in adolescents are scarce (Weaver et al, 1999).

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. 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. 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 and women. Table 1.1 summarises the new recommended intakes of nutrients for Indian girls (7-12 years) and women.

Table 1.1: Recommended dietary allowance (RDA) for Indian females

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>7-9 years</th>
<th>10-12 years</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1690</td>
<td>2010</td>
<td>2230</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>29.5</td>
<td>40.4</td>
<td>60</td>
</tr>
<tr>
<td>Visible Fat (g/d)</td>
<td>30</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>600</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>Phosphorous (mg/d)</td>
<td>600</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>16</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>β-carotene (µg/d)</td>
<td>4800</td>
<td>4800</td>
<td>4800</td>
</tr>
<tr>
<td>Thiamine (mg/d)</td>
<td>0.8</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Riboflavin (mg/d)</td>
<td>1.0</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Niacin equivalent (mg/d)</td>
<td>13</td>
<td>13</td>
<td>1.8</td>
</tr>
<tr>
<td>Ascorbic acid (mg/d)</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Dietary folate (mg/d)</td>
<td>120</td>
<td>140</td>
<td>200</td>
</tr>
<tr>
<td>Vitamin B12 (µg/d)</td>
<td>0.2-1.0</td>
<td>0.2-1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

i) **Energy and protein**

Increased energy intake favours weight gain and higher BMD. The strong positive effect of excess weight on BMD may be due to weight-bearing forces exerted on the skeleton as described in the previous section. Likewise, moderate weight loss of 10% typically results in 1% to 2% bone loss (Hyldstrup et al, 1993; Syendsen et al, 1993; Compston et al, 1992). More severe weight loss or conditions of malnutrition are considered a risk factor for osteoporosis, and there could be many contributing factors: low macronutrient intake (including protein), low micronutrient intake (including Calcium and vitamin D), increased propensity to fall due to poor muscle strength and less protective soft padding on the hip region (Bonjour et al, 1996). On the other hand, over-nutrition may also influence bone formation as overweight children have an increased predisposition to reduced BMD and fracture (Goulding et al, 2000, Goulding et al, 2001). There is increasing evidence that the hormone leptin, secreted by adipocytes or fat tissue, inhibits bone formation (Ducy et al, 2000). Thus, adequate and optimum energy intake is essential to maintain optimum bone health.

Bone matrix contains collagen, elastin, and other proteins which comprise most of the non-mineral composition of bone, and an adequate protein intake is necessary for the synthesis of bone matrix. Insufficient protein intake is detrimental both for the acquisition of bone mass during childhood and adolescents (as bone turnover requires continuous ingestion of new protein) and for the preservation of bone mass with ageing (Weaver, 1999, Holick, 2004). In the Framingham prospective cohort study, elderly men and women with lower total and animal protein intakes had greater rates of hip and spine bone loss than subjects consuming higher amounts of protein (Hannan et al, 2000). However, studies have also indicated that excess protein intake may increase urinary calcium excretion resulting from an increase in calcium resorption especially when calcium intake is low (Heaney 2002). Hence, adequate protein intake along with calcium is essential for optimum bone health.
ii) **Calcium and bone**

Calcium is the most abundant mineral in the body. The adult human body contains about 1000 to 1500 g of calcium (depending on gender, race, and size of the body) of which 99% is found in the bones in the form of hydroxyapatite while the remaining 1% is equally distributed between the teeth and soft tissues. Only 0.1% of calcium is found in the extracellular fluid (ECF). The ECF contains ionized calcium at concentrations of about 4.8mg/100ml (1.20 mmol/l) maintained by the parathyroid–vitamin D system.

**Biological role of calcium**

Calcium salts provide rigidity to the skeleton and calcium ions play a role in many, if not most, metabolic processes. In the human skeleton, rigidity is provided by a form of calcium phosphate in the form of hydroxyapatite [Ca10(OH)2(PO4)6] and is embedded in collagen fibrils. Bone mineral serves as the ultimate reservoir for the calcium circulating in the ECF. Calcium enters the ECF from the gastrointestinal tract by absorption and from bone by resorption. Calcium leaves the ECF via the gastrointestinal tract, kidneys, and skin and enters bone via bone formation. It has two key roles: (1) supporting structural integrity; (2) regulating metabolic function. Calcium is essential for: cellular structure, intercellular and intracellular metabolic function, signal transmission, muscle contractions including heart muscle, nerve function, and activities of enzymes and normal clotting of blood (Lanham-New SA 2008). There is no functional marker of calcium status, since its role in normal blood clotting takes priority and hence plasma calcium is maintained within very narrow limits (Royal College of Physicians 2000).

**Calcium homeostasis**

Figure 1.7 summarizes the hormonal control of calcium homeostasis. The physiology of calcium metabolism is primarily directed towards the maintenance of of ionized calcium concentration in the ECF. The mineralization or deposition of calcium in bone occurs in response to a high level of calcium in the blood via calcitonin. On the other hand, parathyroid hormone (PTH) is involved in the release of calcium from bone in response to low levels of blood calcium. Calcitriol, or the active form of Vitamin D, also plays an important role in calcium homeostasis with
a net action of maintaining bone integrity, thereby preventing osteoporosis (Holick 1996). Active absorption of calcium requires vitamin D (Pansu et al, 1983). With insufficient calcitriol (either from low dietary intake or reduced subcutaneous conversion), intestinal absorption of calcium is reduced. A reduction in calcium absorption stimulates PTH release so that a normal serum calcium concentration, through osteoclastic bone resorption, is maintained (McSheehy and Chambers 1986). In healthy individuals, this circle of calcium homeostasis continues and adjusts as needed to fluctuations in nutrient intakes and blood calcium concentration changes. Thus a diet low in calcium contributes to diminished bone density, early bone loss and an increased risk of fractures.

Figure 1.7: Hormonal control of Calcium homeostasis

![Figure 1.7: Hormonal control of Calcium homeostasis](http://students.cis.uab.edu/elo/finalproject.html)

Calcium requirements are determined from calcium balance studies in healthy adults and using factorial method to estimate calcium intake needed to maintain calcium adequacy in children and adolescents. Information available from clinical trials in which additional calcium was given and then changes in bone
mineral content were measured over time, are used for confirming the requirements in children. In adults, the rate of calcium absorption from the gastrointestinal tract should match the rate of losses from the body. In children, positive calcium balance (i.e., net calcium retention) is required especially during the first two years of life and during adolescent growth spurt. Calcium requirements increase with menopause. Low oestrogen levels lead to an increase in bone resorption, a decrease in the efficiency of intestinal calcium absorption, and a decrease in renal conservation of calcium. By age 65, calcium absorption is 50% of adolescent absorption levels (Straub 2007). Thus, postmenopausal women also require higher calcium intake to maintain bone health. Based on the observations that the body can adapt to different levels of intakes of calcium and maintain a positive calcium balance even at 400 mg/d dietary calcium intake, the previous RDA (1989) suggested a lower level of calcium intake. However recently, based on the evidence from calcium nutrition status of the Indian population, the requirement of calcium for all age groups has been increased. However, the recised Indian recommendations are still lower than the recommendations by FAO/WHO (Table 1.2) even with respect to recommendation given for postmenopausal women.

Table 1.2: Recommendations for Dietary Calcium (mg/d) in female children, adolescents and adults

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>ICMR, 1989</th>
<th>ICMR, 2010</th>
<th>FAO/WHO, 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-9</td>
<td>400</td>
<td>600</td>
<td>700</td>
</tr>
<tr>
<td>10-12</td>
<td>600</td>
<td>800</td>
<td>1300</td>
</tr>
<tr>
<td>19 to menopause *</td>
<td>400</td>
<td>600</td>
<td>1000</td>
</tr>
<tr>
<td>Post menopause</td>
<td>400</td>
<td>800</td>
<td>1300</td>
</tr>
</tbody>
</table>

* Non pregnant and non-lactating

Source: Indian Council of Medical Research: Nutrient requirements and Recommended dietary allowances for Indians, A Report of the Expert Group of Indian Council of Medical Research, 1990
Calcium intake and peak bone mass development

Amongst various nutritional factors, adequate calcium intake is one of the major lifestyle factor believed to have a positive effect on bone accretion, within the limits of genetic potential (Rozen et al, 2003). As 40% of the mineral found in bone is calcium, dietary intake of calcium has a strong association with BMD. A high intake of milk during childhood and adolescence has been associated with increased bone mass at maturity (Cadogan et al, 1997). The amount of dietary calcium consumption exerts a positive dose dependent effect on spinal bone mass in young women (Recker et al, 1992) which is consistent with the known anabolic effect of calcium on the growing skeleton. Peak bone mass can be maximized by raising calcium intake as out of 86 observational epidemiological studies, 64 showed that higher calcium intakes were associated with higher bone mass in children, young adults and postmenopausal women (Heaney, 2000). This is strengthened by the observation that calcium deficiencies early in life can account for as much as 5-10% differences in adult peak bone mass. Such a difference, although small, could potentially contribute to more than 50% to hip fracture rates later in life (Matkovic, 1979; Matkovic, 1995; Stetzer 2011).

Lowest calcium intakes have been reported in developing countries, particularly in Asia (FAO/WHO, 2001). In Indian adolescents girls as well as adult women especially from low income groups, calcium intakes with respect to consumption of dairy foods especially milk and milk products are low (Marwaha et al, 2005; Puri et al, 2008; Harinarayan et al, 2007, Chiplonkar & Tupe, 2010). This indicates a large gap in the requirement and the actual intake of calcium which may lead to achievement of lower peak bone mass in adolescents and inability to make up for the bone loss in women during menopausal years. Further, studies indicate that both calcium absorption and bone calcium deposition rates peak in girls shortly before menarche (Langman 2005). Retrospective studies in postmenopausal women indicate that bone density is associated with childhood and adolescent milk consumption (Sandler et al, 1985). Thus, steps to improve calcium nutrition especially during pubertal years may result in achievement of higher peak bone mass and reduce the risk of future osteoporosis.
iii) Vitamin D and bone

Vitamin D is an essential dietary factor necessary for normal mineralization of osteoid tissue. Vitamin D assists in maintenance of serum calcium concentration and prevention of bone mineral disorders like rickets and osteomalacia (Holicks 1996). Expression of both calbindin (the intracellular calcium binding protein) and the plasma membrane calcium pump are dependent on vitamin D (Johnson & Kumar, 1994). Vitamin D is a pro-hormone responsible for regulating and maintaining intra and extra-cellular calcium levels. It also stimulates bone matrix formation and bone maturation. The Provitamin D3 (25 hydroxy vitamin D) which is the storage form of Vitamin D, located in the skin undergoes conversion to pre-vitamin D in the presence of solar ultraviolet-B (UV-B) photons during sunlight exposure. Pre-vitamin D is then converted to active vitamin D (Figure 1.8).

Figure 1.8: Cutaneous production of Vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth

The vitamin D endocrine system influences calcium and phosphorous metabolism by affecting the target organs: intestine, bone and kidney. Calcium absorption occurs in the small intestine by both active transport and passive diffusion. Normally, the small intestine absorbs 30% of dietary calcium which increases up to 80% during periods of growth, pregnancy, and lactation (Holick 2004). Passive diffusion only occurs with high calcium intake, when the proteins of the active transport mechanism become saturated (Heaney & Weaver, 2003). The biologically active form of vitamin D, 1,25-dihydroxyvitamin D \( (1,25[\text{OH}]2\text{D}3) \), facilitates active transport by increasing calcium uptake at the intestinal mucosal cell’s brush border. Without vitamin D, only 10 to 15% of ingested calcium would be absorbed (Holick 2004). Further, low vitamin D status results in increasing parathyroid hormone (PTH), which in turn mobilizes the necessary amount of calcium from bone. Therefore, a low level of vitamin D results in secondary hyperparathyroidism, leading to increased bone turnover and a subsequent decrease in bone mineral accretion (Lips 2004; McKenna & Freaney 1998; Khaw & Sneyd 1992). Thus, individuals with vitamin D insufficiency may suffer the effects of poor bone mineralization, even with a diet adequate in calcium (Heaney 2002). Recent studies have reported vitamin D deficiency in diverse populations (Shaw & Pal, 2002, Harinarayan et al, 2007) and the need for addressing this issue as a public health problem (Davies et al, 2005, Harinarayan et al, 2011).

**Sources and indicators of Vitamin D status**

The two main sources of vitamin D are through diet and through the skin by exposure to sunlight. Dietary sources of vitamin D are animal foods such as fish, cod liver oil, and dairy products. Due to lack of accurate food composition data for Vitamin D, it is difficult to estimate the dietary intakes of the population. Moreover in vegetarians dietary vitamin D intake is almost meagre unless vitamin D-fortified foods are consumed. Skin synthesis of Vitamin D is also difficult to estimate as it is affected by a variety of factors like age, season, latitude, time of day and skin exposure.

Although 1,25 di-hydroxy vitamin D3 \([1,25(\text{OH})_2\text{D}_3]\) is the most biologically active form of vitamin D, its half-life in circulation of only four to six hours makes it a poor marker of vitamin D status (Gutin et al, 1996). The storage
form of vitamin D, 25 hydroxy vitamin D [25(OH) D], serves as a more valuable index, with a half life of 10 days to three weeks. The measurement of serum or plasma 25 OH-D combines dietary vitamin D with vitamin D synthesized in the skin, serving as a useful assessment of vitamin D status (Hollis 1996). Therefore, many studies have used serum 25 OH-D as a measure of vitamin D status and there is a strong presumptive relationship of 25OH-D with bone status. The definition of the normal range of serum 25 OH-D for adults is a controversial issue, with additional difficulties for children and adolescents. Thus, there are various definitions of vitamin D deficiency, insufficiency and sufficiency in the literature. Recent literature suggests that in adolescents, serum 25 OH-D below 50 nmol/l may be considered the cut-off point for vitamin D deficiency, and between 50-75 nmol may be considered the range for vitamin D insufficiency (Saintonge et al, 2009; Calatayud et al, 2009). However, the most widely used cut-offs are-> 50 nmol/l for normal, 25-50 nmol/l for insufficiency or mild vitamin D deficiency; 12.5-25 nmol/l for moderate deficiency; below 12.5 nmol/l for severe deficiency (Lips 2001, Munns 2006).

Vitamin D and its association with peak bone mass development in girls and fracture risk in women

Since Vitamin D is involved in bone turnover, its deficiency may cause rickets in children (characterized by defective mineralization of bone). There is growing evidence that mild vitamin D insufficiency can also have a detrimental effect on bone mineral mass in adolescent females (Outila et al, 2001) and children (Cheng et al, 2003). The lumbar spine BMD was seen to be 27% higher for subjects in the highest tertile of vitamin D intake compared with those in the lowest tertile (Lehtonen-Veromaa et al, 2002). In a study by Illich et al (1997) baseline calcitriol levels were shown to predict annual change in total body and forearm bone mass of adolescent girls. Also, calcitriol concentration was seen to be highest during peak growth (pubertal stages 3 and 4), probably due to the high skeletal demands for calcium. In another 3-year prospective cohort study in healthy Finnish girls (9–15 years) comparing subjects with severe hypovitaminosis D (25 OHD <20 nmol/l) with those with normal vitamin D status (25 OHD >= 37.5 nmol/l) there was a
difference of 4% in BMD accrual between the two groups (Cheng et al, 2003). Thus, low vitamin D status is a risk factor for achieving peak bone mass (El-Hajj Fuleihan et al, 2001). Studies in Indian adolescent girls have reported high prevalence of vitamin D deficiencies (63%) despite abundant sunlight exposure (Harinarayan et al, 2008) which may further put them at risk for low bone mass. Thus, in addition to improvement in calcium intakes, steps need to be taken to improve vitamin D status during pubertal years to maximize the effect of dietary calcium in the achievement of peak bone mass.

Vitamin D deficiency is also an important risk factor for osteoporosis. Studies have established a relationship between low circulating levels of 25 OH-D and increased secretion of parathyroid hormone which in turn, induces bone loss through increased bone resorption (Khaw et al, 1992). Adequate vitamin D status has been shown to prevent osteoporotic fractures (Chaupuy, 1992). Few Indian studies in post menopausal women indicate a high prevalence of Vitamin D deficiency in adult women (Paul et al, 2008; Harinarayan et al, 2005) indicating the need to assess and improve Vitamin D status in conjugation with bone health status in Indian women.

iv) Zinc and bone

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2g). Skeletal muscle accounts for approximately 60% of the total body zinc content and bone mass, with a zinc concentration of 1.5-3 mmol/g (100-200 mg/g), for approximately 30%. Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and thus contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression.
Role of zinc in bone health

Zinc plays an important role in connective tissue metabolism, acting as a cofactor for several enzymes, such as alkaline phosphatase (necessary for bone mineralization), and collagenase (essential for the development of the collagenous structure of bone) (Beattie, 1992). Zinc deficiency results in impaired DNA synthesis and protein metabolism, which lead to negative effects on bone formation (Beattie, 1992). Bone zinc content is known to decrease with aging and postmenopausal conditions. Low serum levels of zinc and excessive urinary excretion are related to osteoporosis in humans (Herzberg et al, 1990; Atik, 1983). With respect to dietary zinc, higher intakes have been associated with higher lumbar spine BMD in premenopausal women (New et al, 1997) while higher zinc intakes along with energy, protein, calcium, phosphorous and folate have also shown to lower bone loss in women of 35 to 65 yr of age (Freudenheim et al, 1986).

Recent studies indicate that zinc suppresses osteoclast differentiation and promotes osteoblast mineralization in vitro and influences growth and metabolism in growing rats (Yamaguchi & Weitzmann, 2011; Sun et al, 2011). Moreover, zinc inhibits osteoclastic bone resorption by inhibiting osteoclast-like cell formation from marrow cells (Yamaguchi et al, 1998). Studies have also reported effect of zinc on growth potential (Ruz et al, 1997, Brown et al, 2002) but studies examining its effect on bone mineral density are scarce. Thus, the role of zinc in bone development is worth exploring. Considering that recent studies have reported a high prevalence of zinc deficiency (48-74%) in Indian adolescent girls ((Tupe & Chiplonkar et al, 2009; Kapil et al, 2011), there is a need to improve the zinc status and study its role in bone mass accrual during adolescent years.

Requirement and recommended zinc intake

Low levels of zinc can contribute to poor bone formation. Zinc deficiency can lead to impaired DNA synthesis and protein metabolism, both of which can impact bone formation. A study reported that among women with osteoporosis, most had high levels of zinc excretion through the urine. To achieve adequate zinc status, dietary zinc intakes should be optimised to fulfil the physiological requirements. Estimates of zinc requirements are mostly obtained from data using chemical
balance methods or from turnover studies employing radio or stable isotopes in adults. Factorial approach is used to extend the requirements to other age groups. Indian dietary intakes of zinc have been reported to be low (Narasinga & Nageswara, 1980; Tupe & Chiplonkar, 2009). Further, it is well known that intestinal absorption of zinc is markedly inhibited by the phytate and tannin content of diet. Habitual Indian vegetarian (mixed cereal/ pulse) diets are rich in phytate and thus the bio-availability of zinc is found to be poor (Shah & Sachdev 2000, Lonnerdal, 2000, Agte et al, 1995). Considering the diet pattern and the bioavailability of Indian diets, the latest revision of Indian RDA has given the following recommended intake for zinc (Table 1.3).

Amongst dietary sources, zinc is abundant in animal protein foods (red meat, poultry, fish, oysters, eggs), legumes, whole-grain breads and milk. However, the population that may be susceptible to a mild to moderate zinc deficiency are infants and adolescents, due to increased requirements for growth and, in the case of latter, poor eating habits (Skinner et al, 1997; Donovan et al, 1995).

Table 1.3: Recommendations for Dietary zinc (mg/d) in female children, adolescents and adults

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>ICMR, 2010</th>
<th>FAO/WHO, 2004&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-9</td>
<td>8</td>
<td>3.3-11.2</td>
</tr>
<tr>
<td>10-12</td>
<td>9</td>
<td>4.3-14.4</td>
</tr>
<tr>
<td>Adult women*</td>
<td>10</td>
<td>3.0-9.8</td>
</tr>
</tbody>
</table>

* Non pregnant and non-lactating

<sup>a</sup> Based on zinc bioavailability in diet with high bioavailability requiring lower intakes

v) **Other minerals and Vitamins and their association with bone health**

Along with calcium, phosphorous is an essential component of hydroxyapatite. As an inorganic element, phosphorus is second to calcium in abundance in the human body with 85% of the body’s phosphorus bound to the skeleton. Phosphorus is widely distributed in foods including meat, poultry, fish, eggs, dairy products, nuts, legumes, cereals, grains and cola beverages. It is also added in processed foods. The primary purpose of dietary phosphorous is to support growth and to replace losses. Phosphorous deficiency is unlikely to occur in the types of diets consumed in India, ensuring an adequate intake. It has been suggested that an elemental Ca:P ratio of 1:1 should be maintained in most age groups. Thus based on this, the new Indian RDA has revised the recommended intakes of phosphorous in light of the increased recommendation of calcium (Table 1.1).

In addition to calcium, phosphorous and vitamin D, there is increasing recognition of the role of other vitamins and minerals on adult bone health. Ascorbic acid (Vitamin C) is necessary for hydroxylation of protein, and copper is a cofactor for lysyl oxidase (an enzyme required in forming collagen cross-links). Low intakes of vitamin C have been associated with a faster rate of BMD loss in adults (Hall & Greendale, 1998; Sahni et al, 2008). Vitamin K is a cofactor of γ-carboxylase, an enzyme necessary for the γ-carboxylation of glutamic acid residues in proteins, including osteocalcin, the principal non-collagenous protein of bone. Plant protein, calcium, phosphorous, iron, thiamin, riboflavin and vitamin C intakes were related with BMD in all age groups of men (Yu et al, 2004). B complex vitamins have association with bone density through their effect on lowering homocysteine levels which has been associated with lower BMD and increased hip fracture risk in elderly (McLean et al, 2004; Morris et al, 2005). Homocysteine levels in the blood may also rise if there is inadequate intake of vitamin B6, vitamin B12 and folic acid.

However, the role of these micronutrients in bone health of children and adolescents’ is unclear and thus needs to be explored.
1.8 Adolescence: An opportune time for nutritional intervention to improve bone health

Adolescence is considered as a nutritionally critical period of life for several reasons. Firstly, the dramatic increase in physical growth and development puts greater pressure on the need for nutrients. Ninety percent of the adult reference height and 53% of the adult reference total body bone mineral content (TBBMC) are achieved during the premenarchal period in girls, with as much as 86% of BMD gained before 14 years of age (Lloyd et al, 1992; Bailey, 1997). Secondly, there may be socio-cultural factors or change of lifestyle and food habits of adolescents that can affect both nutrient intake and needs (Spear, 2002). For example, gender discrimination plays an important role in intra-household distribution of food allocation as described in previous sections. Thirdly, adolescence can be the second opportunity to catch up growth if environmental conditions, especially in terms of nutrient intake are favourable (Gopalan, 1989). Thus, adolescence provides a window of opportunity to improve bone health through nutritional intervention since under-nutrition during growing years can result in reduced bone density later in life (WHO, 1995).

1.9 Strategies to improve peak bone mass

Peak bone mass is influenced to a large extent by nutrition especially in terms of calcium and vitamin D. Pubertal years are associated with rapid bone mass accrual making these years a likely target for any intervention for improving bone health. Considering the high prevalence of nutritional deficiencies seen during this period especially in children from low income groups, school based intervention is one of the strategies that can be adopted. This intervention can target populations where requirement of nutrients is otherwise difficult to achieve through normal diets (Sivakumar, 2006). Calcium intervention studies in children and adolescents have shown that daily supplementation through either food-based or calcium tablet supplements can produce significant increase in the percentage gain of bone mass (French et al, 2000). The common forms of calcium supplement used are calcium carbonate, food based supplements (milk extract and other dairy products)
or calcium-citrate malate. Considering the feasibility of supplementation in a school set-up, tablets in the form of calcium carbonate (which is one of the highly concentrated forms of calcium and is also most widely used) is a suitable option adopted by many supplementation trials in the past (Rozen et al, 2003; Stear et al, 2003; Cameron et al, 2003).

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 (Cameron et al, 2004, Courteix et al, 2005, Johnston et al, 1992, Lloyd et al, 1993, Matkovic et al, 2005, Molgaard et al, 2004). The dosage of supplementation in these studies ranged from 300-1200mg/d. The few studies that have investigated the effect of calcium supplementation in girls with habitually low intakes of calcium, have supplemented enough calcium in addition to the dietary intake to meet the required RDA (Lee et al, 1994) resulting in increase in BMD. In a recent study by Yin et al (2010) in Chinese adolescents (12-15 years) with low dietary intake of calcium (380±163 mg/d), a dosage of 230mg/d and 500mg/d of calcium (in the form of calcium carbonate) showed significant improvement in bone mineral accretion. Also, it has been established that calcium intake above the threshold does not provide any additional benefit. Thus, based on the Indian RDA, 500mg/d of calcium supplement in addition to the dietary calcium may be sufficient to show positive results in Indian adolescents as calcium absorption is known to be maximum during this period.

To observe the effect of supplementation on bone mass accrual requires some time period. Most of the calcium supplementation trials have shown positive effect of 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). Longer supplementation trials range from 2-3 years (Lloyd et al, 1993; Cameron et al, 2004; Johnston et al, 1992) to 7 years (Matkovic et al, 2004). Rozen et al (2003) in their calcium intervention trial for 1 year showed that at 6 months follow-up there were no significant differences between the calcium (2.23%) and placebo (1.73%) group (p>0.1) while at 12 months follow-up calcium group showed significantly higher TBBMD (3.8%) than the placebo group (3.07%). Similarly Molgaard et al (2004) also showed significantly higher TBBMD after 12
months of supplementation. This indicates that 12 months may be a sufficient period to see any effect of supplementation on bone mass accrual.

A review by Winzenberg et al. (2006) on the effect of calcium supplementation on bone mass accrual in children indicated gaps in certain areas of research. For example, though it is known that calcium accumulation in the skeleton accelerates during puberty, there is absence of supplementation data in the peripubertal period which need further research (Abrams 1996; Bonjour 1991). Other gaps observed were related to ethnicity with relatively few studies in non-Caucasian populations. Thus calcium supplementation trials to study the effect on bone mass accrual in premenarchal Indian girls are warranted.

Though both calcium and vitamin D are recognised for their importance in bone health, few studies have explored the combined effect of supplementing both (Cheng et al, 2005; Moyer-Mileur et al, 2003). In a study on 212 adolescent girls who were calcium replete (mean age 11.4 years), bone mineral augmentation at the femur was found to be 14.3 and 17.2% higher in the groups receiving the vitamin D supplementation for a period of 1 year (at either 5 mg/d or 10 mg/d) in comparison with the placebo group (Viljakainen et al, 2006). However, similar studies in girls with habitual low calcium intake and high prevalence of vitamin D deficiency to see the combined effect of calcium and vitamin D supplementation on maximising peak bone mass attainment are lacking (Lanham-New, 2008).

Further, though calcium and vitamin D are most widely studied nutrients with respect to bone health, latest research has established a positive association between micronutrients like zinc, ascorbic acid and vitamin B12 with bone health (Freudenheim et al, 1986; Hyun et al, 2004; Hall & Greendale, 1998; Tucker et al, 2004). Studies in adults have indicated beneficial effect of Ascorbic acid supplementation in preventing or aiding the treatment of age-related osteoporosis (Ruiz-Ramos et al, 2011) while normal level of vitamin B12 have been related to a decrease of femur neck bone loss in older men (Naharci et al, 2010). However, similar studies to see the effect on bone mass accrual of adolescents are lacking. Zinc supplementation has been shown to have restorative effect on bone loss under various pathophysiological conditions including aging, calcium- and vitamin D-deficiency, oestrogen deficiency and fracture healing thus suggesting that zinc
compounds may be designed as new supplementation factor in the prevention and therapy of osteoporosis (Yamaguchi 2010). Supplementation studies have also indicated the positive effect of zinc on bone health of postmenopausal women with habitual low intakes of zinc (Nielsen et al, 2011). As zinc is also known to have beneficial effect on growth, it is worthwhile to study effect of zinc supplementation along with calcium and vitamin D on bone mass.

The effect of supplementing multiple micronutrients on bone mass accrual giving due consideration to the interaction between these nutrients, has not been explored. Most of the studies on zinc supplementation address the effect of zinc in improving growth parameters in children (Friis et al, 1997; Ebrahimi et al, 2006) and reducing morbidity especially in children below 5 years of age (Larson et al, 2008), with scarcely any studies to explore its effect on bone mass accrual during pubertal years. The studies that have reported the effect of micronutrient supplementation (containing zinc as one of the constituents) on bone health are in healthy children (Shatrugna et al, 2006). This study in healthy residential Indian school children sufficient in their macronutrient intakes showed significantly higher total body bone mineral content and total body bone area in the supplemented group compared to the control group after 14-months of supplementation with micronutrient fortified drink (containing 400mg of calcium, only 2.3 mg zinc along with iron and both fat and water soluble vitamins in a single preparation) (Shatrugna et al, 2006). However, many of these nutrients show interactions and may lead to lower absorption. For example, calcium is known to reduce the absorption of simultaneously ingested zinc (Lowe et al, 2002) and iron in the diet (Hallberg 1998). Also, a study by Wang et al (2003) in Chinese 8-12 year old children demonstrated a positive effect of multi-micronutrient supplementation on BMD and BMC of mid ulna. A study in 16-36 year old Bangladeshi women has also demonstrated an increase of 1.5%-2.2% in femoral neck BMD post supplementation with micronutrients (containing 600mg calcium and 15 mg zinc along with other vitamins and minerals) along with calcium and vitamin D supplementation (Islam et al, 2010). However, studies in young adolescents especially who are at increased risk of low bone mass need to be undertaken to explore the effect of micronutrient supplementation on their bone health.
Thus, supervised supplementation programmes during premenarchal period may provide an opportunity for substantial contribution towards the achievement of peak bone mass in children from low income groups having low intakes of calcium and micronutrients.

To summarize, the literature review suggests that,

- Women are at a higher risk of developing osteoporosis than men as a result of low bone density compared to men and accelerated bone loss especially during menopausal years. Considering the ethnicity and gender discrimination in a country like India, Indian women are at greater risk.
- The two main determinants of bone health are: peak bone mass attained during early adulthood and rate of subsequent loss during menopause. Puberty is a crucial period for bone mass accrual especially the premenarchal years in girls. Therefore, attainment of adequate peak bone mass during adolescence and early adulthood may provide protection against bone loss related osteoporosis in later years of life.
- In order to formulate strategies to improve bone health during these phases of rapid bone turnover, there is a need to identify the influence of various factors on bone health during premenarchal and postmenopausal phases.
- Nutrition is one of the important modifiable factors known to affect bone health especially during critical years of bone mass accumulation. However, widespread nutritional deficiencies of both macro as well as micronutrients have been reported in Indian children and adolescent girls.

Therefore, the specific aims and objectives of the present research are:

**Aim:** To study the nutritional aspects of bone health in premenarchal girls and postmenopausal women and examine the effect of calcium, vitamin D, zinc and multivitamin supplementation on bone health of premenarchal girls.

**Objectives:**

1. To assess role of anthropometric, nutritional and other lifestyle factors in bone mineral accrual in a cross sectional cohort of premenarchal girls.
2. To investigate the effect of calcium along with multivitamin and zinc supplementation on bone mineral content, bone area and bone mineral density in premenarchal girls.

3. To evaluate relative importance of anthropometric, nutritional and other lifestyle factors in bone loss in a cross sectional cohort of post-menopausal women.

During the course of the present work, it has been possible to publish a major part of results in terms of 5 articles in international peer-reviewed journals and few more articles are in the process of publication. The thesis is presented in the form of 6 chapters. In addition, there is a chapter on summary and conclusions of the entire study along with the implications and scope for future research.