CHAPTER 7

Summary and Conclusions

Bone development and maintaining bone health is a lifelong process that begins at birth and continues in childhood and adulthood (National Osteoporosis Foundation, 2009). Peak bone mass (PBM) is achieved by early adulthood and is one of the two main determinants of osteoporosis, the other being rate of bone loss during adult years. Of the total adult body bone mineral content (TBBMC), 53% is achieved during premenarchal period in girls making pubertal years important from long term bone health point of view. Women have lower bone mass than men at all ages and as a result of menopausal bone loss, are at higher risk of osteoporosis related fractures. Furthermore, Asians are known to have lower bone mass than their Western counterparts. Therefore, there is a need to target bone health of women especially during years of rapid bone mass accrual i.e. adolescence and rapid bone loss during post menopausal years.

Various non-modifiable (genetics, puberty, ethnicity, etc) and modifiable factors (nutrition, physical activity, etc) are known to affect bone health. High prevalence of nutritional deficiencies especially those of calcium, vitamin D and micronutrients such as zinc and ascorbic acid have been reported in both Indian adolescent girls (Venkaiah et al, 2002; Tupe & Chiplonkar, 2010) and women (NNMB, 2002; Agte et al, 2004). Calcium and vitamin D are known to influence bone health. The role of other micronutrients like zinc Vitamin B complex and ascorbic acid in maintaining bone health has been established in adults (New et al, 1997; Righetti 2008; Hall & Greendale, 1998). As yet, similar studies in adolescent girls to assess the role of these nutrients in bone mass accrual during growth are lacking. One of the strategies to improve bone health in children is through improving nutritional deficiencies by means of supplementation. This intervention can target vulnerable population groups especially from lower socioeconomic strata where prevalence of macro & micronutrient deficiencies is high suggesting overall poor growth with adverse effects on bone health. Supervised supplementation through school-based programmes can provide this opportunity. Therefore, in the present study effect of supplementation of calcium and vitamin D along with
multivitamins and zinc on bone mass accrual was assessed in premenarchal girls in a school set-up.

With the view to assess factors influencing bone health in premenarchal girls, a cross sectional study was carried out in 214 premenarchal girls (8-12 yr) from two public schools in Pune city, India, catering to children from low socioeconomic strata (LSES). Power of the study was estimated to be 90% at 5% level of significance to detect a difference of more than 10% between the groups. A written informed consent was obtained before start of the study. Exclusion criteria consisted of any condition or use of drugs known to adversely affect bone metabolism, any major illness or attainment of menarche. Study protocol was approved by the ethics committee of the Hirabai Cowasji Jehangir Medical Research institute (HCJMRI) and Jehangir Clinical development center (JCDC), Pune, India. Data were collected on socio-demographic variables (education and occupation of parents, family size, monthly income), body measures (height, weight, waist, hip circumference), blood pressure, physical activity, sunlight exposure and dietary intakes (by 24-hour recall method). Blood levels of calcium, phosphorous, parathyroid hormone, 25 hydroxy vitamin D (25OH-D), zinc and hemoglobin were estimated using standard methods. Bone parameters at total body viz. bone mineral content, bone area and fat% were measured in girls using Dual energy X-ray absorptiometry (DXA).

As per World Health Organization (WHO) criteria, the height-for-age and weight-for-age Z scores were within normal range (above -2) for majority of the girls (75% and 61% of the total girls respectively) indicating that they were apparently healthy. Mean dietary intakes of energy and protein were 74.5% and 79% of Indian recommended dietary allowances (RDA). The intakes of calcium, zinc and iron were 28-33% of the RDA respectively implying dietary deficiency of minerals. Seventy one percent girls had ionized calcium concentration below the reference range (<1.12 mmol/l) while high concentrations of PTH (>6.4pmol/l) were observed in 39.5% of the girls. Vitamin D insufficiency (25OH-D concentration <50nmol/l) and serum zinc deficiency (<0.7mg/l) were observed in 34.2% and 55.5% of the girls respectively indicating biochemical deficiencies of micronutrients. Mean total body bone mineral content (TBBMC) and total body bone area (TBBA) were...
809±148g and 1047±150 cm² respectively. Compared to Indian reference-database, 15.6% girls had TBBMC-for-age Z scores below -2 (normal >-2), and 37.5% had Z scores between -2 and -1. Further, 20% of the girls had TBBMC-for-TBBA Z scores below -2 indicating under-mineralized bones in the study population. Association of bone parameters with anthropometric, lifestyle and diet parameters revealed that height (r=0.80), weight (r=0.87), moderate physical activity (r=0.24), serum 25OH-D (r=0.17), dietary intakes of calcium (r=0.14) and zinc (r=0.16) were positively associated with TBBMC (p<0.05). Multiple regression analysis revealed that weight, Tanner stage, energy adjusted dietary intakes of protein, calcium and zinc along with serum 25OH-D concentrations were significant factors affecting TBBMC (p<0.05).

In comparison to published reference databases it was observed that the mean total body bone mineral density (TBBMD) of the present study population at each age group was lower as compared to Indian, Polish and Caucasian reference population. Further, the mean rate of increase in TBBMC with age was lower in the study population (11.6%) compared to the Indian reference database (14.2%), Polish population (13.6%) and Caucasian populations (13%). Thus, a large number of premenarchal girls from low income groups suffer from micronutrient deficiencies and are “at risk” for achieving low bone mass especially due to lower rate of bone mass accrual during critical years of growth.

To assess the effect of socioeconomic status on bone health, cross-sectional data on height, weight and bone parameters in 93 age-matched girls was collected from private schools [catering to higher socioeconomic strata (HSES)] and compared with the girls from the present study (LSES). Comparison between age-matched girls from LSES and HSES revealed that the mean TBBMC (811±171g) and TBBA (1051±168cm²) in LSES girls was significantly lower than that of age-matched HSES girls [TBBMC(1057±292g), TBBA(1229±252cm²)] (p<0.01). There was a significant effect of age and puberty on all bone parameters. Further, results indicate that girls from LSES had significantly lower TBBMC at Tanner stage III (1008±164g) compared to HSES girls (1258±190g) (p<0.05). Thus, girls from LSES are at risk for attaining lower PBM by adulthood and steps to improve bone health particularly during pubertal years are warranted.
Therefore, a one year intervention trial to study the effect of calcium, zinc, multivitamin and vitamin D supplementation on bone mass accrual in these girls was carried out. The 214 apparently healthy premenarchal girls (8-12 yr) from the cross sectional study were randomly allocated to one of the three intervention groups: Calcium group (Ca+D)-500 mg of Calcium 6d/wk (n=72) and vitamin D; Calcium and multivitamin-zinc group (Ca+Mzn+D)- 500 mg of calcium and multivitamin tablet containing 15 mg of Zinc with a gap of at least 2 hours (to prevent interference in the absorption) (n=73) and vitamin D; Multivitamin group (M+D) (n=69)-multivitamin tablet and vitamin D. Vitamin D was given every 3 months in the form of cholecalciferol (Vitamin D3). Anthropometry, lifestyle factors (physical activity, sunlight exposure, dietary intakes), blood and bone parameters were assessed at baseline and at the end of study period. Compliance to intervention was 80% throughout the year for all three groups. Post intervention, mean percent increase in TBBMC was significantly higher in the Ca+D group (23.1%) and Ca+Mzn+D group (21.5%) compared to M+D group (19.4%) even after adjustment for Tanner stage and TBLBM (total body lean body mass) (p<0.05). Mean percent increase in TBBMC in Ca+Mzn+D group and Ca+D group were similar (p>0.1). Improvement in TBBMC-for-age Z scores was higher in the two calcium supplemented groups (13.6%-22%) compared to the M+D-group (0.0%, no improvement). Also, shift in distribution pattern of TBBMC towards the right was higher in both the calcium supplemented groups (Ca+Mzn+D and Ca+D group) compared to the multivitamin group (M+D). Results thus indicate that one year calcium, vitamin D supplementation with or without zinc+multivitamin supplementation was useful in improving bone health of underprivileged premenarchal girls.

Furthermore, to compare the effect of supplementation on height velocity of the study population (LSES), with girls from higher socioeconomic group (HSES), one year longitudinal data on anthropometry (height, weight, BMI) and pubertal stage of 119 age-matched girls from HSES (who were not given any supplementation for that duration) was compiled from available database and treated as the Control group (C1, age-matched controls for Ca+Mzn+D group; C2, age-matched controls for Ca+D group and C3, age-matched controls for M+D group). Comparison between LSES (supplemented groups-Ca+Mzn+D, Ca+D and M+D
groups) and HSES (C1, C2 and C3 group) girls revealed that change in height velocity adjusted for Tanner stage was significantly higher in the Ca+MZn+D group (6.7±0.4 cm/yr) as compared age-matched C1 group (5.3±0.2 cm/yr), Ca+D group (5.6±0.4 cm/yr) and M+D group (6.1±0.2 cm/yr) (p<0.05). Also, height-for-age Z scores adjusted for Tanner stage were significantly higher in the Ca+MZn+D group (0.32±0.06) compared to age-matched C1 group (0.02±0.02), Ca+D group (0.18±0.06) and M+D group (0.14±0.05) (p<0.05). Improvement in TBBMC and TBBMD-for-age Z scores was also observed. The shift in the Z score from “<-2 or stunting” category to “between -2 to -1” category was considered as improvement. It was seen that while 22.2% of the girls from Ca+MZn+D, 9.1% from Ca+D group and 16% girls from M+D group showed improvement in height-for-age Z scores. However, for the control group of age-matched girls from HSES, no change in the height Z score status was observed. This suggests the beneficial effect of zinc+multivitamin in improving height-for-age Z scores in the study population of LSES premenarchal girls. An Indian study has reported that peak height velocity was achieved around 11 years of age for both LSES and HSES girls without any supplementation. In the present study population, Ca+MZn+D group showed continued higher height velocity even at age 12 as compared to the HSES unsupplemented controls (C1 group). Thus, calcium and multivitamin+zinc supplementation showed an enhancing effect on height velocity as well as improvement in height Z scores. This may result in catch-up growth as seen in our population of underprivileged premenarchal girls.

In older women, it has been reported that prevalence of osteoporosis increases with age as a result of bone loss during menopause ranging from 1% to 6% per year (Falch & Sandvik, 1990). Low bone mass has been reported in Indian women above 40 years of age (Kadam et al, 2010; Bhalerao-Gandhi & Shukla, 2005) along with low intakes of calcium and high prevalence of vitamin D deficiency (Harinarayan et al, 2005). Bone loss during menopausal transition is influenced by a combination of factors such as genetics, lifestyle factors (like diet and physical activity, sunlight exposure) and hormonal factors. Therefore, the present study examines relative importance of factors affecting bone health in women (>40 yr). A cross-sectional study was undertaken in women (above 40 years of age) attending routine health check in a tertiary care hospital in Pune city, India.
In all, 319 women were randomly recruited for the study during 2008-2009. Power of the study was 90% at level of significance 5% for detecting a difference of means more than 5%. Ethical approval was obtained from the ethics committee of HCJMRI and JCDC, Pune before the start of the study. The participants were classified into two groups—Premenopausal (n=104) and postmenopausal (n=215) based on their menopausal status as defined by WHO. Exclusion criteria included any condition or use of drugs known to adversely affect bone metabolism, any major illness or age below 40 years. Data on socio-demographic factors, anthropometry, lifestyle factors, (physical activity, sunlight exposure, diet) and biochemical parameters (including lipid profile) were assessed using methods described in the cross sectional study in girls. Also, skin fold thickness (using Harpenden calipers at two sites (triceps and supra-illiac) was measured to calculate fat percentage (fat %). Since in adults, spine and hip are the most common sites for fractures, bone mineral content (BMC), bone area (BA), bone mineral density (BMD) of lumbar spine, total hip and femoral neck was measured using DXA.

In postmenopausal women, average age at menopause was 47.1±3.3 yr and average years since menopause were 7.7±7.1 yr. Majority of premenopausal and postmenopausal women had adequate moderate physical activity of more than 30min/d (76.5% and 67.7% pre and postmenopausal women respectively). Mean dietary intakes of macronutrients as also calcium and micronutrients were below the Indian RDA in more than 90% of the pre & postmenopausal women. Serum Ionized calcium deficiency was seen to be higher in postmenopausal women than in premenopausal women. Majority of both premenopausal (96.2%) and postmenopausal (84.7%) women showed Vitamin D (25OH-D) insufficiency (<50nmol/l) while more than 50% of both the groups of women also showed serum zinc deficiency. BMC, BMD and bone mineral apparent density (BMAD) at all sites (Lumbar spine, total hip and femoral neck) were significantly lower in postmenopausal than premenopausal group (p<0.001). Prevalence of osteoporosis was highest at the lumbar spine (25.8%) in post-menopausal women, while prevalence of osteopenia was high in pre-menopausal women (44.3%) indicating that bone loss is seen even in women nearing menopause. Partial correlation coefficients adjusting for age and energy intake revealed that lumbar spine BMD was positively associated with dietary intake of calcium (r=0.28), phosphorus
(r=0.27), riboflavin (r=0.25) and calcium:phytate:zinc ratio (r=0.21)(p<0.05). Regression analysis indicated that age, weight, height and body fat% were the determinants of BMD in premenopausal women while in post menopausal women, height, weight and years since menopause were the factors affecting BMD. With increasing stage of menopause (perimenopausal, premenopausal, post menopausal but less than 5 years since menopause and more than 5 years since menopause), higher decline was observed in BMD with the rate of decline ranging from 4 to 5.7% from pre to post menopausal stage. Pooled regression after adjusting for known factors affecting BMD (age, weight and height) revealed that percent change of 2.1% - 4.5% may be attributed to menopause, the other major determinant being height. The present findings thus indicate the importance of improving dietary intakes of calcium, vitamin D status and sunlight exposure in women above 40 years of age and especially in premenopausal women as menopause explains a large portion of bone loss in these women.

Main Conclusions:

- Bones of girls from lower socioeconomic stratum (LSES) were undermineralised as compared to age-matched girls from higher socioeconomic stratum (HSES) and both LSES and HSES girls had lower bone mass compared to Caucasian reference database.

- With increasing pubertal stages, increased difference in bone mass accrual by LSES as compared to HSES girls was demonstrated.

- Underweight status, vitamin D insufficiency and low dietary intakes of protein, calcium and zinc adversely affect bone health of underprivileged girls.

- One year supplementation with calcium, vitamin D and multivitamin+zinc was useful in improving bone mass accrual in premenarchal girls.

- One year supplementation with multivitamin+zinc with calcium improved height-for-age Z scores and height velocity in underprivileged premenarchal girls than calcium supplementation alone.
In women, of the total decrease in bone mineral density, menopause explained 2.1%, 2.5% and 4.5% percent change for lumbar spine, femoral neck and total hip respectively after accounting for factors such as age, height and weight.

Age, weight, height, menopause, low intakes of calcium and low vitamin D status along with poor sunlight exposure were factors contributing to bone loss in Indian women above 40 years of age.

Implications:

- The present study indicates the need to focus on improvement of bone health during pubertal years especially in premenarchal girls.
- School-based supplementation with calcium and multivitamin+zinc along with vitamin D can be used as a strategy for improving bone health of adolescent girls.
- Zinc+multivitamin+calcium supplementation during premenarchal years can be used as an effective strategy to improve height deficits experienced by girls by increasing height velocity during this critical phase of growth.
- In relation to known factors (like age, weight and height) affecting bone health in women, menopause is an important contributor to the bone loss experienced by women above 40 years of age. Thus, additional focus should be directed also at premenopausal women to combat the loss during and post menopause.
- Based on the factors affecting bone loss in women above 40 years of age, specific intervention strategies for improving bone health can be formulated.

Future scope:

- Calcium, zinc+multivitamin and Vitamin D supplementation on bone health in premenarchal girls after cessation of supplementation needs to be studied to see the sustainability and long term benefits on bone health.
• Effect of zinc+ multivitamin supplementation needs to be investigated in older age group girls (above 12 years) to see if the enhancing effect on height velocity is seen and also to determine at what age peak height velocity is achieved in presence of supplementation.

• Zinc supplementation in premenarchal girls with calcium and vitamin D sufficiency can be undertaken to assess if zinc has any enhancing role in bone mass accrual as seen in the case of height velocity.

• Similar to the study in premenarchal girls, effect of calcium, vitamin D along with multivitamin+zinc supplementation on decreasing bone loss and maintaining bone health in women above 40 years of age needs to be investigated.