CHAPTER 6
Risk Factors for Low Bone Status in Women above 40 Years of Age

The ability of bone to adapt to mechanical loads is brought about by continuous bone resorption and bone formation. In a homeostatic equilibrium, resorption and formation are balanced which is defined as bone remodeling (Frost 1990). Until women reach the age of 30, the building of bone outweighs breakdown (Kadam et al, 2009). If, as in osteoporosis, bone breakdown predominates, bone substance and structure are lost. This loss seems to accelerate in many women during the menopausal transition. Therefore, the crucial period that needs utmost care for optimal bone status is the few years before and the few years after menopause. There is a need to better understand potential bone mineral density loss during this time since this period may include the initiation of interventions.

Bone mineral density loss around the years surrounding menopause depends on a combination of factors including heredity, diet, physical activity, and the hormonal system. Relatively little is known about risk factors in women from Indian subcontinent who, along with low calcium intake and high prevalence of Vitamin D deficiency (Harinarayan et al, 2005), have been reported to have lower bone mineral density compared to their Caucasian counterparts (Hafeez et al, 2009). Since oestrogen is not the only factor responsible for bone health during the menopause transition, more studies are required in women around menopause to identify factors affecting bone health. This would be helpful to devise appropriate intervention strategies aimed at decreasing the rate of decline and prevent severe consequences of the same. Therefore, the relative importance of anthropometric, nutritional and other lifestyle factors in bone loss in a cross sectional cohort of Indian premenopausal and postmenopausal women above 40 years of age was investigated.
6.1 Material and Methods

A cross sectional study was carried out in women above 40 years of age. Based on the variation in bone mineral density (BMD) as observed from a previous study in premenopausal and postmenopausal women (Salamat et al, 2008), a sample size of 300 was estimated to obtain the power of the study to be 90% at level of significance 5% for detecting the difference of means more than 5%.

6.1.1 Study participants

Women above 40 years of age visiting the health check clinic of a tertiary care hospital in Pune city, India for routine check up between March and May 2008 (summer season) were contacted.

6.1.2 Exclusion criteria

- Age below 40 years.
- History of fracture within last 12 months
- Prolonged immobilization in the past 12 months
- Any disease that may reduce bone mineral density (BMD) like renal disease, hyperthyroidism, hypoparathyroidism, thyroid disease, adrenal disease, cancer, chronic gastrointestinal disease, liver disease, diabetes, Paget’s disease, hypogonadism, cystic fibrosis, celiac disease.
- History of consumption of drugs affecting calcium metabolism and BMD like glucocorticoids, oestrogen, anticonvulsant drugs, heparin, thyroid hormone, calcium supplementation and vitamin D therapy.

Amongst those willing to participate and fulfilling the inclusion criteria, 319 women were randomly selected for the study. All the women gave written informed consent after being explained the study protocol. The study protocol was approved by the ethical committee of Jehangir Clinical Development center and Hirabai Cowasji Jehangir Medical Research Institute.

Women were categorised into two groups: premenopausal and post menopausal women. Premenopause was defined as women above 40 years of age with regular menstruation. Post menopause was defined as permanent cessation of
menstrual periods that occurs naturally or is induced by surgery in accordance with the definition by World Health Organization (WHO) (NIH, 2005).

6.2 **Outcome parameters**

Data on anthropometry (height, weight), bone parameters [Bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) at lumbar spine] and biochemical parameters (lipid profile and haemoglobin) were collected. Additionally, on a subset (80 premenopausal and 92 postmenopausal), skinfold measurements, waist & hip circumference, age at menarche, clinical signs and symptoms of nutritional deficiencies, lifestyle factors (diet and physical activity), biochemical parameters (calcium, phosphorous, PTH, zinc, 25OH-D) and bone parameters at dual femurs (BMD, BMC and BA) were recorded.

For the subset of 172 women, based on previous study in women (Paul et al, 2008), the power of the study was found to be 80% at 5% level of significance to detect a difference of more than 5% in the study parameters.

6.2.1 **Anthropometric measurements**

Weight was measured on an electronic digital scale (Libra Industries, Mumbai, India) to the nearest 0.1 kg in the morning with women in light indoor clothes without shoes. Standing height was measured using a portable stadiometer (Leicester Height Meter, Child Growth Foundation, UK, range 60-207 cm). Body mass index (BMI) was computed by the following formula: BMI = Weight (kg) / Height$^2$ (m$^2$). Women were categorized as normal, overweight and obese as per the Asian cutoff for BMI in Indians (Misra, 2003).

Waist and hip circumferences (cm) were measured using a non-stretchable measuring tape to the nearest 1 mm. Waist circumference was measured midway between the lowest rib and the iliac crest at the end of gentle expiration and hip circumference was measured as maximal circumference at the level of the trochanters. Waist to hip ratio was calculated.
The skinfold thickness at two sites (triceps and supra iliac) was measured using a Harpenden skinfold caliper (CMS Weighing Equipment Ltd, London, UK) to the nearest accuracy of 0.2mm. Body fat percentage was calculated from these skinfold measurements by the method given by Durnin & Womersley (1974), as:

\[
%\ Fat = \left[\frac{(4.95/Density)}{4.5}\right] \times 100
\]

(Siri’s (1956) equation)

Density in the above equation is calculated using formula,

\[
Density = (c - m) \times \log Skinfold
\]

Where, c and m are estimates for different age-sex groups. Values for triceps+supra-iliac skinfold thickness for women between 40-49 years of age are \(c=1.1383\), \(m=0.0660\) and for women above 50 years of age, \(c=1.1415\), \(m=0.0718\) were used for the present study cohort.

6.2.2 Bone parameters

Since in adults, the most likely sites of fractures are lumbar spine and hip, bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) was measured at two sites: at the posteroanterior (PA) lumbar spine (L1-L4, L2-L4) and dual femurs (femoral neck, wards, trochanters and total hip) by Lunar DPX-PRO total body pencil beam Densitometer (GE Healthcare, Wisconsin, USA) using a medium mode scan (software encore 2005 version 9.30.044) (photograph 6.1, 6.2). The precision of the lunar DPX for lumbar spine BMD and femoral neck BMD is 1.04% and 2.13% respectively (Johnson J and Dawson-Hughes, 1991).

DXA does not represent the true bone mineral density as it calculates area of the bone and not volume. Therefore, to correct bone density for bone size, apparent volumetric BMD (BMAD g/cm3) of the lumbar spine and dual femurs was calculated using the following formula,

\[
BMAD = \frac{BMC}{BA^{3/2}}
\]

Where, BMC is the bone mineral content and BA is the projected area (Carter et al, 1992).
Photograph 6.1: DXA scan for lumbar spine

![Image of DXA scan for lumbar spine]

Photograph 6.2: DXA scan for total hip and femoral neck

![Image of DXA scan for total hip and femoral neck]
DXA machine provides T scores for each individual. T score is defined as number of standard deviations above or below the BMD of a young healthy adult of the same sex.

\[
T \text{ score} = \frac{\text{Subject’s BMD value} - \text{mean young normal BMD value}}{\text{Young normal BMD standard deviation}}
\]

The DXA machine software computes T scores using reference database consisting of cross-sectional age and gender specific adult data on lumbar spine and dual femur bone mineral density of ambulatory healthy Caucasian men (2880) and women from USA (3000) and Europe (8000-9000) (Mc Mohan et al, 2003; Faulkner et al, 1996; Kroger et al, 1992; Mazess et al, 1999 and Burger et al, 1994). According to WHO definition, a person with T score above -1 is considered “normal”, between -1 and -2.5 is classified as “osteopenia” (low bone mass) and a T score below -2.5 is classified as “osteoporosis” (Figure 6.1).

It is important to have ethnicity and machine specific reference database in order to avoid false positive or false negative labelling of a patient as having osteoporosis. However, in absence of published normative Indian reference database for Lunar DPX-PRO for adults, T scores computed by the DXA machine software were used to assess women’s bone health status.

According to The International Society for Clinical Densitometry (ISCD), in postmenopausal women, osteoporosis can be diagnosed if the BMD is ≤−2.5 standard deviations (SD) from the T score in the lumbar spine, total hip or femoral neck (Figure 6.2). For lumbar spine they recommend using the mean value of the lumbar spine (L1-L4 or L2-L4) analysis and state clearly that the categorization of the patients must not be done from the analysis of a single vertebrae (Martínez-Aguilà et al, 2008). While some papers have described lumbar spine bone health in adults on the basis of L1-L4 other studies have also used L2-L4. In the present study though both L1-L4 and L2-L4 measures (BMC, BA, BMD and T score) have been described, L2-L4 measures were used for further analysis throughout the chapter.

All scans were performed by me after a 3-day training session at HCJMRI.
Figure 6.1: Bone health status based on T scores as defined by WHO

Figure 6.2: Interpretation of DXA scan for lumbar spine and femur
6.2.3 Physical activity

Data on habitual physical activity was collected using a validated structured questionnaire (Chiplonkar et al, 2004) (Appendix F) Information about duration in minutes of major daily activities like sleep, sitting, standing, walking, exercise, recreation and occupational activity were used to classify an individual into level of physical activity groups: inactivity, light and moderate activity (CDC, 1999). Personal activity (which included bathing, eating, office work, reading and commuting) was considered as light activity. Time spent in exercise and sports activities (e.g., yoga, walking, jogging, swimming and gym) was considered as moderate activity. Watching television and other leisurely activities were categorized as inactivity.

6.2.4 Sunlight exposure

Information about sunlight exposure in minutes per day for each participant was recorded along with type of clothing to estimate the body surface area exposed to sunlight (Appendix B). The “rule of nines” (Knaysi et al, 1968) was used to represent adult body surface area (BSA) (front and back torso and each of the legs were counted as 18%, the arms and head as 9%) and usual skin exposure was estimated according to each subject’s combination of clothes and sleeve length. An index adapted from Barger-Lux & Heaney (2002) was used combining measure of time outdoors during daylight and BSA usually exposed during that time, as given below:

\[
\text{Sun index} = \text{Hours of sun exposure per week \times Fraction of BSA exposed to sunlight}
\]

Sun index was further categorised as low, medium or high sun exposure using percentiles of the sun index.
6.2.5 Dietary intake

Dietary intake was assessed by 24-hour recall using the multiple pass approach (Rutishauser, 2005; Guenther et al, 1996) (Appendix C). Information on portion size per consumption was also collected with the help of standard serving plates, cups and spoons (Photograph 6.3). Each participant was asked to give information about the recipes, ingredients and amount of dish consumed through interview method.

Estimation of nutrient intake

Nutrient intakes were estimated from 24 hour diet recall using the methods described in Chapter 2, section 2.2.5.

6.2.6 Biochemical parameters

A venous blood sample was collected at 8.30 am after an overnight fast (not less than 10 hours and not more than 12 hours) from each participant using ethylenediaminetetraacetic acid (EDTA) coated vacutainers for hemoglobin and plain mineral free vacutainers (BD Franklin Lakes, NJ USA) for serum estimations. Samples in plain vacutainers were immediately centrifuged at 2500 rpm for 15 minutes and the serum separated and frozen at –70°C until analysis.

Haemoglobin, ionized calcium (iCa), serum concentrations of inorganic Phosphorous (iP), alkaline phosphatase (ALP), intact parathyroid hormone (PTH), 25-hydroxyvitamin D (25 OH-D) and zinc were estimated using the methods as described in Chapter 2, section 2.2.6. Serum zinc was estimated using atomic absorption spectrometer (Spectra AA, model Varian 220, Russia) (Photograph 6.4).

Additionally, lipid profile was estimated on a Siemens analyzer (Date Dimension RXL Max.) with enzymatic procedures for measurement of cholesterol, triglycerides and HDL. The LDL cholesterol level was calculated by using the formula \[ LDL = TC - (HDL + TG /5) \] (Friedewald equation, Manual of laboratory operation, 1974).
Reference serum (Biorad Laboratories, India) was used as the standard for each batch of blood estimations. All biochemical analyses were done by me at the Biochemistry Unit of the hospital under the supervision of the laboratory In-charge.

6.2.7 Health status and clinical signs and symptoms of nutritional deficiencies

A structured questionnaire was designed to assess health status and clinical signs and symptoms of nutritional deficiencies especially those of zinc, iron and multivitamins (NNMB, 1994; Shils et al., 2005; Chiplonkar and Agte, 2007) (Appendix G) in consultation with a lady physician. The questionnaire was filled under the physician’s supervision to record clinical signs and symptoms of nutritional deficiencies and also any pre-existing morbidities and current health complaints. Data on menopausal status was also recorded as the month and year of attainment of menopause (as defined by WHO) and time since menopause was calculated.

Blood pressure was measured in the left arm after 10 min of rest with the participant lying down quietly. Measurements were made by auscultation with a mercury-column sphygmomanometer and a cuff appropriately sized for the arm size of the subject. The onset of the first Korotkoff phase was used to determine systolic blood pressure, and the onset of the fifth Korotkoff phase was used to determine diastolic blood pressure. Mean of two measurements was recorded.
Photograph 6.3: Assessment of dietary intake in women

Photograph 6.4: Serum Zinc estimation on Atomic absorption spectrometer
6.2.8 Socio-demographic observations

The date of birth was obtained from each participant and age was calculated from the survey date. A structured questionnaire adapted from National family health survey (NFHS, 2005-06) was used to collect information on social and demographic characteristics of the women. Data on education, occupation, family size, marital status and number of children (Appendix H) was collected. The test retest reliability coefficient of the questionnaire was 0.99 (p<0.01) which was tested on a pilot sample of 20 women.

6.3 Statistical analysis

Analyses were performed using SPSS software for Windows (version 11.0, 2001, SPSS Inc, Chicago, IL). Normality of the variables was tested by Kolmogorov-Smirnov test. Differences between pre and postmenopausal women were tested using Student’s t test for normal variables and by Mann-Whitney test for non-normal variables. Association between categorical variables was tested using chi-square test. Partial correlations controlling for age, BMI and energy intake were computed to estimate the association of various anthropometric and lifestyle parameters with BMD (Daniel, 1991). Generalized linear model was used to examine relative influence of various factors on BMD at all three sites for the entire cohort (Lindsey JK 1997). Stepwise multiple regression analysis was used to identify significant factors affecting BMD in premenopausal and postmenopausal women separately for each site, lumbar spine, femoral neck and total hip. For postmenopausal women an additional variable of years since menopause was also included in the analysis. General linear model was used to estimate percent change in bone mineral density with age, anthropometric parameters and menopause.
6.4 Results

Of the 319 women who fulfilled all the selection criteria, 104 women were identified as premenopausal and 215 as post-menopausal women. The two groups were similar with respect to geographic region (Pune, India) and ethnicity.

6.4.1 General characteristics of the study population

Table 6.1 describes the general characteristics of the study population. The age of premenopausal women ranged from 40-54 years and 43-75 years for post menopausal women. There were no significant differences between mean height, weight, skinfold thickness (triceps and suprailliac), waist and hip measurements of the two groups (p>0.1). Abdominal obesity as indicated by waist to hip ratio >0.8 (Mishra et al, 2003) was seen in 85.5% and 80% of premenopausal and postmenopausal women respectively. The mean body fat% as estimated from skinfold thickness of two sites (triceps and suprailliac region) was also similar in premenopausal (39.0±4.2%) and postmenopausal women (39.2±5.2%) (p>0.1).

Socio-demographic parameters i.e. family size and education of the two groups were similar between the two groups with the average family size being 4±1 and number of children 2±1. Majority of the women (68%) were graduates or held a higher degree. Percent of retired women from postmenopausal group was higher than the premenopausal group as was expected. Mean age at menarche was 13.6±1.2 years and 13.5±1.5 years in premenopausal and postmenopausal women respectively, the difference being not statistically significant (p>0.1). In postmenopausal women, the average age at menopause was 47.1±3.3 years and average years since menopause was 7.7±7.1 years.
Table 6.1: General characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal (n=104)</th>
<th>Postmenopausal (n=215)</th>
<th>Total (n=319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>45.0±4.4</td>
<td>55.0±6.7*</td>
<td>51.5±7.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.7±5.1</td>
<td>155.3±6.5</td>
<td>155.5±6.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.8±11.7</td>
<td>65.0±11.1</td>
<td>64.9±11.3</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>26.7±4.6</td>
<td>26.9±4.4</td>
<td>26.9±4.5</td>
</tr>
<tr>
<td>Waist (cm)*</td>
<td>91.3±9.8</td>
<td>91.5±10.8</td>
<td>91.4±10.3</td>
</tr>
<tr>
<td>Hip (cm)*</td>
<td>105.0±10.6</td>
<td>105.9±10.8</td>
<td>105.5±10.7</td>
</tr>
<tr>
<td>Waist:hip ratio*</td>
<td>0.87±0.06</td>
<td>0.86±0.06</td>
<td>0.87±0.06</td>
</tr>
<tr>
<td>SFT-tricep (mm)*</td>
<td>22.4±7.3</td>
<td>21.6±8.0</td>
<td>22.0±7.7</td>
</tr>
<tr>
<td>SFT-Supra iliac (mm)*</td>
<td>25.4 (13)</td>
<td>25.5 (15.7)</td>
<td>28.6±11.5</td>
</tr>
<tr>
<td>Body fat % a</td>
<td>39.0±4.2</td>
<td>39.3±5.2</td>
<td>39.1±4.8</td>
</tr>
<tr>
<td>Systolic BP (mm/Hg)</td>
<td>120 (14)</td>
<td>130 (20)*</td>
<td>120 (20)</td>
</tr>
<tr>
<td>Diastolic BP (mm/Hg)</td>
<td>80 (10)</td>
<td>80 (18)*</td>
<td>80 (14)</td>
</tr>
<tr>
<td>Age at menarche (yrs)*</td>
<td>13.6±1.2</td>
<td>13.5±1.5</td>
<td>13.5±1.4</td>
</tr>
<tr>
<td>Age at menopause b</td>
<td>-</td>
<td>47.0±3.3</td>
<td>-</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>-</td>
<td>5.7 (11)</td>
<td>-</td>
</tr>
<tr>
<td>Education (% women)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upto 10th</td>
<td>17.9</td>
<td>29.2</td>
<td>24</td>
</tr>
<tr>
<td>Diploma/12th</td>
<td>7.7</td>
<td>9.0</td>
<td>8.4</td>
</tr>
<tr>
<td>Graduation and above</td>
<td>74.4</td>
<td>61.8</td>
<td>67.7</td>
</tr>
<tr>
<td>Occupation (% women)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>51.3</td>
<td>52.2</td>
<td>51.8</td>
</tr>
<tr>
<td>Service</td>
<td>38.5</td>
<td>33.3</td>
<td>35.7</td>
</tr>
<tr>
<td>Retired</td>
<td>5.1</td>
<td>11.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Business/Self employed</td>
<td>5.1</td>
<td>3.3</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Values are Mean±SD
BMI, Body Mass Index; SFT, Skin fold thickness
*Significantly different from premenopausal women
a Data on 80 premenopausal women and 92 postmenopausal women
b If natural menopause
When women were classified as per the Asian cutoff for BMI in Indians (Mishra et al, 2003), 18.5% of premenopausal and 17.4% postmenopausal women were found to be overweight while 61% premenopausal and 66.7% postmenopausal women were obese (Table 6.2).

<table>
<thead>
<tr>
<th>BMI categories</th>
<th>Premenopausal (n=104)</th>
<th>Postmenopausal (n=215)</th>
<th>Total (n=319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;23)</td>
<td>20.4</td>
<td>15.9</td>
<td>17.5</td>
</tr>
<tr>
<td>Overweight (23-25)</td>
<td>18.5</td>
<td>17.4</td>
<td>17.8</td>
</tr>
<tr>
<td>Obese (&gt;25)</td>
<td>61.1</td>
<td>66.7</td>
<td>64.7</td>
</tr>
</tbody>
</table>

### 6.4.2 Bone parameters

Mean BMC, BMD and BMAD at both L1-L4 and L2-L4 were significantly lower in postmenopausal women than the premenopausal group (p<0.05) whereas the mean BA showed no significant difference between the two groups (p>0.1) (Table 6.3). At the lumbar spine, the mean T score was significantly lower in postmenopausal women than premenopausal women indicating higher bone loss after menopause.

The dual femurs were divided into two regions namely femoral neck and total hip since the most common site of fractures of hip is the femoral neck region. The mean BMC, BMD and BMAD of postmenopausal women was significantly lower than in premenopausal women (p<0.05) at both femoral neck and total hip region (Table 6.4).
Table 6.3: Bone parameters at lumbar spine in the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal women (n=104)</th>
<th>Postmenopausal women (n=215)</th>
<th>Total (n=319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC, L1-L4 (g)</td>
<td>50.3±9.2</td>
<td>45.8±10.6*</td>
<td>47.4±10.3</td>
</tr>
<tr>
<td>BMC, L2-L4 (g)</td>
<td>38.9±7.4</td>
<td>35.3±8.2*</td>
<td>37.0±8.1</td>
</tr>
<tr>
<td>BA, L1-L4 (cm²)</td>
<td>46.1±4.8</td>
<td>46.1±5.0</td>
<td>46.1±4.9</td>
</tr>
<tr>
<td>BA, L2-L4 (cm²)</td>
<td>35.4±3.6</td>
<td>35.2±3.9</td>
<td>35.3±3.7</td>
</tr>
<tr>
<td>BMD, L1-L4 (g/cm²)</td>
<td>1.09±0.13</td>
<td>0.99±0.16*</td>
<td>1.02±0.16</td>
</tr>
<tr>
<td>BMD, L2-L4 (g/cm²)</td>
<td>1.10±0.15</td>
<td>1.00±0.16*</td>
<td>1.04±0.16</td>
</tr>
<tr>
<td>BMAD, L1-L4 (g/cm³)</td>
<td>0.16±0.02</td>
<td>0.15±0.02*</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>BMAD, L2-L4 (g/cm³)</td>
<td>0.18±0.02</td>
<td>0.17±0.02*</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>T score, L1-L4</td>
<td>-0.90 (1.68)</td>
<td>-1.70 (1.7)*</td>
<td>-1.30 (1.80)</td>
</tr>
<tr>
<td>T score, L2-L4</td>
<td>-1.00 (1.8)</td>
<td>-1.90 (1.86)*</td>
<td>-1.30 (1.97)</td>
</tr>
</tbody>
</table>

All values are mean±SD

* Significantly lower than premenopausal women (p<0.05)

SD, standard deviation; BMC, Bone Mineral Content; BA, Bone Area; BMD, Bone Mineral Density; BMAD, Bone Mineral Apparent Density.

Table 6.4: Bone parameters of dual femurs in the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal women (n=80)</th>
<th>Postmenopausal women (n=92)</th>
<th>Total (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Femoral Neck</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>4.1±0.6</td>
<td>3.6±0.6*</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>4.4±0.7</td>
<td>4.3±0.3</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>BMD (g/cm³)</td>
<td>0.92±0.14</td>
<td>0.85±0.11*</td>
<td>0.88±0.13</td>
</tr>
<tr>
<td>BMAD (g/cm³)</td>
<td>0.44±0.07</td>
<td>0.41±0.05*</td>
<td>0.43±0.06</td>
</tr>
<tr>
<td>T score</td>
<td>-0.88 (1.40)</td>
<td>-1.35 (1.05)</td>
<td>-1.10 (1.20)</td>
</tr>
<tr>
<td><strong>Total Hip</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>27.3±4.3</td>
<td>25.4±4.0*</td>
<td>26.3±4.2</td>
</tr>
<tr>
<td>BA (cm²)</td>
<td>28.2±2.0</td>
<td>28.2±2.1</td>
<td>28.2±2.0</td>
</tr>
<tr>
<td>BMD (g/cm³)</td>
<td>0.97±0.12</td>
<td>0.90±0.13*</td>
<td>0.93±0.13</td>
</tr>
<tr>
<td>BMAD (g/cm³)</td>
<td>0.18±0.02</td>
<td>0.17±0.03*</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>T score</td>
<td>-0.30 (1.58)</td>
<td>-0.80 (1.15)</td>
<td>-0.60 (1.35)</td>
</tr>
</tbody>
</table>

All values are mean±SD

* Significantly lower than premenopausal women (p<0.05)

SD, standard deviation; BMC, Bone Mineral Content; BA, Bone Area; BMD, Bone Mineral Density; BMAD, Bone Mineral Apparent Density.
Further, T scores were used to classify women as having osteopenia or osteoporosis as per WHO criteria (Figure 6.3 & 6.4).

**Figure 6.3: Prevalence of osteopenia and osteoporosis at Lumbar spine**

At L1-L4 and L2-L4, higher prevalence of osteoporosis (21-26%) was seen in postmenopausal women (T score < -2.5) compared to premenopausal women (Figure 6.3). However, at both L1-L4 and L2-L4, prevalence of osteopenia (T score between -1 and -2.5) was found to be similar between premenopausal and postmenopausal women (41-48%) indicating that a large proportion of the
premenopausal women were equally “at risk” for osteoporosis especially after achieving menopause.

Similarly, at the femoral neck, osteoporosis was observed in 8% postmenopausal women while osteopenia was seen in 57.5% postmenopausal and 41% premenopausal women. At the total hip, 1.2% of postmenopausal women had osteoporosis while 42.4% had osteopenia and 26.3% of the premenopausal women had osteopenia (Figure 6.4) indicating that even at the dual femurs, a large section of both premenopausal as well as postmenopausal women were at risk of osteoporosis in future.

6.4.3 Lifestyle factors

Based on the bone parameters it was seen that there was a higher loss experienced by postmenopausal women compared to premenopausal women. Further, to examine the role of lifestyle factors on bone mineral density of these women, data on physical activity, diet, sunlight exposure were analysed.

a) Physical activity pattern of the study population

Daily activity was classified under 3 levels considering the type and intensity of various activities reported by the women (Table 6.5). Inactivity included time spent in watching television. Light activity included activities such as personal work, household/office-desk work, reading and commuting by scooter/car/train. Moderate activity covered time spent in walking, yoga, jogging, exercises (CDC 1999). Since the daily activity data was non-normally distributed, values are expressed as median and inter-quartile range (IR).
Median time spent in sleep, personal work, household/office work, reading and commuting was similar in both premenopausal and postmenopausal women (p>0.1). However, time spent in watching television was significantly higher in postmenopausal women than premenopausal women (p<0.05). Moderate activity (exercising, walking, yoga, exercise) and light activity did not show any difference between premenopausal and postmenopausal women (p>0.1). Percentage of women with moderate activity less than 30 min/d was 23.5% in premenopausal women and 32.3% in postmenopausal women.

b) **Sunlight exposure in the study population**

Sunlight exposure in the study population was classified into 4 categories viz. Less than 15 min/d, 15-30 min/d, 30-60 min/d and 60-90 min/d. There were no significant differences observed between the premenopausal women and postmenopausal women with respect to the sunlight exposure in minutes per day (p>0.1). Majority of the women (77.8% premenopausal and 83.3% postmenopausal women) had sunlight exposure less than 30 min/d.
Based on the duration of sunlight exposure and total body surface area exposed to sunlight, sun index was computed. The median (IR) sun index was 35.4 (24.1) in pre and 23.6 (19.7) in postmenopausal women, the difference being statistically not significant (p>0.1). Sun index was further categorized into low, medium and high using percentiles of the sun index (Figure 6.5). It was seen that in both premenopausal and postmenopausal women, majority of the women were in the low and medium sun index indicating low sunlight exposure in the study population considering both the duration and exposure to sunlight.

**Figure 6.5: Sunlight exposure (sun-index) in pre and postmenopausal women**

![](image)

**c) Dietary intake**

Mean energy and protein intakes in premenopausal women were not significantly different than those of postmenopausal women (Table 6.6). Ninety percent of the premenopausal women and 97% of the postmenopausal women had their total energy intake below the Indian recommended dietary allowance (RDA, 2010) while protein intakes below RDA were found in 93% premenopausal and 97% postmenopausal women. Excess fat intake above the RDA was seen in 92.6% and 96.7% of premenopausal and postmenopausal women respectively.

In micronutrients, the mean calcium intake for premenopausal and postmenopausal women was similar (p>0.1). Eighty nine percent of premenopausal and
86% of the post menopausal women had calcium intake below RDA. Comparatively, the mean phosphorous intake in premenopausal and postmenopausal women was almost double the Indian RDA. The mean intakes of iron, zinc, β-carotene, thiamine and riboflavin for all the premenopausal and postmenopausal women were below the RDA while 79.3% and 62% of premenopausal and postmenopausal women had their ascorbic acid intake below the RDA respectively. The mean calcium: phytate: zinc molar ratio was similar in premenopausal and postmenopausal women respectively.

### Table 6.6: Nutrient intakes of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal women (n=80)</th>
<th>Postmenopausal women (n=92)</th>
<th>Total (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1574±441</td>
<td>1546±417</td>
<td>1559±428</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>36.1±12.2</td>
<td>35.0±10.6</td>
<td>35.5±11.3</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>52.5±17.2</td>
<td>50.4±18.5</td>
<td>51.4±17.8</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>239±69</td>
<td>238±64</td>
<td>239±66</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>416±154</td>
<td>434±160</td>
<td>426±160</td>
</tr>
<tr>
<td>Phosphorous (mg/d)</td>
<td>813±251</td>
<td>784±215</td>
<td>797±235</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>4.1±1.5</td>
<td>4.0±1.2</td>
<td>4.1±1.3</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>8.1±2.6</td>
<td>7.4±2.3</td>
<td>7.7±2.5</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>337±154</td>
<td>315±129</td>
<td>325±132</td>
</tr>
<tr>
<td>Copper (mg/d)</td>
<td>1.70±0.72</td>
<td>1.75±0.71</td>
<td>1.73±0.71</td>
</tr>
<tr>
<td>β-carotene (µg/d)</td>
<td>1255±803</td>
<td>1369±993</td>
<td>1316±909</td>
</tr>
<tr>
<td>Thiamine (mg/d)</td>
<td>590±220</td>
<td>554±215</td>
<td>571±213</td>
</tr>
<tr>
<td>Riboflavin (µg/d)</td>
<td>395±164</td>
<td>415±183</td>
<td>405±174</td>
</tr>
<tr>
<td>Niacin (mg/d)</td>
<td>10.2±3.5</td>
<td>9.4±3.2</td>
<td>9.8±3.4</td>
</tr>
<tr>
<td>Folic acid (µg/d)</td>
<td>83±31</td>
<td>75±22</td>
<td>79±27</td>
</tr>
<tr>
<td>Ascorbic acid (mg/d)</td>
<td>33±16</td>
<td>33±15</td>
<td>33±15</td>
</tr>
<tr>
<td>Phytate (mg/d)</td>
<td>139±55</td>
<td>135±44</td>
<td>136±49</td>
</tr>
<tr>
<td>Phytate/calcium molar ratio</td>
<td>0.074±0.035</td>
<td>0.074±0.036</td>
<td>0.074±0.035</td>
</tr>
<tr>
<td>Phytate/zinc molar ratio</td>
<td>11.5±2.4</td>
<td>11.6±2.8</td>
<td>11.5±2.6</td>
</tr>
<tr>
<td>Calcium:phytate:zinc molar ratio</td>
<td>119±43</td>
<td>121±40</td>
<td>120±41</td>
</tr>
</tbody>
</table>

*All values are Mean±SD*

The differences between premenopausal and postmenopausal women were statistically not significant for all the nutrient intakes (p>0.1)
When intakes were expressed as percent RDA, it was observed that mean intakes of energy and protein were 70% and 64.5% of the RDA in both premenopausal and postmenopausal women (Figure 6.6). In micronutrients, intake of calcium in both premenopausal and postmenopausal women was 70% of the RDA while intakes of zinc, iron, β-carotene, riboflavin and folic acid were below 50% of the RDA. Similarly, intakes of thiamine, niacin and ascorbic acid were 50-85% of the RDA indicating micronutrient deficiencies in both premenopausal and postmenopausal women (Figure 6.7).

Figure 6.6: Energy and macronutrient intakes as percent RDA in the study population

Figure 6.7: Micronutrients intakes as percent RDA in the study population
6.4.4 Clinical status of nutritional deficiency signs and symptoms in the study population

Furthermore, clinical sign and symptoms of nutritional deficiencies in these apparently healthy women were recorded (Table 6.7). For dietary calcium deficiency, unlike in children, there are no obvious signs and symptoms in adults until bone thinning occurs and fractures develop in weakened bones. In adults, symptoms can be vague, take years to develop, and may not be noticeable until advanced osteoporosis has developed.

Table 6.7: Clinical signs and symptoms in the study population

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early greying of hair</td>
<td>41.2</td>
<td>34.7</td>
<td>37.6</td>
</tr>
<tr>
<td>Reduced sense of smell</td>
<td>11.2</td>
<td>7.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Reduced sense of taste</td>
<td>30</td>
<td>30.6</td>
<td>30.3</td>
</tr>
<tr>
<td>Blackening of nails</td>
<td>8.8</td>
<td>11.2</td>
<td>10.1</td>
</tr>
<tr>
<td>White spot on nails</td>
<td>16.2</td>
<td>9.2</td>
<td>12.4</td>
</tr>
<tr>
<td>Delayed wound healing</td>
<td>22.1</td>
<td>28.9</td>
<td>25.9</td>
</tr>
<tr>
<td>Skin: Dermatitis</td>
<td>0</td>
<td>2.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Eyes: Burning of eyes/ Photophobia / eye infections/impaired vision in dark</td>
<td>26.2</td>
<td>24.5</td>
<td>25.3</td>
</tr>
<tr>
<td>Gastrointestinal: Loss of appetite / Acidity / Constipation / Frequent diarrhea / Worm infection / Vomiting</td>
<td>50</td>
<td>55.1</td>
<td>52.8</td>
</tr>
<tr>
<td>Oral: Mouth ulcers/ red tongue / Swollen Gums/ Bleeding gums</td>
<td>13.8</td>
<td>26.5</td>
<td>20.8</td>
</tr>
<tr>
<td>Heart: Fatigue / Palpitation / Lack of stamina / Breathlessness</td>
<td>47.5</td>
<td>58.2</td>
<td>53.4</td>
</tr>
<tr>
<td>Central nervous system: Forgetfulness / Lack of sleep / Lack of confidence / Irritability / Nervousness</td>
<td>52.5</td>
<td>48</td>
<td>50</td>
</tr>
</tbody>
</table>
Loss of height and stooped postures which are some of the symptoms of calcium deficiency in adults were not observed in any of the women. Symptoms like early greying of hair, decreased sense of smell, addition of extra salt, nail blackening, delayed wound healing, nervousness, irritability, lack of confidence were present in 67% of the study population which may indicate zinc deficiency. Signs of iron deficiency such as white spots on nail, fatigue, pallor, breathlessness, were present in 73% of the total women. Moreover, signs and symptoms associated with vitamin deficiencies (Vitamin A and B) were observed in 25.1 and 20.7% of the women respectively (Table 6.7). Thus, presence of the above signs and symptoms also indicated prevalence of nutritional deficiencies in the study population.

6.4.5 Biochemical parameters

There were no significant differences between mean serum levels of 25 OH-D, PTH, ionized calcium and serum zinc between premenopausal and postmenopausal women (p>0.1) (Table 6.8). Hemoglobin was significantly lower in premenopausal women as is expected due to menstruation (p<0.05). Total cholesterol and triglyceride levels were also significantly higher in postmenopausal women compared to premenopausal women (p<0.05).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal women (n=104)</th>
<th>Postmenopausal women (n=215)</th>
<th>Total (n=319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-hydroxy vitamin D (nmol/l)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2±11.4</td>
<td>26.9±17.0</td>
<td>25.6±14.7</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/l)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6±2.0</td>
<td>3.0±1.9</td>
<td>3.3±1.9</td>
</tr>
<tr>
<td>Ionized calcium (mmol/l)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03±0.11</td>
<td>1.07±0.12</td>
<td>1.05±0.12</td>
</tr>
<tr>
<td>Serum zinc (mg/dl)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.2</td>
<td>0.71±0.16</td>
<td>0.71±0.18</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.5±1.3</td>
<td>13.1±1.2*</td>
<td>12.9±1.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>192±40</td>
<td>205±39*</td>
<td>200±40</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>46.5±10.3</td>
<td>48.8±11.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.0±11.2</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>113.9±66.0</td>
<td>130.3±65.8*</td>
<td>125±66</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>123.8±32.6</td>
<td>130.6±33.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>128.2±33.0</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>23.1±13.5</td>
<td>25.8±12.6</td>
<td>24.8±13.0</td>
</tr>
</tbody>
</table>

All values are Mean±SD; ! p<0.1, * p<0.05; SD, standard deviation

<sup>a</sup> Data on 80 premenopausal women and 92 postmenopausal women

Reference range: 25-hydroxy vitamin D: >30ng/ml; Parathyroid hormone: 0.8-3.9 pmol/l; Ionized calcium: 1.13-1.18 mmol/l; Serum zinc: 0.7-1.2 mg/l; Hemoglobin: 12-14 g/dl; Total cholesterol: <200 mg/dl, Triglyceride: <150 mg/dl

Vitamin D insufficiency (<50nmol/l) (Lips 2001) was found in 96.2% of premenopausal and 84.7% of postmenopausal women. Percentage of postmenopausal women who had their ionized calcium concentration below the normal range (< 1.13 mmol/l) was higher than that of premenopausal women (Figure 6.8). Serum zinc deficiency was seen in 55.4 and 54.2% of premenopausal and postmenopausal women respectively. The prevalence of anemia was 31.5% and 14.3% in premenopausal and postmenopausal women respectively (Figure 6.8). Hypercholesterolemia was observed in 42.7% premenopausal and 57% postmenopausal women (Figure 6.9).
Figure 6.8: Percent prevalence of biochemical deficiencies in the study population

Figure 6.9: Percent prevalence of lipid abnormalities in the study population
6.4.6 Association of bone parameters with anthropometric and lifestyle factors in women

Results so far indicate micronutrient deficiencies of calcium, vitamin D and zinc in both premenopausal as well as postmenopausal women. Also, a high prevalence of osteoporosis was seen in postmenopausal women while a large section of premenopausal women showed osteopenia. To further examine the relative influence of diet, anthropometry and lifestyle factors along with menopausal status on bone mineral density, correlation analysis was carried out. Associations of various factors with bone mineral density were studied in the entire study cohort of women by considering menopausal status and age at menarche as independent variables.

Partial correlation coefficients controlling for age revealed significant positive associations between BMD (at all three sites i.e. lumbar spine, femoral neck, total hip) and height, weight, BMI, waist and hip circumference (p<0.05) (Table 6.9).

Table 6.9: Correlation coefficients of bone parameters with anthropometric factors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L2-L4 BMD</th>
<th>Total Hip BMD</th>
<th>Femoral Neck BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>0.49**</td>
<td>0.60**</td>
<td>0.51**</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.42**</td>
<td>0.51**</td>
<td>0.44**</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>0.30*</td>
<td>0.48**</td>
<td>-0.33**</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>0.23*</td>
<td>0.35*</td>
<td>0.47*</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>0.35*</td>
<td>0.46*</td>
<td>0.41*</td>
</tr>
<tr>
<td>Body fat %</td>
<td>0.23!</td>
<td>0.34*</td>
<td></td>
</tr>
<tr>
<td>Age at menarche (yr)</td>
<td>-0.25**</td>
<td>-0.17</td>
<td>-0.03</td>
</tr>
<tr>
<td>Years since menopause (yr)</td>
<td>-0.33**</td>
<td>-0.2</td>
<td>-0.34**</td>
</tr>
</tbody>
</table>

Adjusted for age
! p<0.1, * p<0.05, ** p<0.01

Age at menarche was found to be negatively associated with lumbar spine BMD (r = -0.25, p<0.01) while similar correlations at femoral neck and total hip though negative were not statistically significant (p>0.1). A significant negative association was obtained between years since menopause and lumbar spine BMD.
and femoral neck BMD (p<0.01). Years since menopause were also negatively associated with total hip BMD (r =-0.2), although the difference was only marginally significant (p<0.1).

Correlation coefficient between physical activity and BMD after controlling for age and BMI were positive for moderate activity and negative for inactivity, though not significant at all three sites (p>0.1). Partial correlations of BMD and nutrient intakes controlling for energy intake and age revealed that lumbar spine BMD was significantly 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). BMD of the femoral neck and total hip were positively correlated with calcium (r=0.14), phosphorus (r=0.14), riboflavin (r=0.15) and calcium: phytate: zinc ratio (r=0.14) though statistically not significant (p>0.1). All other nutrient intakes were not associated with BMD at any of the three sites.

In biochemical parameters, partial correlations of BMD controlling for age at lumbar spine, femoral neck and total hip with serum 25 OH-D, ionized calcium and zinc were positive and of the order of 0.1, though statistically not significant (p>0.1). Similarly a negative but non-significant correlation of PTH was obtained with BMD at all three sites. However, correlation coefficient of total hip Z score with serum 25 OH-D levels (r = 0.16) was statistically significant (p<0.05) though not with lumbar spine and femoral neck Z scores.

**Sunlight exposure and its association with 25 OH-D levels and bone status**

The median (IR) sun index was 35.4 (24.1) in premenopausal women and 23.6 (19.7) in postmenopausal women, the difference being statistically not significant (p>0.1). Therefore, the association of sunlight exposure with 25 OH-D status or bone status was explored in the pooled cohort. Levels of 25 OH-D showed an increase from 26.6±17.3 nmol/l to 30.5±20.2 to 38.1±19.9 nmol/l with increasing sunlight exposure categories (low, medium and high) (F=112.93, p<0.001), the Spearman correlation coefficient between sunindex and 25 OH-D being marginally significant (r=0.13, p<0.1) (Figure 6.10) indicating association of sunlight exposure with Vitamin D.
To assess the relationship between sunlight exposure and BMD, Spearman rank correlation between sun index and BMD were computed (Figure 6.11). Association of sun index and BMD was examined between presence of osteopenia and osteoporosis using T-scores.

**Figure 6.10**: Association of Vitamin D status across sun index categories

![Vitamin D status across sun index categories](image)

**Figure 6.11**: Percent prevalence of osteopenia and osteoporosis with respect to sun light index

![Percent prevalence of osteopenia and osteoporosis](image)

*Normal, T score > -1; Osteop, T score < -1*

*L2-L4, Lumbar spine; FN, Femoral neck; TF, Total Hip*
Percentage of women with high sun index was higher in normal women (16.1%) than the women with osteopenia and osteoporosis (6.1%) at lumbar spine, the difference was marginally significant (p=0.09). At other sites, i.e., femoral neck and total hip, such differences were not statistically significant (p>0.1)

6.4.7 Association of menopausal status with bone loss

To explore the change in BMD with menopausal stage, premenopausal stage was sub classified into two groups; women between 40 to 45 years of age as premenopausal and above 45 years of age as perimenopausal stage (Bainbridge et al, 2002). Similarly the postmenopausal group was divided as those with less than 5 years since menopause and those with more than 5 years since menopause because the rates of bone loss are reported to be highest in the early postmenopausal period (Ahlborg et al, 2001).

Figure 6.12: Changes in BMD at three sites with menopausal stage

When changes in mean lumbar spine BMD were examined from premenopausal years to increasing stages of menopause, a significant decline was observed in BMD from first group of premenopausal women to the last group of
postmenopausal women (F=10.729, p<0.001) (Figure 6.12) giving an average decrease of 5.7%. Similar average decrease was observed for BMD at femoral neck (4.7%) and total hip (4.0%) from premenopausal to postmenopausal stage (p<0.01) indicating increase in bone loss with increasing menopausal stages.

6.4.8 Determinants of bone mineral density

So far results revealed that age, height, weight, fat%, sunlight exposure, age at menarche, dietary nutrient intakes and increasing menopausal stages had a significant influence on bone loss. Therefore, to examine relative significance of these parameters on BMD at each site, generalized linear model was fitted for the entire cohort of women (Table 6.10).

Results indicated that weight and increasing menopausal stage were significant factors influencing BMD in women at all three sites. Additionally, for lumbar spine age, height and age at menarche were other factors influencing BMD. For femoral neck BMD, increasing sun index category as well as advancing stage of menopause were the major factors. For total hip region, fat% and increasing menopausal stage were the major determinants. Moderate activity, inactivity and energy adjusted intake of calcium though included in the model were not found to be significant at any of the three sites (p>0.1). Thus, anthropometric parameters along with menopause were the major factors influencing BMD in women above 40 years of age.
Table 6.10 Significant factors influencing bone mineral density at the three sites using generalized linear model analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lumbar Spine (L2-L4) BMD</th>
<th>Femoral Neck BMD</th>
<th>Total hip BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β±S.E (95% CI)</td>
<td>β±S.E (95% CI)</td>
<td>β±S.E (95% CI)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>-0.006±0.003* (-0.011-0.00)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.007±0.002** (0.003-0.011)</td>
<td>0.005±0.002** (0.001-0.008)</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.003±0.001** (0.001-0.006)</td>
<td>0.004±0.001** (0.002-0.006)</td>
<td>0.004±0.001** (0.002-0.006)</td>
</tr>
<tr>
<td>Fat%</td>
<td>-</td>
<td>-</td>
<td>0.005±0.002! (0.000-0.009)</td>
</tr>
<tr>
<td>Age at menarche (yr)</td>
<td>-0.017±0.008* (-0.032-(-0.002))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sunindex low</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sunindex medium</td>
<td>-</td>
<td>0.050±0.031! (-0.010-0.11)</td>
<td>-</td>
</tr>
<tr>
<td>Menopausal stage I</td>
<td>-</td>
<td>0.094±0.039* (0.017-0.172)</td>
<td>0.091±0.293* (0.033-0.148)</td>
</tr>
<tr>
<td>Menopausal stage II</td>
<td>0.088±0.037* (0.015-0.162)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constant</td>
<td>0.134±0.40 (-0.653-0.920)</td>
<td>-0.280±0.314 (-0.895-0.336)</td>
<td>0.168±0.34 (0.835-0.243)</td>
</tr>
</tbody>
</table>

Variables included in the analysis were: age, height, weight, fat percentage, age at menarche, moderate activity, inactivity, energy adjusted intakes of calcium, Menopause stages, sun index categories. *p<0.05, **p<0.01

Moderate activity, inactivity, energy adjusted intake of calcium, and postmenopause less than 5 years were not found to be significant for any of the three sites (p>0.1)

To investigate the contribution of menopause towards decrease in BMD in women after accounting for the significant factors from generalized linear model, general linear model was used to estimate adjusted means of BMD in premenopausal and postmenopausal women. Total percent change in BMD was observed to be 7%, 8.8% and 7.3% in lumbar spine, femoral neck and total respectively. Of the total change, height accounted for major share of 3.9% at the
lumbar spine, 0.9% at femoral neck and 2.6% at total hip. After adjusting for all the factors, age, weight and height, it was revealed that percent change of 2.1%, 2.5% and 4.5% for lumbar spine, femoral neck and total hip respectively remained which may be attributed to menopause.

Further, to examine factors other than menopause that are responsible for bone loss in women above forty years of age, separate stepwise multivariate regression models were fitted for premenopausal and postmenopausal women so as to assess different factors (if any), in the two stages (Table 6.11). In premenopausal women, height, weight and age were significant factors associated with BMD at lumbar spine ($R^2=0.265, p<0.05$). Height, weight and fat % were found to be predictors of BMD at femoral neck region while height and weight were found to be associated with BMD at the total hip. In postmenopausal women, height, weight and years since menopause were found to be important predictors of BMD at the lumbar spine and femoral neck region. At the total hip, weight alone was significantly associated with BMD.

Table 6.11: Anthropometric factors as determinants of bone mineral density

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Premenopausal</th>
<th></th>
<th>Postmenopausal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar Spine BMD (L2-L4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.0087*</td>
<td>0.004</td>
<td>0.007*</td>
<td>0.003</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.0031*</td>
<td>0.001</td>
<td>0.0032*</td>
<td>0.001</td>
</tr>
<tr>
<td>Years since menopause (yr)</td>
<td>-</td>
<td>-</td>
<td>-0.0043*</td>
<td>0.002</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>-0.0078*</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Femoral Neck BMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.0095*</td>
<td>0.004</td>
<td>0.0047*</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.0058**</td>
<td>0.002</td>
<td>0.0036**</td>
<td>0.001</td>
</tr>
<tr>
<td>Years since menopause (yr)</td>
<td>-</td>
<td>-</td>
<td>-0.0047*</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat %</td>
<td>-0.01*</td>
<td>0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Hip BMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.0077*</td>
<td>0.004</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.0043**</td>
<td>0.001</td>
<td>0.0038**</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* $p<0.05$, ** $p<0.01$

BMD, Bone Mineral Density

Age at menarche, sun index, moderate physical activity, inactivity and dietary intakes of calcium included in the model but were not found significant
6.5 Discussion

In this cross-sectional study on urban Indian women over 40 years of age, all the participants were apparently healthy with no known clinical history of osteoporosis or any other bone-related disorders. Prevalence of osteopenia was found to be high in premenopausal women at all three sites (26-42%) with osteoporosis seen only at the lumbar spine in 6.2% women. This indicates that rapid bone loss starts few years before onset of menopause. In postmenopausal women, osteoporosis was more at the lumbar spine (26.1%) than at the femoral neck (8%) or total hip (1.2%).

Similar high prevalence of osteoporosis at the lumbar spine in postmenopausal women (17.8%) and high prevalence of osteopenia (44%) in premenopausal women has been reported by Mittal et al (2011) in apparently healthy urban Indian women. Another study in Indian women above 50 years of age also reported the prevalence of osteoporosis and osteopenia to be 42.5% and 45% respectively (Marwaha & Tandon, 2011). The current findings also agree well with those reported in a study from Tehran on early postmenopausal women, wherein 36% women were found to be osteopenic and 26% were osteoporotic at lumbar spine, while at the total hip 42.5% were osteopenic, and 1.4% osteoporotic (Rassouli et al, 2001). However, prevalence of osteoporosis and osteopenia in the present study are higher than those reported in premenopausal Dutch women (27.3% for osteopenia and 4.1% for osteoporosis) (Smeets-Goecaers et al, 1998) and in Canadian women aged ≥50 years (12.1% at the lumbar spine and 7.9% at the femoral neck for osteoporosis) (Tenenhouse et al, 2000). However, prevalence of osteoporosis and osteopenia in the present study are higher than those reported in premenopausal Dutch women (27.3% for osteopenia and 4.1% for osteoporosis) (Smeets-Goecaers et al, 1998) and in Canadian women aged ≥50 years (12.1% at the lumbar spine and 7.9% at the femoral neck for osteoporosis) (Tenenhouse et al, 2000).

In the present study on apparently healthy women above 40 years of age, the prevalence of obesity based on body mass index was found to be 61% in premenopausal women and 67% in postmenopausal women respectively. Similar high prevalence of obesity has been reported in a study from North India where the prevalence of obesity in premenopausal and postmenopausal women was 70.3 and 75% respectively (Khokhar et al, 2010). Considering the dietary intakes, it was observed that majority of the women had low intakes of calcium, zinc, iron, β-carotene, thiamine and riboflavin compared to Indian RDA. Similar results were reported by Mittal et al (2011) in healthy pre and postmenopausal Indian women.
where the mean intake of calcium was 515.7 ± 145.2 mg/d and 442 ± 157.7 mg/d in pre and post menopausal women respectively. In addition, low intakes of micronutrients in Indian women has been reported in National surveys (NNMB, 2002). Also, the diet of women in the present study was high in phytate/calcium ratio. Intake of a diet which is rich in phytate, retards the absorption of calcium from the gut. Panwar and Punia (2000) have shown that the calculated values for all nutrients are significantly higher than the analytical values. Therefore, with 89% pre and 86% postmenopausal women having their calcium intake already below the RDA along with high deficiency of vitamin D, the implications would be detrimental for their bone health.

The mean serum 25 OH-D concentration was seen to be 48 nmol/L in premenopausal women from Indonesia and Malaysia (Mithal et al, 2009) which were higher than that found in premenopausal women in the present study (24.2 nmol/l) indicating higher prevalence of 25 OH-D insufficiency in the study population. Also, almost half of the women exhibited 25 OH-D insufficiency in spite of the availability of adequate sunlight which is one of the major sources of vitamin D. Similar high prevalence of 25 OH-D deficiency was reported by Harinarayan et al (2008) in Indian post menopausal women from South India. The current estimates of vitamin D deficiency also agree well with those reported in postmenopausal women from South- East Asian countries; i.e., 47% in Thailand, 49% in Malaysia, 90% in Japan and 92% in South Korea (Mithal et al, 2009). Furhermore, though 25 OH-D deficiency was observed in both the groups, the PTH levels in both pre and post menopausal women were within the normal range. Harinarayan et al (2005) in their study on Indian postmenopausal women have showed that the intact PTH levels were relatively equal in the pre and postmenopausal women on the background of 25 OH-D deficiency suggesting the possibility of PTH-mediated bone resorption to be absent. The present study results are in line with these findings.

A weak association of sunlight exposure and serum vitamin D levels was observed in the study population. This may be because mean sunlight exposure of women was less than 30 minutes/day in 83.3% postmenopausal and 77.5% of premenopausal women. In a study from Hawaii, despite abundant sun exposure,
51% of the healthy adults had low vitamin D status (Binkley et al, 2007) indicating variable responsiveness to UVB radiation evident among individuals.

Considering the influence of various factors on bone health of women above 40 years of age, it was seen that in addition to age, height, weight and fat %, age at menarche, years since menopause was found to be an important predictor of BMD in postmenopausal women. The findings are in agreement with those reported by other studies where that time elapsed after the menopause onset as one of the main determinants for osteoporosis in postmenopausal women (Akdeniz et al, 2009) with increased weight being significantly associated with increased bone mineral status in both premenopausal and postmenopausal women (Kroger et al, 1994; Harris et al, 1992). Further, of the total decline in bone mineral density, 3% of the change was attributable to menopause with height being the other major determinant besides age and weight. Further generalized linear model revealed that women nearing menopause and those in early years of menopause are at greater risk of low BMD. Thus, even in premenopausal women, bone loss is evident and in light of the above findings, steps to improve bone health should target women before the attainment of menopause.

Also, it was observed that age at menarche was negatively associated with BMD at lumbar spine. Similar results have been reported by Ito et al (1995) in their study where they found a significant inverse relationship between early menarche and BMD. A weak correlation between serum 25 OH-D levels with lumbar spine BMD has been reported in early postmenopausal women from Tehran (2001). Our study also reports weak association of 25 OH-D with BMD at all the three sites. Similarly, Roy et al (2004) have reported that in South Asian women, a decrease in serum 25 OH-D level <15 ng/ml is associated with a progressive reduction in bone mass at the hip and wrist. The rate of decline in BMD in our study population ranged from 4% - 5.7% from pre to post menopausal stage. Similar accelerated BMD loss at both the hip and spine has been reported during the time of transition from peri- to post-menopause in US women (Guthrie et al, 1998). A study conducted in Moroccan women showed an annual rate of decline in BMD ranging from 0.35% to 0.6% from 40 -79 years (El-Maghraoui et al, 2006).
In conclusion, our study reveals that there was high prevalence of osteopenia in premenopausal women, while the prevalence of osteoporosis was higher in post menopausal women. Except for the menopausal state, other factors determining bone loss such as age, height, weight, low calcium intake and poor sunlight exposure were similar in both pre and post menopausal women, suggesting the need to improve upon these factors in all women above 40 years of age. It is crucial for postmenopausal women to increase their calcium and vitamin D intake, as well as sunlight exposure to combat bone loss due to menopausal state. Deficiency of 25(OH) D along with low dietary intake of calcium even in the high income groups is an alarming finding of our study. It is thus necessary to create awareness in women about risk of osteoporosis especially in the early years of menopause and need for taking preventive measures for avoiding future risk of fractures.

Part of these results are published under two research articles:


- Nidhi Kadam, Shashi Chiplonkar, Anuradha Khadilkar, Uma Divate and Vaman Khadilkar. Low bone mass in urban Indian women above 40 years of age: prevalence and risk factors Gynecol Endocrinol 2010; 26(12):909-17