INTRODUCTION

Bones provide structural support for muscles, protect vital organs, and store the calcium essential for body processes, bone density and strength. Human body continually removes and replaces small amounts of calcium from its bones. If the body removes more calcium than it replaces, the bones become weak and have a greater chance of fracturing. Osteoporosis is a metabolic bone disorder characterized by low bone mass and micro-architectural deterioration, with a subsequent increase in bone fragility and susceptibility to fracture. The most typical sites of fractures related to osteoporosis are the hip, spine, wrist and ribs, although the disease can affect any bone in the body. Areal bone mineral density (BMD), the amount of mineral per cubic centimetre of bones, is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk.

The foundation of bone health is laid during childhood and adolescence and acquisition of optimal bone mass during growing years is vital for reducing the future risk of fractures and osteoporosis. Adequate bone growth is influenced by various factors with genetic setup having a major role in bone mass accrual. Vitamin D receptor gene (VDR) polymorphisms are shown to be associated with BMD (Morrison et al. 1994; Arabi et al. 2010; Abrams et al. 2005). Ethnic differences are also reported in bone density; black children and adolescents have higher bone mineral content and density than white Caucasians of comparable age and body weight (Wang et al. 1999) and Asians have lower bone mineral density than white Caucasians (Boot et al. 1997). Lifestyle factors such as physical activity and nutrition are the important determinants of a child’s bone development (Bacciottini et al. 2004; Ilich & Kerstetter, 2000). Calcium is the most important mineral affecting bone mass accrual (Cadogan et al. 1997; Lytle 2002). Dietary calcium intake has been reported to be low in Indian children and adolescents with low bioavailability (Puri et al. 2008; Sanwalka et al. 2010). Therefore, there is a need to increase absorbable calcium intake in Indian diets.

Assessment of bone status during childhood years is important to monitor adequacy of bone mass accrual. This requires appropriate ethnicity-matched reference standard to judge the bone status (Nelson et al. 1997; Bachrach et al. 1999).
1.1: Importance of Measuring Bone Mineral Density in Childhood and Adolescence

Bone or osseous tissue can be defined as a special form of connective tissue with a collagen framework impregnated with calcium and phosphate salts, particularly hydroxyapatites (Ganong 2003). At least 26% of the adult bone mineral is acquired during the 4 year period surrounding peak height velocity, with up to 60% acquired in the remaining peripubertal years (Loud and Gordon 2006). Proper acquisition of bone mass till adulthood is crucial for avoiding risk of osteoporotic fractures. Various paediatric diseases such as osteogenesis imperfecta, chronic disorders (inflammatory diseases, neuromuscular disorders, long-term immobilization after injuries, celiac disease) or pharmaceutical interventions (glucocorticoid therapy) may also result in fragility fractures (Antoniazzi et al. 2011; Mager et al. 2011; Hill et al. 2011; Lims et al. 2011; Tasdemir et al. 2001; Bianchi et al. 2003; Munoz and Argente 2002; Shouman et al. 2010). Bone mass measurement identifies bone mass, detects bone loss, or determines bone quality. Thus, it is important to assess bone mineral density during childhood and adolescence.

1.1.1: Assessment of Bone Status in Children

Various non-invasive techniques such as photon absorptiometry, dual photon absorptiometry, single X-ray absorptiometry, dual energy X-ray absorptiometry (DXA), peripheral quantitative computerised tomography and quantitative ultrasound have been developed and used over the years to assess bone mineral density. Of these, DXA is one of the most preferred methods for assessment of bone mineral status in children due to higher precision, shorter scanning time, low radiation dose, and improved calibration stability in the clinical environment (Blake and Fogelman 1997). BMD can be measured at the wrist, spine, hip, or calcaneum (heel) by DXA. According to a recent Position statement by International Society of Clinical Densitometry (ISCD), posterior-anterior lumbar-spine and total body are the most accurate and reproducible skeletal sites for BMD measurements as also proximal femoral region in children and adolescents (Gordon et al. 2008). To assess whether the bone density measured by DXA is normal for a particular child, the measurement needs to be compared with age and gender specific reference database of normal healthy population. The comparison yields a value, Z score, which is the number of
standard deviations away from the average value of the reference group of the same age and gender. According to WHO, a child having an age-gender specific Z score $\leq -2$ of bone mineral density is at risk of having low bone mineral density (Adams and Shaw 2004). The DXA machine calculates age and gender matched Z scores for BMD using Caucasian database. In a review on ethnic and genetic differences in bone mass accrual, Pollitzer and Anderson (1989) have highlighted influence of ethnicity on bone mineral density and thus, emphasized the need to use ethnicity specific reference database for comparison of bone mineral density in children (Nelson et al. 1997; Bachrach et al. 1999). Thus, development of Indian reference database for assessment of bone mineral density of Indian children and adolescents is important.

1.2: Genetics of bone mass accrual

The Vitamin D endocrine system plays a central role in calcium and phosphate homeostasis as well as bone mineralization by promoting the dietary absorption of calcium and by facilitating bone resorption and mineralization (Quesada et al. 2004). The secosteroid hormone vitamin D, its receptor (VDR), and the metabolizing enzymes involved in the formation of the biologically active form of the hormone, together are major players in the vitamin D endocrine system (Uitterlinden et al. 2004). Vitamin D receptor gene located on chromosome 12q13.1 is responsible for encoding vitamin D receptor which in turn affects the expression of active form of vitamin D and thereby is a candidate gene for bone mineral density.

In 1992, Morrison et al. for the first time described the association of bone turnover and VDR gene polymorphisms in adults. Thereafter, several association studies have been performed in children and elderly, to study VDR gene polymorphisms and their relationship with BMD, however the results are conflicting. Some studies have illustrated an association of VDR gene polymorphisms with BMD (Tokita et al. 1996; Spector et al. 1995; Lorentzon et al. 2001) whereas others show no such associations (Hansen et al. 1998; Jørgensen et al. 1998). In a recent study, Arabi et al. (2010), demonstrated that VDR gene polymorphisms were associated with BMD in elderly Lebanese populations whereas no such associations were found in Lebanese children and adolescents emphasising the influence of age on VDR gene polymorphisms and their associations with bone mass. Studies in Indian elderly women, have demonstrated an association of BMD with VDR gene polymorphisms of
Bone metabolism is of crucial importance in a woman’s life cycle, with postmenopausal women having greater risk of osteoporosis than men. Moreover, gender bias is also prominent in familial food distribution (Puri et al. 2008) resulting in higher risk of low bone mass in girls. However, in absence of studies in adolescents, knowledge of associations of VDR gene polymorphisms and bone mass in Indian adolescents may provide an opportunity to promote bone health during growing period and thereby, incur a health benefit.

1.3: Calcium – An Important Macro-Mineral for Life

Calcium is the most abundant mineral in the human body and makes up about 1.5-2% of the body weight and 39% of the total body minerals. Ninety-nine percentage of the body calcium exists in the bones and teeth and the remaining 1% of calcium is in the blood, extra-cellular fluids and in the tissues (Beyer 2000).

Calcium is essential for various functions in the body. The most important role of calcium is mineralization of bones. Adequate intake of calcium is necessary for optimum mineralization of bones especially during the pre-pubertal and adolescent years. Peak calcium retention during prepubertal and pubertal years lays the foundation for strong bones thereby reducing the risk of osteoporotic fractures in old age (Bonjour 1994).

1.3.1: Dietary Habits and Calcium Absorption

Dietary calcium intake in children and adolescents from developing countries including both rural and urban India has been reported to be less than the recommended dietary allowances (Salamoun et al. 2005; Rozen et al. 2001; Ahmed 1998; NNMB 2002, Puri et al. 2008, Sanwalka et al. 2010) emphasising the need to devise strategies to improve calcium intake during growing years. Various government and health professional organizations including 1994 National Institute of Health (NIH) consensus panel on Optimal calcium intake, National Institute for Child Health and Human Development (NICHD), United States Department of Health and Human Services, American Dietetic Association (ADA) and American Medical Association (AMA) recommend food as a preferred source of calcium intake (Miller et al. 2001).
Various nutritional and non-nutritional factors affect calcium absorption. Vitamin D is one of the most essential nutrients required for adequate calcium absorption. Certain carbohydrates like lactose, glucose, fructose and proteins like whey protein have been shown to promote calcium absorption (Guéguen et al. 2000). Certain fatty acids like acetic acid, butyric acid, oleic acid, linoleic acid have also been shown to be beneficial for calcium absorption (Cashman 2003, Kimura et al. 2001; Jewell and Cashman 2003). Supplementation with indigestible carbohydrates or prebiotics has been beneficial to increase calcium absorption (Cashman 2003). On the other hand, phytate (in whole grains, nuts, and seeds), and oxalate (in foods like spinach) have been shown to reduce calcium absorption (Ma et al. 2005; Pankaja and Prakash 1994). Foods which are very high in fibre content may also reduce calcium availability in the body (James et al. 1978). Thus, formulation of recipes containing calcium rich foods, incorporating dietary factors that increase calcium absorption and using processing methods that decrease the inhibitory factors would be beneficial to alleviate calcium status of youth.

1.4: Rationale and significance of the study

Comparison with age, height, weight and ethnicity specific reference dataset is crucially important for accurate interpretation of DXA measured bone densitometry in children (Kate et al. 2007). In view of lack of a paediatric reference database for the clinical assessment of bone status of Indian children and adolescents, there is a need for development of gender and age specific reference data on bone parameters for assessment of bone status in Indian children.

Along with ethnicity, studies have shown that there is a great influence of genetic makeup on BMD in children and adolescent. Studies on vitamin D receptor (VDR) gene polymorphisms have shown that loci within VDR gene are associated with BMD (Arabi et al. 2010; Lorentzon et al. 2001). Thus, analysis of associations of VDR gene polymorphisms and bone status in adolescents may provide an opportunity to take additional care through modifiable lifestyle factors to promote bone mass accrual in adolescents.

Diets of Indian adolescents are plant-based and contain low amounts of milk and milk products (NNMB 2002). The major source of calcium in their diets are
cereals, pulses, green leafy vegetables which are high in phytates and oxalates that bind with calcium and decrease its absorption (Sanwalka et al. 2010; Ahmed et al. 1998). Thus, to increase the intake of bio-available calcium and thereby to promote bone mass accrual in vegetarian children and adolescents, devising recipes from non-dairy based food sources using processing methods like malting and fermentation (processing methods increasing calcium extractability) would be beneficial (Idris et al. 2007; Weaver et al. 1991).

Dietary factors such as certain short chain, medium chain and long chain fatty acids and a low pH have shown been to promote dietary calcium absorption (Cashman 2003; Kimura et al. 2001; Jewell and Cashman 2003). Fortification with prebiotics has been shown to increase intestinal fatty acid content and thereby increase intestinal calcium absorption (Cashman 2003). To the best of our knowledge, no study has evaluated the effect prebiotic fortification on fatty acid profile and pH of plant food during fermentation and thereby increase calcium absorption of a fermented-malted cereal – pulse based snack naturally high in calcium.

Therefore, the aim of the present research work was to develop a reference database for bone mass status in Indian children and adolescents, and study the association of bone status with Vitamin D Receptor gene polymorphism. The study also aimed to examine the effect of prebiotic fortification on calcium absorption from fermented-malted non-dairy based calcium rich products in adolescent girls. The study was conducted in children and adolescents (5 – 17 years) from schools and colleges in Pune city, Maharashtra, Western India. The work in the thesis is presented in following chapters:

**Chapter 2** reviews the existing literature and assesses the need for research in the area of bone health, genetic factors affecting bone status and calcium absorption in Indian adolescents

**Chapter 3** describes the development of reference database for bone mass and presents percentile curves for bone status in Indian boys and girls aged 5 – 17 years

**Chapter 4** portrays the association of vitamin D receptor gene polymorphism and bone status in adolescent girls aged 15 – 17 years
Chapter 5 describes dietary calcium intakes in Indian boys and girls aged 6 to 19 years and development of non-dairy based calcium rich recipes.

Chapter 6 illustrates the effect of fermentation and malting along with prebiotic fortification on fatty acid composition of non-dairy based foods and thereby on calcium absorption in adolescent girls.

Chapter 7 highlights the summary and conclusions of the study.

Bibliography of the literature cited in these chapters is been provided after chapter 7. Sample questionnaires of general information, clinical signs and symptoms, diet and physical activity and Hedonic Rating are given in appendices A to F.