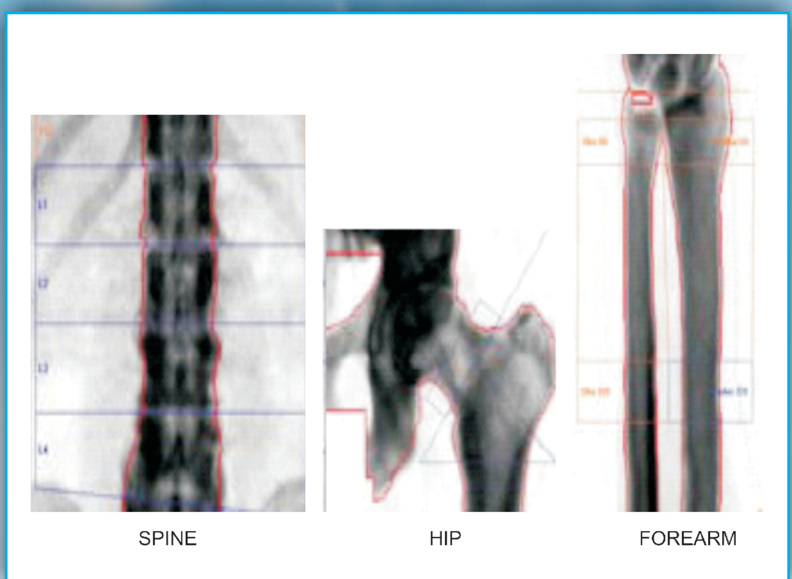
POPULATION BASED REFERENCE STANDARDS OF PEAK BONE MINERAL DENSITY OF INDIAN MALES AND FEMALES - AN ICMR MULTI-CENTRE TASK FORCE STUDY



INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

Population based reference standards of Peak Bone Mineral Density of Indian males and females – an ICMR multi-centre task force study

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INDIAN COUNCIL OF MEDICAL RESEARCH
New Delhi
2010

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Published by:
Director-General
Indian Council of Medical Research
New Delhi 110 029

March, 2010

ISBN : 978-81-910091-0-1

Production Controller:
J.N. Mathur, Press Manager, ICMR, New Delhi

Printed at: Aravali Printers & Publishers Pvt. Ltd., W-30, Okhla Industrial Area, Phase-II, New Delhi - 110 020

Preface

Osteoporosis is emerging as a major public health problem, especially among the elderly population in India. Both males and females are afflicted by osteoporosis. The disease progresses silently and weakens the bones to the extent that even an innocuous fall may result in fracture of the hip, spine or the forearm. The diagnosis of osteoporosis is based on assessment of inadequate peak bone mass. Most of the standards used for the diagnosis of osteoporosis are drawn from the western population. It is therefore prudent and necessary to have reference values of our own population to avoid false positive and false negative findings in patients evaluated for osteoporosis. Keeping this need in view, the ICMR undertook a community based multi-centre study from January 2001 to July 2006 to determine the Peak Bone Mineral Density (PBMD) values at hip, forearm and lumbar spine of healthy Indian males and females aged 20-29 years so that estimates of prevalence of osteoporosis and osteopenia in India could be examined in a better fashion or in the right perspective.

I am happy that the study had not only established standards of peak bone mineral density (BMD) of Indian males and females but had also evaluated various other factors affecting BMD of the Indian population. In common with the West, Indian population is also rapidly graying throwing up new challenges to the already overburdened health services.
The findings of the study should therefore go a long way in diagnosing osteoporosis in India and help researchers to develop preventive strategies as well as to look for effective drugs and other interventions to combat this serious emerging health problem.

(Vishwa Mohan Katoch)
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Executive Summary

Osteoporosis, a silently progressing metabolic bone disease that leads to loss of bone mass, is expected to be widely prevalent in India and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women. The diagnosis of osteoporosis is the end result of an inadequate peak bone mass or a rapid bone loss in people of age 40 years or more. It is known that peak bone mass or calcium accretion in the bones is reached in the early twenties and no future increase is possible after that. Reports of the age at peak bone mass vary from 20 to 30 years. Most of the standards used for the diagnosis of osteoporosis are based on the Western population. Therefore ICMR undertook a community based multi-centre study from January 2001 to July 2006 to determine the Peak Bone Mineral Density (PBMD) values at hip, forearm and lumbar spine for healthy Indian males and females aged 20-29 years so that estimates of prevalence of osteoporosis and osteopenia in India could be revised. A total of 808 subjects including 404 males and 404 females were enrolled in the study. The reference standards of BMD obtained in the present study at the three sites namely, total hip, forearm and lumbar spine were 0.988 0.131, 0.611 0.052 and 0.976 0.105 gm/cm^2 respectively in males and 0.901 0.111, 0.538 0.044 and 0.954 0.095 gm/cm^2 respectively in females. The estimates obtained in the present study were found to be significantly lower than the corresponding NHANES III and Hologic reference standards currently under use. However, the impact of racial differences in bone shape and height on BMD is very important issue and remains to be explored further. The impact of various demographic, physical, clinical and biochemical factors on BMD at the three sites was also assessed. Amongst the demographic parameters, the nature of physical activity performed had a strong impact on BMD. Amongst the physical parameters, height, weight and BMI all significantly affected BMD with weight alone contributing maximum to the BMD. Amongst the biochemical parameters, serum albumin, serum alkaline phosphatase, serum vitamin D and serum PTH had all affected BMD at one site or the other.

Contents

1. Introduction

Osteoporosis, a silently progressing metabolic bone disease that leads to loss of bone mass, is expected to be widely prevalent in India[1] and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women. It is a systemic skeletal disease characterized by low bone density and micro architectural deterioration of bone tissue. The consequent increase in bone fragility results in an increased risk of fractures of the spine, hip and the forearm. The diagnosis of osteoporosis is the end result of an inadequate peak bone mass or a rapid bone loss in post menopausal women. It is known that peak bone mass or calcium accretion in the bones is reached in the third decade of life. Reports of the age at peak bone mass vary from 20 to 30 years. It is also related to growth and dietary intake of calcium, minerals, protein and other nutrients necessary for bone formation and calcium accretion during the growth spurts. It is possible that Total Bone Mineral Content (TBMC) and peak bone mass may be compromised in Indians as evidenced by their short stature, a feature of skeletal growth retardation in the absence of adequate nutrition.

Further, it is believed that the major determinant of peak bone mass is genetic[2] but it is now known that calcium and vitamin D nutrition may have a greater effect both during the growth phase and also during adulthood and even in the older age group.[3, 4] Studies in postmenopausal women indicate lower rates of bone loss with calcium supplementation of approximately 1000 to 1500mg/day[5] and decreased fracture rates have also been demonstrated with calcium supplementation.[6]

With low dietary intakes of calcium in Indian women from the low socio-economic group, (250-350mg/day against an RDA of over 800mg to 1gm in the West it may be assumed that in spite of growth retardation, Indians would have lower bone mineral densities at all age groups. In addition foods rich in calcium such as milk and cheese are expensive or used in small quantities in our diets.

Reports suggest that vitamin D deficiency may be a problem in certain parts of the country contributing to a lowered BMD though osteomalacia is not known to be associated with osteoporotic fractures. Some workers[7] suggest that vitamin D deficiency osteomalacia with secondary hyperparathyroidism may be the most important differential diagnosis. However, biochemical markers of vitamin D deficiency may be of use. Adequate vitamin D levels in the presence of low calcium intakes may result in increased osteoclastic activity for the normal maintenance of serum calcium levels. Low serum calcium levels due to poor calcium in the diet increase parathormone secretion which enhances the conversion of 25 (OH)D3 to 1,25(OH) D3, which in turn destroys 25 (OH) D3 leading to further reduced absorption of calcium from the gut.

The role of oestrogens has been acknowledged in the rapid fall of BMD during the menopausal age in Western studies. Indian women from the poor socio-economic group have had low circulating levels of oestrogens during their pregnancies, with possibly reduced bone turnover, resulting in lowered BMD in the reproductive age group. In addition, low body weights in the menopausal age are not conducive to the peripheral conversion of androstenidione to oestrogens. Therefore with mean weights of 42kg it is possible that circulating levels of oestrogens are not adequate to maintain the BMD. The interaction of low oestrogens with low calcium levels may further worsen bone thinning.

Most of the standards used for the diagnosis of osteoporosis are based on the Western population. Moreover, geographic, ethnic and socio-economic factors are known to affect bone mass significantly.[8-15] It, therefore, seems prudent to obtain and use regionally derived BMD reference values. Further, the locally derived reference values are important to avoid false positive and false negative findings in patients evaluated for osteoporosis. Realizing this need, ICMR undertook a community based multi-centre study to determine the Peak Bone Mineral Density (PBMD) values at hip, forearm and spine for healthy Indian males and females aged 20-29 years so that estimates of prevalence of osteoporosis and osteopenia in India could be revised based on these new standards. The present study was carried out during January 2001- July 2006.

2. Methodology

For establishing reference values of BMD, men and women aged 20-29 years who “had no constraints” to growth and bone mineralization during childhood and adolescence were enrolled. The following check list was used to define the 'no constraints' group.

Subjects from the upper socio-economic group with normal BMI (18.5-25.0 Kg/m^2) were recruited from a locality which is known to have people from the upper socio economic group i.e. Grade A colony and where the selected subjects have been living for more than ten years, or else the investigator ensured that the family belonged to Higher Income Group (HIG) by other objective criteria.

The following exclusion criteria were followed for enrolling the subject into the study:

Chronic Diseases:

History- of epilepsy, asthma, high blood pressure, diabetes
- of other metabolic diseases like hyper and hypothyroidism
- Malabsorption syndrome
- of fractures

Drug intakes:

- On antitubercular/antiepileptic drugs (last two years)
- On steriods
- History of any other drugs intake like contraceptives etc.

Life style factors:

- Current smoker
- Regular consumption of alcohol
- State level representation in sports events
- Childhood allergy to milk (lactose intolerance)
- Having high fluoride levels in drinking water
- Dental mottling
- Resident in fluorotic area during childhood

History of

- Delayed menarche (>18 years)
- Delayed puberty in boys
- Pregnant or breastfeeding within last 12 months
- More than 3 live births in women
- Hysterectomy
- Amenorrhea (more than 3 months duration or irregular cycles)
- Polio or obvious physical disabilities
- Chronic immobilization
- Joint problems like rheumatoid arthritis etc.
- Use of any radio isotope for clinical investigation in the last one month

2.1 Participating centres:

The following four centres participated in the study

1. National Institute of Nutrition (NIN)
   Jamai-Osmania
   Hyderabad – 500 007

2. Department of Endocrinology & Metabolism
   All India Institute of Medical Sciences (AIIMS)
   Ansari Nagar
   New Delhi-110029

3. Department of Endocrinology
   Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS)
   Raibareli Road
   Lucknow – 226 014

4. National Institute for Research in Reproductive Health (NIRRH)
   Jehangir Merwanji Street
   Parel, Mumbai – 400 012

2.2 Sample size:

Assuming a mean BMD of 0.9 gm/cm^{2}and SD of 0.2 gm/cm^2 with a confidence level of 95% and with a relative error of 5%, a sample size of 100 was expected to provide a true estimate of BMD. Hence each centre enrolled 100 men and 100 women.

2.3 Selection of area and enrollment of subjects:

After selecting an HIG area having a minimum of 1000 households covering a diverse population (possibly of different castes), it was divided in to four segments. Twenty five subjects of each sex aged 20-29 years from each of the four segments were then enrolled in the study following the above exclusion criteria.

2.4 Type of information collected

In addition to the information on demographic, physical and clinical characteristics, the following information was collected from each individual enrolled in the study.

(i) Dietary assessment of calcium intake by a food frequency questionnaire.

(ii) Biochemical tests (on all subjects):

The following biochemical parameters were assessed:

- Haemoglobin
- Serum albumin
- Serum calcium
- Serum phosphorus
- Serum creatinine
- Serum alkaline phosphatase
- Serum 25 hydroxy vitamin D3
- Serum parathormone
- Random urine fluoride levels

The quantitative determination of 25-hydroxyvitamin D was done using a 125-I based radioimmunoassay 25-OH-D. The kits for these assays were obtained from Dia Sorin Inc., Stillwater, Minnesota, U.S.A.

The quantitative determination of intact human parathyroid hormone (PTH 1-84) was done using an immunoradiometric assay (IRMA) for PTH. The kits for these assays were obtained from DSL Inc., Webster, TX, USA.

(iii) Bone Mineral Density measurements at the following sites using DEXA

- Femoral neck (Right)
- Left forearm for right hander and vice versa
- Spine (L1-L4) AP
- Whole body
- Left femur and non-dominant forearm was scanned unless technically not feasible

The sunlight exposure and physical activity scores were also assessed as per the protocol developed.

2.5 Quality assurance of biochemical and DEXA investigations:

Inter and intra laboratory quality assurance assays for the above-mentioned biochemical parameters were performed. All the laboratory methods were standardized with the coefficient of variation (CV) for most of the biochemical parameters varying from 1-5 % amongst the centres. All the assays were carried out at the centres and CV calculated. Thirty samples of sera were also exchanged from AIIMS to other centres and the results were found to be satisfactory with CV varying between 1-7%.

Similarly, a phantom was taken to all the participating centres and the Hologic DXA machine 'QDR-4500' used by each centre (series A – AIIMS, SGPGIMS; series B – NIN, NIRRH) for estimating BMD was standardized. Four scans at each site were taken and the coefficient of variation amongst all the participating centres was found to vary between 0.5-1.1 percent.

The third National Health and Nutrition Examination Survey (NHANES III, 1988-94) based on the nationally representative sample of US adults provided reference standards for diagnosing osteoporosis at Hip and Hologic reference standards (Hologic, Waltham, Mass.) for diagnosing osteoporosis at Lumbar spine and Forearm were used by the DXA machine at each centre. These reference standards are as given below:[16-18]

	Sex	Total Hip	Total Forearm	Total Lumbar Spine
NHANES III	Male	1.041 ± 0.144	-	-
	Female	0.942 ± 0.122	-	-
Hologic	Male	-	0.679 ± 0.054	1.120 ± 0.110
	Female	-	0.564 ± 0.051	1.084 ± 0.111

All the information was collected and recorded in pre-coded proformae developed for the present study.

3. Results and discussion

A total of 808 subjects including 404 males and 404 females were enrolled in the study. Table1 describes the number of subjects enrolled at each centre sex wise.

Table 1. Number of subjects enrolled at each centre

Centre	Number of subjects enrolled		Total
	Male	Female	
NIN, Hyderabad	104	107	211
AIIMS, New Delhi	100	98	198
SGPGIMS, Lucknow	100	99	199
NIRRH, Mumbai	100	100	200
All	404	404	808

All the data were analysed by various sets of parameters for males and females separately. The correlation analysis followed by one way and two way analysis of variance (ANOVA) was carried out to obtain evidence of their impact, if any on BMD. Post-hoc analysis was also used to elicit centre wise differences. P-values below 0.05 were considered significant. A multiple regression analysis was also attempted using BMD at the three sites (namely, hip forearm and spine) as outcome (dependent) variable and various other factors as identified during the course of correlation and ANOVA analysis as independent variables. Results are described in the following sections:

3.1 Bone Mineral Density (BMD) at hip, forearm and spine and at other sub-sites

The mean BMD, BMC (Bone Mineral Content) and area at hip, forearm, lumbar spine and whole body and at

Table 1.1a. Mean BMD SD (gm/cm^2) at Hip, Forearm and Spine and at other sub-sites of healthy normal males

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	0.887±0.120	0.878±0.134	0.882±0.123	0.929±0.141	0.894±0.131
Trochenta	0.748±0.105	0.721±0.126	0.690±0.103	0.735±0.110^{a}	0.723±0.113
Intertrochenta	1.171±0.126	1.116±0.148	1.126±0.140	1.160±0.133^{a}	1.142±0.138
Hip	0.999±0.114	0.971±0.155	0.961±0.117	1.022±0.127^{a}	0.988±0.131
Forearm-					
1/3	0.755±0.049^{b}	0.698±0.061	0.747±0.051^{c}	0.700±0.060	0.725±0.062
Mid	0.653±0.044^{b}	0.611±0.049	0.646±0.048^{c}	0.609±0.044	0.630±0.050
UD	0.485±0.052^{b}	0.449±0.051	0.478±0.049^{c}	0.448±0.053	0.465±0.054
Forearm	0.638±0.049^{b}	0.589±0.050	0.626±0.045^{c}	0.590±0.043	0.611±0.052
Spine-					
L_1	0.921±0.110	0.917±0.109	0.903±0.108	0.877±0.104^{e}	0.904±0.109
L_2	1.003±0.105	0.998±0.118	0.982±0.103	0.950±0.113^{d}	0.983±0.112
L_3	1.017±0.118	1.024±0.118	1.004±0.106	0.969±0.103^{d}	1.004±0.113
L_4	1.006±0.101	1.027±0.118	1.002±0.116	0.976±0.100^{f}	1.003±0.110
Spine	0.987±0.095	0.996±0.116	0.976±0.102	0.946±0.099^{d}	0.976±0.105
Wholebody	1.170±0.083 (101)	-	1.104±0.063 (62)	1.116±0.082 (99)	1.134±0.083 (263)

Figures in parenthesis indicate number of observations

$^{a}p<0.05$ – compared to AIIMS and SGPGIMS; $^{b}p<0.0001$ – compared to AIIMS and NIRRH; $^{c}p<0.0001$ – compared to AIIMS and NIRRH; $^{d}p<0.05$ – compared to NIN and AIIMS; $^{e}p<0.05$ – NIN vs. NIRRH; $^{f}p<0.05$ – AIIMS vs. NIRRH other sub-sites in males and females is as given in tables 1.1a-1.3a and 1.1b-1.3b respectively.

The mean BMD at total hip of males in NIRRH was significantly higher (P<0.01) than that of males in AIIMS and SGPGIMS. The mean BMD at total forearm of males in NIN was significantly higher (p<0.0001) than that of males in AIIMS and NIRRH. Further, the mean BMD at total forearm of males in SGPGIMS was significantly higher (p<0.0001) than that of males in AIIMS and NIRRH. The mean BMD at total lumbar spine of males in NIRRH was significantly lower (P<0.03) than that of males in NIN and AIIMS.

In females, the mean BMD at total hip in NIRRH was significantly higher (P<0.02) than that of females in the other three centres. Further, the mean BMD at total hip of females in NIN was significantly higher (P<0.0001) than that of females in AIIMS and SGPGIMS. The mean BMD at total forearm of females in NIRRH was significantly lower (P<0.0001) than that of females in NIN and SGPGIMS. Further, the mean BMD at total forearm of females in AIIMS was significantly lower (p<0.001) than that of females in the other three centres. The mean BMD at total lumbar spine of females in NIN was significantly higher (P<0.008) than that of females at AIIMS. No other significant differences were observed with regard to mean BMD at total spine of females amongst other centres.

Levene's test of homogeneity of variances was applied and no centre wise significant difference in variances of BMD at any of the three sites was observed thereby enabling pooling of data of all the centres. The absolute relative differences in BMD at hip with regard to pooled BMD at hip were found to vary between 1.1-3.4 % in males over the four centres. Similarly, the absolute relative differences in BMD at forearm and lumbar spine with regard to pooled BMD at forearm and lumbar spine were found to vary between 2.5-4.5 % and 0-3.1 % respectively in males over the four centres. Further, the absolute relative differences in BMD at hip, forearm and lumbar spine with regard to pooled BMD at hip, forearm and lumbar spine were found to vary between 2.7-7.3 %; 1.9-5.6 % and 0-2.6 % respectively in females over the four centres.

Table 1.2a. Mean BMC ± SD (gm) at Hip, Forearm and Spine and at other sub-sites of healthy normal males

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	4.785±0.711	4.774±1.023	4.516±0.680	5.110±0.935	4.797±0.875
Trochenta	8.884±1.537	8.420±2.061	8.197±2.021	7.821±1.873	8.328±1.920
Intertrochenta	24.572±3.984	22.960±5.964	24.835±4.737	31.615±5.550	25.979±6.107
Hip	38.239±5.737	36.199±8.204	37.549±6.569	44.684±9.600	39.149±8.339
Forearm-					
1/3	3.992±0.398	3.599±0.447	3.869±0.392	3.578±0.488	3.755±0.467
Mid	9.230±1.371	6.844±1.098	7.280±1.517	7.790±1.350	7.765±1.606
UD	2.774±0.332	2.721±0.512	2.707±0.318	2.588±0.710	2.697±0.500
Forearm	15.996±1.970	12.995±1.905	13.855±2.050	13.684±1.868	14.104±2.239
Spine-					
L_1	12.337±2.322	12.070±2.099	11.713±2.380	11.598±2.020	11.926±2.219
L_2	14.655±2.233	14.046±2.268	14.038±2.323	13.470±2.331	14.044±2.318
L_3	16.268±2.497	15.797±2.474	15.500±2.567	15.138±2.427	15.669±2.516
L_4	18.626±5.654	18.521±6.149	16.670±2.907	16.811±2.781	17.652±4.707
Spine	61.390±9.176	59.343±9.796	57.995±9.681	56.670±10.002	58.817±9.793
Wholebody	2467.544 ±284.956	-	2261.294 ±265.434	2198.039 ±274.457	2313.370 ±300.690

Table 1.3a. Mean area ± SD (cm²) at Hip, Forearm and Spine and at other sub-sites of healthy normal males

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	5.402±0.426	5.442±0.792	5.124±0.343	5.417±0.409	5.348±0.541
Trochenta	11.858±1.171	11.638±1.325	11.784±1.706	10.640±1.867	11.478±1.614
Intertrochenta	20.959±2.535	20.482±4.286	22.095±3.466	27.226±2.921	22.683±4.313
Hip	38.290±3.526	37.305±5.293	39.002±4.315	42.894±5.867	39.361±5.280
Forearm-					
1/3	5.284±0.349	5.142±0.589	5.172±0.388	4.987±0.497	5.145±0.479
Mid	14.146±1.740	11.135±1.569	11.254±2.111	12.254±1.728	12.172±2.152
UD	5.757±0.503	5.901±0.497	5.682±0.524	5.470±0.906	5.704±0.649
Forearm	25.188±2.366	22.091±2.691	22.111±2.710	22.902±2.288	23.047±2.809
Spine-					
L_1	13.430±1.412	13.112±1.288	12.886±1.513	13.173±1.189	13.147±1.363
L_2	14.680±1.335	14.035±1.306	14.259±1.485	14.124±1.246	14.268±1.363
L_3	16.158±1.561	15.392±1.520	15.405±1.687	15.577±1.461	15.626±1.583
L_4	13.430±1.412	13.112±1.288	12.886±1.513	13.173±1.189	13.147±1.363
Spine	62.365±5.382	58.791±7.161	59.142±5.934	59.660±6.703	59.967±6.467
Wholebody	2121.905 ±190.590	-	2043.645 ±158.312	1978.162 ±155.545	2047.363 ±180.600

Table 1.1b. Mean BMD ± SD (gm/cm²) at Hip, Forearm and Spine and at other sub-sites of healthy normal females

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	0.826±0.098[a]	0.750±0.101	0.786±0.095	0.898±0.111*	0.816±0.115
Trochenta	0.693±0.078	0.624±0.082	0.636±0.081	0.673±0.084[b]	0.658±0.085
Intertrochenta	1.087±0.108	0.996±0.136	1.023±0.119	1.089±0.107[b]	1.050±0.124
Hip	0.925±0.091[a]	0.838±0.106	0.869±0.098	0.967±0.105*	0.901±0.111
Forearm-					
1/3	0.666±0.035	0.608±0.058[d]	0.656±0.044	0.621±0.054[c]	0.639±0.054
Mid	0.577±0.034	0.534±0.047[d]	0.570±0.039	0.549±0.041[c]	0.558±0.044
UD	0.433±0.042	0.379±0.044[d]	0.423±0.043	0.395±0.043[c]	0.408±0.048
Forearm	0.561±0.034	0.508±0.045[d]	0.551±0.037	0.528±0.039[c]	0.538±0.044
Spine-					
L_1	0.878±0.087[e]	0.822±0.131	0.841±0.098	0.847±0.094	0.848±0.105
L_2	0.977±0.088[e]	0.914±0.125	0.950±0.107	0.942±0.095	0.947±0.106
L_3	1.014±0.091[e]	0.975±0.116	0.997±0.108	1.000±0.090	0.997±0.102
L_4	1.013±0.090	0.971±0.111	1.005±0.099	1.000±0.089	0.998±0.098
Spine	0.976±0.083[e]	0.929±0.111	0.955±0.096	0.954±0.084	0.954±0.095
Wholebody	1.132±0.066 (105)	-	1.032±0.069 (62)	1.096±0.083 (100)	1.095±0.083 (267)

Figures in parenthesis indicate number of observations; *p<0.05; [a]p<0.0001 – compared to AIIMS and SGPGIMS respectively; [b]p<0.05 - compared to AIIMS and SGPGIMS; [c]p<0.003 – compared to NIN and SGPGIMS; [d] p<0.001 – compared to other three centres; [e]p<0.05 – compared to AIIMS

Table 1.2b. Mean BMC ± SD (gm) at Hip, Forearm and Spine and at other sub-sites of healthy normal females

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	3.865±0.511	3.477±0.533	3.620±0.512	4.366±0.835	3.839±0.697
Trochenta	6.422±1.080	5.706±1.091	5.871±1.303	5.231±1.178	5.820±1.240
Intertrochenta	17.826±2.682	14.860±3.309	16.861±3.791	23.811±5.908	18.395±5.257
Hip	28.112±3.854	24.043±4.485	26.351±4.925	33.109±7.363	27.979±6.242
Forearm-					
1/3	3.029±0.290	2.686±0.353	2.938±0.366	2.705±0.324	2.845±0.364
Mid	5.848±0.874	4.480±0.991	5.005±1.215	5.526±0.835	5.241±1.111
UD	2.133±0.250	1.979±0.266	2.071±0.288	1.857±0.230	2.002±0.340
Forearm	11.004±1.309	9.154±1.463	9.972±1.658	10.096±1.306	10.090±1.575
Spine-					
L_1	9.952±1.780	8.633±1.620	8.916±1.662	9.336±1.587	9.237±1.733
L_2	12.374±1.673	10.504±1.807	11.204±1.979	11.433±1.892	11.419±1.950
L_3	14.428±1.925	12.429±1.980	13.134±2.200	13.532±1.982	13.424±2.141
L_4	16.179±2.387	14.368±2.362	14.700±2.279	15.129±2.255	15.131±2.412
Spine	52.982±7.114	45.631±8.057	47.953±7.670	49.418±7.198	49.161±7.927
Wholebody	2027.866±206.829	-	1798.848±235.479	1827.351256.268	1899.587±254.375

Table 1.3b. Mean area ± SD (gm) at Hip, Forearm and Spine and at other sub-sites of healthy normal females

Site	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Hip-					
Neck	4.690±0.399	4.641±0.388	4.565±0.511	4.842±0.531	4.686±0.471
Trochenta	9.266±1.106	9.027±1.375	9.203±1.554	7.775±1.448	8.821±1.501
Intertrochenta	16.414±1.731	14.794±2.260	16.449±2.961	21.764±4.180	17.386±3.917
Hip	30.364±2.503	28.539±2.809	30.257±3.914	33.941±5.734	30.809±4.386
Forearm-					
1/3	4.544±0.314	4.404±0.403	4.469±0.385	4.356±0.420	4.445±0.387
Mid	10.139±1.296	8.325±1.469	8.672±1.732	10.040±1.160	9.331±1.632
UD	4.932±0.412	5.197±0.392	4.901±0.571	4.715±0.470	4.930±0.495
Forearm	19.620±1.856	17.914±1.918	18.042±2.448	19.105±1.794	18.703±2.136
Spine-					
L_1	11.356±1.025	10.448±1.159	10.557±1.168	10982±1.040	10.855±1.151
L_2	12.658±1.057	11.431±1.209	11.752±1.275	12.062±1.174	12.002±1.259
L_3	14.208±1.235	12.682±1.255	13.157±1.505	13.506±1.268	13.421±1.428
L_4	15.939±1.548	14.698±1.564	14.601±1.554	15.100±1.618	15.108±1.655
Spine	54.164±4.376	48.903±6.164	50.070±5.093	51.659±4.627	51.316±5.417
Wholebody	1789.029±124.730	-	1738.048±147.852	1669.950±133.928	1732.592±143.216

Comparison with NHANES III and Hologic reference standards

The pooled estimates of BMD at total hip, forearm and lumbar spine both for males and females were compared with the corresponding NHANES III and Hologic normative values (Table 1.11). All the above estimates of BMD were found to be significantly lower ($p<0.001$) than the corresponding NHANES III and Hologic normative values. Some studies done in the younger population of Denmark[16] and Lebanon[17] had also reported significant differences in BMD when compared to NHANES III and Hologic normative values.

3.1 Demographic profile and its impact on BMD

Distribution of subjects by various demographic parameters is given in tables 2a and 2b. Majority of males (87%) and females (86%) were Hindus followed by 2-5% males and 1-7% females being Muslim/Christian/Sikh and others. Amongst the females, about 12% in AIIMS and 10% in NIRRH centres were Christians. No significant differences were observed in BMD at the three sites namely, hip, forearm and spine with respect to religion.

In majority of males (15-31%) and females (12-29%), father's occupation during childhood was reported to be office, manager, professional or business. The other occupations of father namely, skilled/unskilled worker, farmer and teacher varied from 2-6% in both males and females. No significant impact of father's occupation on BMD at three sites was observed.

About 82% of the subjects belonged to the general category followed by 14% belonging to OBC/BC and about 4% belonging to SC/ST category.

About 20% of the male subjects and 30% of the female subjects were married. Interestingly, while marital status had no significant impact on BMD of the males, it had statistically significant impact on the BMD of the females at the forearm and spine. In fact, mean BMD of the married females at forearm (0.550 ± 0.039) and spine (0.977 ± 0.090) was significantly higher ($p<0.003$, $p<0.001$) than that of the unmarried females at the two corresponding sites (0.531 ± 0.043, 0.946 ± 0.095). However, a post-hoc analysis by the centres indicated statistically significant differences between BMD of married and unmarried females at forearm in Lucknow and Mumbai centres ($p<0.02$, $p<0.05$) only and at spine ($p<0.05$) in Lucknow only.

About 78% of the males and 80% of the females were graduate/postgraduate. Further, about 16 and 14% of males and females respectively were professionally qualified. The Graduate males were found to be having a significantly higher ($p<0.008$) BMD at forearm (0.621±0.053) compared to other male subjects (0.604±0.049). However, this difference did not persist amongst the centres.

About 55% of both males and females were unemployed/student. Around 11-12% of males and 6-7% of the females were either holding a managerial or some professional position. Further, about 11% of males were in business and 15% of females were housewives. No significant impact of occupation was observed on BMD at any of the three sites.

A majority of 47% of males and 64% of the females were found performing walking and equivalent activities. Further, about 34% of males and 22% of females were

Table 1.11. Comparison of BMD of young Indian males and females at total hip, forearm and lumbar spine with the corresponding NHANES III and Hologic normative values

Site	Sex	Mean BMD±SD (gm/cm²)	
		Indian	NHANES III/Hologic
Hip	M	0.988±0.131*	1.041±0.144
	F	0.901±0.111*	0.942±1.122
Forearm	M	0.611±0.052*	0.820±0.050
	F	0.538±0.044*	0.690±0.060
Lumbar spine	M	0.976±0.105*	1.120±0.110
	F	0.954±0.095*	1.084±0.111

*P<0.001

Table 2a. Demographic profile of healthy normal males (%)

Variable	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Religion-					
Hindu	90.4	83.0	93.0	82.0	87.1
Muslim	4.8	5.7	2.0	6.0	4.6
Christian	2.9	7.5	2.0	2.0	3.7
Sikh	1.9	1.9	2.0	1.0	1.7
Others	-	1.9	1.0	9.0	2.9
Father's Occupation-					
Skilled/unskilled workers	1.0	7.6	4.0	5.0	4.4
Office goers	17.3	33.0	47.0	28.0	31.2
Teacher	2.9	11.3	1.0	10.0	6.3
Manager	28.8	3.8	16.0	12.0	15.1
Professional	15.4	21.7	17.0	18.0	18.0
Businessmen	28.8	14.2	11.0	25.0	19.8
Farmer	5.8	6.6	4.0	2.0	4.6
Caste-					
General	79.8	87.7	93.0	68.0	82.2
OBC/BC	18.3	7.5	6.0	19.0	12.7
SC/ST	2.0	4.6	1.0	13.0	3.9
Marital status-					
Married	21.2	22.6	21.0	16.0	20.2
Single	78.8	77.4	79.0	84.0	79.8
Education-					
Illiterate/R/W	-	-	-	-	-
Primary/middle	-	0.9	-	-	0.2
Matric/Sec./Sr. Sec.	3.8	5.7	11.0	-	5.1
Graduate/PG	80.8	68.9	73.0	90.0	78.0
Professional/Others	15.4	21.7	16.0	10.0	15.8
Self Occupation-					
Skilled/unskilled	-	6.6	-	-	1.7
Office goer	7.7	13.2	4.0	-	6.3
Teacher	1.0	3.8	1.0	-	1.5
Manager	7.7	31.1	4.0	5.0	12.2
Professional	11.5	22.6	10.0	2.0	11.7
Businessmen	17.3	1.9	22.0	3.0	11.0
Farmer	1.0	-	-	-	0.2
Unemployed/Student	53.8	18.8	58	90.0	54.7
Status of activity-					
Normal walking	20.8	-	1.0	11.0	8.2
Walking and equivalent activities	33.3	79.6	13.0	64.0	47.0
Brisk walking and equivalent activities	32.3	19.4	62.0	21.0	33.9
Vigorous/bone loading equivalent	13.5	1.1	24.0	4.0	10.8
Dental mottling-					
Yes	1.0	-	5.0	1.0	1.8

Table 2b. Demographic profile of healthy normal females (%)

Variable	NIN, Hyderabad	AIIMS, New Delhi	SGPGIMS, Lucknow	NIRRH, Mumbai	Pooled
Religion-					
Hindu	88.8	81.6	88.9	84.0	85.9
Muslim	4.7	2.0	6.1	1.0	3.5
Christian	5.6	12.2	1.0	10.0	7.2
Sikh	0.9	1.0	3.0	-	1.2
Others	-	3.1	1.0	5.0	2.2
Father's Occupation-					
Skilled/unskilled workers	0.9	4.0	1.0	6.0	2.9
Office goers	12.1	30.6	42.4	32.0	29.0
Teacher	3.7	12.2	1.0	4.0	5.2
Manager	22.4	3.1	12.1	9.0	11.9
Professional	21.5	17.3	22.2	27.0	22.0
Businessmen	33.6	26.5	18.2	21.0	25.0
Farmer	5.6	6.1	3.0	1.0	4.0
Caste-					
General	76.6	89.8	87.9	84.0	84.4
OBC/BC	17.8	4.1	7.1	10.0	9.9
SC/ST	2.0	3.7	1.0	13.0	4.9
Marital status-					
Married	43.0	22.4	33.3	23.0	30.7
Single	57.0	77.6	65.7	77.0	69.1
Education-					
Illiterate/R/W	-	-	-	-	-
Primary/middle	-	3.0	-	-	0.7
Matric/Sec./Sr. Sec.	4.7	5.1	10.1	-	4.9
Graduate/PG	83.1	84.7	76.8	77.0	80.5
Professional/Others	12.1	7.1	13.1	22.0	13.6
Self Occupation-					
Skilled/unskilled	-	2.0	-	-	0.5
Office goers	10.3	9.2	2.0	2.0	5.9
Teacher	1.9	2.0	7.1	1.0	3.0
Manager	5.6	16.3	1.0	8.0	7.7
Professional	12.1	3.1	8.1	14.0	9.4
Businessmen	0.9	-	-	-	0.2
Farmer	0.9	9.2	-	-	2.5
Unemployed/Student	44.8	51.0	56.6	70.0	55.4
Housewife	23.4	6.1	25.3	5.0	15.1
Status of activity-					
Normal walking	14.7	-	3.1	11.0	7.3
Walking and equivalent activities	42.2	93.9	45.9	75.0	64.1
Brisk walking and equivalent activities	21.6	6.1	48.0	13.0	22.1
Vigorous/bone loading equivalent	21.6	-	3.1	1.0	6.5
Dental mottling-					
Yes	2.8	-	8.1	-	2.7

Table 2c. Nature of activity performed and its impact on BMD

Nature of activity	Male			Female		
	Mean BMD±SD			Mean BMD±SD		
	Forearm*	Spine*	Hip	Forearm**	Spine**	Hip
Normal walking	0.619±0.057 (32)	0.939±0.092 (32)	0.978±0.121 (31)	0.546±0.042 (28)	0.964±0.097 (26)	0.930±0.077(28)
Walking and equivalent activities	0.597±0.050 (177)	0.959±0.100 (171)	0.976±0.117 (175)	0.532±0.045 (245)	0.943±0.097 (247)	0.892±0.115(248)
Brisk walking and equivalent activities	0.618±0.051 (129)	0.985±0.108 (128)	0.988±0.143 (127)	0.544±0.038 (88)	0.979±0.080 (87)	0.917±0.107(88)
Vigorous/bone loading equivalent	0.626±0.047 (41)	1.001±0.107 (42)	1.009±0.114 (41)	0.559±0.040 (25)	0.995±0.089 (26)	0.930±0.101(26)
Total	0.609±0.051 (379)	0.971±0.103 (373)	0.984±0.127 (374)	0.537±0.044 (386)	0.956±0.095 (386)	0.903±0.110(390)

*p<0.009, **p<0.004; Figures in parentheses indicate number of observations

reportedly performing brisk walking and equivalent activities and 11% of males and 6.5% of females were found performing vigorous/bone loading activities. Only about 8% of males and 7% of females were doing normal walking activities.

A one way ANOVA done on the pooled data indicated that the nature of activities performed, significantly affected BMD at forearm and spine of both male (p<0.009) and female (p<0.004) subjects (Table 2c). A two-way ANOVA also indicated statistically significant centre wise differences in BMD at forearm in both males (p<0.0001) and females (p<0.0001) indicating further that the nature of activity performed did really affect the BMD at forearm regardless of the centre. Further, difference in proportion of married (11.3%) and unmarried (4.4%) females doing vigorous bone loading activities was found to be statistically significant (p<0.01). This might explain higher BMD of the married females as against unmarried/single females as reported earlier.

About 2-3% of both males and females were reportedly having dental mottling, which was found to have no impact on BMD.

3.2 Physical and clinical profile and its impact on BMD

Distribution of subjects by various physical and clinical parameters is given in tables 3a and 3b. Mean age of males and females was respectively 24.8±2.7 and 24.4±2.9 years. The mean BMD at hip, forearm and spine of male and female subjects at each point of age is tabulated in tables 3c and 3d respectively as well as in figures 1a & 1b respectively. A one-way ANOVA done on the pooled data indicated no statistically significant differences in BMD at any of the three sites by age. Mean age of the married females (26.6±2.3 years) was significantly high as compared to that of the unmarried females (23.5±2.5 years). But since age has no impact on BMD, the higher age of the married females can not be attributed to their significantly higher BMD as compared to unmarried females.

It could be clearly seen that the peak bone mineral density (PMBD) of the males at total hip and lumbar spine was attained at the age of 25 years and that at forearm at the age of 28 years (Figure 1a). These were respectively 1.0±0.103, 0.619±0.059 and 1.027±0.102 gm/cm^2 at total hip, forearm and lumbar spine. Similarly, the peak bone mineral density of the females at total hip, forearm and lumbar spine was attained at the age of 28, 29 and 20 years (Figure 1b). These were respectively 0.989±0.090, 0.548±0.038 and 0.934±0.099 gm/cm^2 at total hip, forearm and lumbar spine. Comparing the PBMD of healthy males and females with the corresponding NHANES III and Hologic standards, it was observed that except the PBMD at hip of females, all other values of PBMD were found to be significantly lower (p<0.001) than the corresponding NHANES III and Hologic values. The PBMD at hip of

Table 3a. Physical and clinical profile (Mean ± SD) of healthy normal males

Centre	Age (years)	Height*** (cms.)	Weight*** (kg.)	BMI*** (Kg/m^2)	SBP*** (mm of Hg)	DBP (mm of Hg)	Av. Ca intake (mg/day)	Age at puberty (yrs)
NIN, Hyderabad	24.1±3.1 (104)	174.1±6.2 (104)	69.7±8.1 (104)	23.0±2.0 (104)	113.8±11.5 (104)	77.1±8.4 (104)	1074.7±379.0 (103)	16.4±1.8 (103)
AIIMS, New Delhi	25.6±2.3 (100)	170.5±6.0 (99)	67.1±7.4 (99)	23.1±2.2 (99)	118.7±6.6 (98)	78.2±5.4 (98)	1575.1±548.4*** (88)	13.9±1.4 (89)
SGPGI, Lucknow	24.9±2.8 (100)	169.8±6.3 (100)	66.1±6.8 (100)	22.9±1.7 (100)	121.5±10.1 (100)	78.5±7.7 (100)	1186.0±527.9 (95)	16.5±1.4 NS (99)
NIRRH, Mumbai	24.9±2.3 (100)	173.3±5.3 (100)	64.4±6.3 (100)	21.4±1.6 (100)	117.3±6.9 (100)	75.9±6.2 (100)	991.1±401.7 (100)	17.7±1.5*** (100)
Pooled	24.9±2.7 (404)	171.9±6.2 (403)	66.9±7.4 (403)	22.6±2.0 (403)	117.8±9.4 (402)	77.4±7.1 (402)	1194.5±513.1 (386)	16.2±2.0 (391)

***$p<0.0001$; NS – Not significant with respect to NIN; Figures in parentheses indicate number of observations

Table 3b. Physical and clinical profile (Mean ± SD) of healthy normal females

Centre	Age (years)	Height*** (cms.)	Weight*** (kg.)	BMI*** (Kg/m^2)	SBP*** (mm of Hg)	DBP (mm of Hg)	Av. Ca intake (mg/day)	Age at menarche (yrs)	Age at first birth (yrs)	No. of births
NIN, Hyderabad	24.2±3.1 (107)	159.9±5.6 (107)	56.7±6.7 (107)	22.1±2.0 (107)	105.6±10.6 (107)	71.1±8.0 (107)	996.4±350.8 (106)	12.9±1.0 (106)	23.5±3.6 (29)	1.4±0.6 (27)
AIIMS, New Delhi	24.5±2.8 (98)	154.4±6.1 (98)	51.7±6.4 (98)	21.7±2.5 (98)	117.0±6.3 (98)	76.5±5.4 (98)	1248.1±412.6*** (98)	12.5±1.7 (96)	23.5±3.2 (12)	1.6±0.7 (13)
SGPGI, Lucknow	24.8±2.8 (99)	155.8±6.6 (99)	53.6±6.7 (99)	22.0±2.0 (98)	113.4±8.6 (99)	73.3±7.3 (99)	997.5±471.6 (95)	13.4±1.2 (99)	23.2±1.9 (26)	1.4±0.6 (25)
NIRRH, Mumbai	24.2±2.6 (100)	159.8±5.3 (100)	55.1±5.5 (100)	21.5±1.6 (100)	113.4±7.8 (100)	74.6±6.1 (100)	900.7±347.2 (100)	13.3±1.1 (100)	24.1±2.8 (10)	1.1±0.3 (9)
Pooled	24.4±2.9 (404)	157.5±6.4 (404)	54.3±6.6 (404)	21.8±1.9 (404)	112.2±9.5 (404)	73.8±7.1 (404)	1034.5±415.8 (399)	13.0±1.3 (401)	23.5±2.9 (77)	1.4±0.6 (74)

***$p<0.0001$; Figures in parentheses indicate number of observations

females was slightly but insignificantly higher than the NHANES III value.

Mean height, weight and BMI of the males were respectively 171.9±6.2 cm, 66.9±7.4 kg. and 22.6±2.0 kg./m^2 and that of the females were 157.5±6.4 cm., 54.3±6.6 kg. and 21.8±1.9 kg./m^2. Statistically significant differences were observed ($p<0.0001$) between the centres with respect to these three variables in males. However, in females, statistically significant differences were observed ($p<0.0001$) between the centres with respect to height and weight only. A correlation analysis done on the pooled data indicated significantly positive correlations of weight with BMD at hip, forearm and spine and these were of the order of respectively 0.253, 0.227 and 0.341 in males and 0.372,0.288 and 0.381 in females all with $p<0.0001$. Similarly, significantly positive correlations of height with BMD at hip, forearm and spine were found to be of the order of respectively 0.176, 0.156 and 0.222 in males and 0.336, 0.219 and 0.254 in females all with $p<0.0001$. BMI, which is derived from weight and height also showed significantly positive correlations with BMD at hip, forearm and spine and these were of the order of respectively 0.175, 0.152 and 0.247 in males and

Table 3c. Mean BMD ± SD (gm/cm²) at Hip, Forearm and Spine of healthy normal males

Age (yrs)	Mean BMD ± SD (n)		
	Hip	Forearm	Spine
20	0.967±0.097 (29)	0.611±0.06 (29)	1.002±0.144 (27)
21	0.977±0.107 (31)	0.598±0.054 (32)	0.958±0.116 (31)
22	0.978±0.121 (27)	0.620±0.040 (28)	0.990±0.154 (27)
23	0.999±0.108 (43)	0.610±0.050 (43)	1.013±0.167 (42)
24	0.939±0.110 (44)	0.605±0.046 (45)	0.986±0.120 (45)
25	1.0±0.103 (44)	0.618±0.052 (43)	1.027±0.102 (43)
26	0.975±0.101 (51)	0.608±0.045 (51)	1.0±0.125 (51)
27	0.951±0.096 (41)	0.599±0.053 (44)	0.967±0.125 (44)
28	0.987±0.098 (50)	0.619±0.059 (52)	0.971±0.105 (52)
29	0.969±0.091 (32)	0.611±0.043 (32)	0.936±0.093 (32)
Total	0.975±0.104 (392)	0.610±0.051 (399)	0.986±0.126 (394)

Figures in parentheses indicate number of observations

Table 3d. Mean BMD ± SD (gm/cm²) at Hip, Forearm and Spine of healthy normal females

Age (yrs)	Mean BMD ± SD (n)		
	Hip	Forearm	Spine
20	0.941±0.074 (40)	0.538±0.040 (42)	0.934±0.099 (41)
21	0.936±0.091 (35)	0.527±0.047 (34)	0.896±0.102 (36)
22	0.918±0.111 (46)	0.530±0.043 (45)	0.893±0.119 (47)
23	0.961±0.110 (42)	0.532±0.041 (43)	0.911±0.119 (43)
24	0.936±0.085 (39)	0.548±0.050 (39)	0.896±0.124 (40)
25	0.971±0.091 (46)	0.536±0.039 (45)	0.903±0.111 (46)
26	0.966±0.075 (39)	0.535±0.050 (38)	0.890±0.095 (39)
27	0.949±0.100 (30)	0.533±0.050 (30)	0.885±0.127 (29)
28	0.989±0.090 (39)	0.547±0.039 (38)	0.904±0.113 (38)
29	0.978±0.095 (39)	0.548±0.038 (41)	0.896±0.100 (40)
Total	0.954±0.095 (395)	0.538±0.044 (395)	0.901±0.111 (399)

Figures in parentheses indicate number of observations

0.192, 0.181 and 0.288 in females all with $p<0.0001$. It is important to note that weight alone had much higher correlations with BMD at the three sites as against height and BMI in both males and females. The above trend of correlations continued even within each of the centres indicating that weight of subjects had a much larger impact on BMD at hip, forearm and spine in both males and females.

The mean dietary calcium intake in males and females were 1194.5±513.1 and 1034.5±415.8 mg/day respectively. In AIIMS, however, the mean dietary calcium intake in both males (1575.5±548.4 mg/day) and females (1248.1±412.6 mg/day) were found to be significantly high ($p<0.0001$) compared to other centres. A significantly negative correlation ($r=-0.137$, $p<0.007$) between dietary intake of calcium and BMD at hip was observed in females. A one–way ANOVA further confirmed that rising levels of dietary Ca were associated with significant fall in BMD at hip ($p<0.02$) in females. These significant differences persisted even amongst the centres ($p<0.0001$) in case of females.

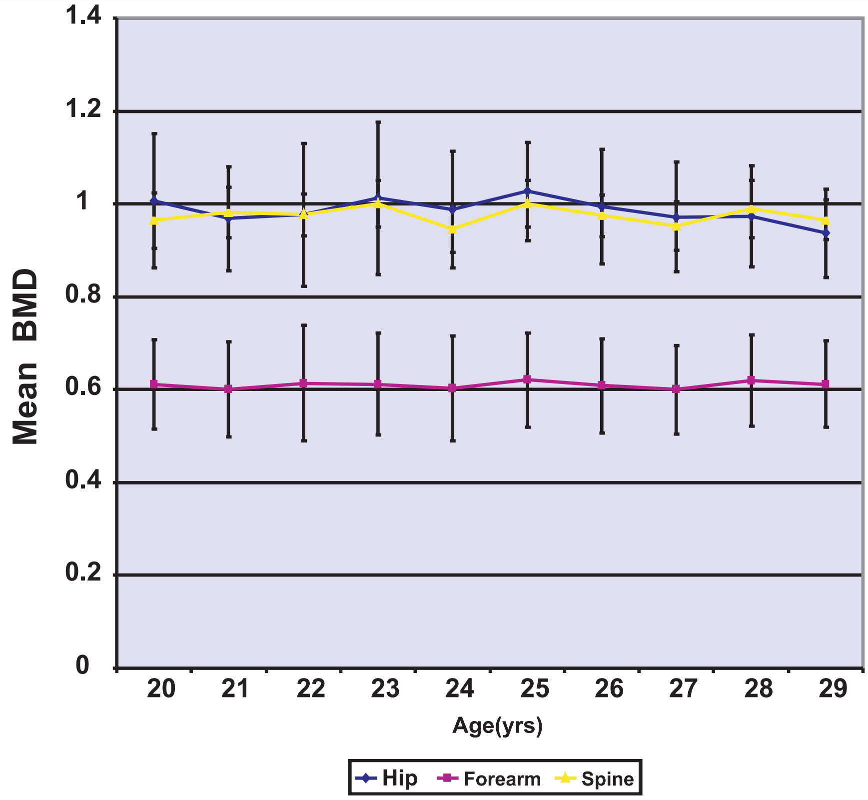

Figure 1a. Mean BMD of healthy Indian Males

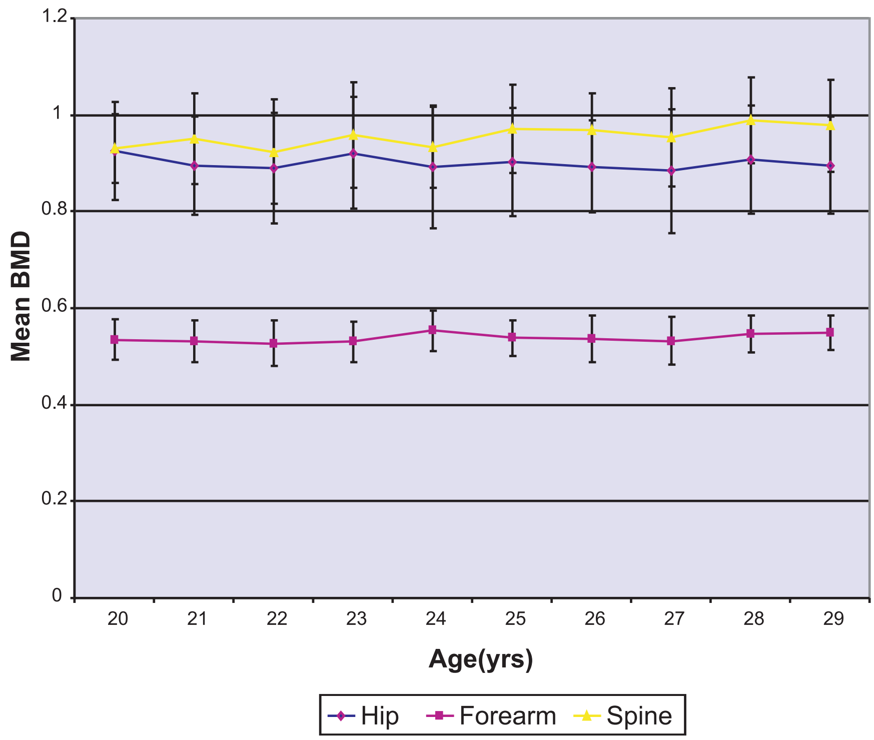

Figure 1b. Mean BMD of healthy Indian females

The mean age at menarche, at first birth and mean number of births in females were respectively 13.0±1.3 years, 23.5±2.9 years and 1.4±0.6. There were no centre wise significant differences with regard to mean age at menarche, mean age at first birth and mean number of births. The number of births showed a significantly negative correlation (r = -0.215, p<0.04) with BMD at hip. A one-way ANOVA further confirmed significant decline in BMD at hip (p<0.04) with increasing number of births.

3.3 Biochemical profile and its impact on BMD

Distribution of subjects by various biochemical parameters is given in tables 4a and 4b.

Mean haemoglobin level of males and female was 14.3±1.0 and 12.2±1.4 g/dl respectively. No centre wise significant differences were noted in mean haemoglobin level in males. However, females in Lucknow centre had

Table 4a. Biochemical profile (Mean ± SD) of healthy normal males

Centre	Hb (g/dl)	S.alb. (g/dl)	S.Ca (mg/dl)	S.phos (mg/dl)	S.alk (iu)	S.creat*** (mg/dl)	Vit.D*** (ng/ml)	PTH (pg)	U.fl. (ppm)
NIN, Hyderabad	14.3±0.7 (98)	4.6±0.4 (98)	10.0±0.7 (98)	4.5±0.4*** (98)	194.8±60.1 (98)	1.4±0.3 (98)	24.3±14.6 (98)	36.5±23.4 (98) Median-32.0	0.6±0.2 (101)
AIIMS, New Delhi	14.5±1.6 (100)	5.0±0.4 (100)	10.0±0.7 (100)	3.6±0.8*** (100)	119.1±65.2*** (100)	1.0±0.1 (100)	8.6±3.7 (91)	87.3±110.1*** (90) Median-70.0	1.0±0.6 (94)
SGPGI, Lucknow	14.2±0.8 (100)	5.1±0.6 (100)	9.6±0.8 (100)	4.1±0.6 (100)	194.7±111.7 (95)	0.7±0.2 (100)	14.9±7.7 (100)	75.9±39.6 (99) Median-69.6	1.3±0.6** (94)
NIRRH, Mumbai	14.1±0.6 (100)	4.1±0.2*** (100)	9.2±0.5*** (100)	4.0±0.4 (100)	183.8±44.5 (100)	0.9±0.1 (100)	19.3±4.6 (100)	30.5±8.0^{NS} (100) Median-31.0	0.7±0.5^{NS} (98)
Pooled	14.3±1.0 (398)	4.7±0.6 (398)	9.7±0.8 (398)	4.1±0.7 (398)	172.7±80.4 (393)	1.0±0.3 (398)	16.9±10.5 (389)	56.8±62.8 (387) Median-41.0	0.9±0.6 (387)

***p<0.0001 – ANOVA; **p<0.001; NS – Not significant compared to NIN; Figures in parentheses indicate number of observations

Table 4b. Biochemical profile (Mean ± SD) of healthy normal females

Centre	Hb (g/dl)	S.alb. (g/dl)	S.Ca (mg/dl)	S.phos (mg/dl)	S.alk (iu)	S.creat (mg/dl)	Vit.D (ng/ml)	PTH (pg)	U.fl. (ppm)
NIN, Hyderabad	12.5±1.5 (103)	4.4±0.4 (103)	9.7±0.6 (103)	4.4±0.4 (103)	165.2±44.7 (103)	1.4±0.9## (103)	18.5±15.0$ (103)	40.4±27.3 (103) Median-34.0	0.6±0.2 (103)
AIIMS, New Delhi	12.0±1.5 (98)	4.8±0.7 (98)	10.0±0.8 (98)	4.0±1.5# (98)	136.7±62.5*** (98)	0.9±0.6 (98)	8.8±7.5 (98)	78.2±38.7 (98) Median-66.5	0.7±0.5 (98)
SGPGI, Lucknow	11.5±1.1*** (97)	4.6±0.6 (98)	9.5±0.7 (98)	3.9±0.5 (98)	195.8±109.1 (94)	0.7±0.2 (97)	9.8±5.6 (95)	114.7±100.7** (93) Median-94.3	1.2±0.7** (95)
NIRRH, Mumbai	12.9±0.9 (100)	4.0±0.2*** (100)	9.2±0.6*** (100)	4.0±0.4 (100)	170.3±36.4 (100)	0.8±0.2 (100)	17.1±4.4$$ (100)	30.3±6.7^{NS} (100) Median-30.0	0.6±0.5^{NS} (95)
Pooled	12.2±1.4 (398)	4.5±0.6 (399)	9.6±0.7 (399)	4.1±0.8 (399)	166.7±71.1 (395)	1.0±0.6 (398)	13.6±10.2 (396)	64.8±63.6 (394) Median-44.4	0.8±0.6 (391)

***p<0.0001; #p<0.0001 vs. SGPGIMS; ##p<0.0001 with respect to SGPGIMS and NIRRH; $p<0.0001 – with respect to AIIMS and SGPGIMS; $$p<0.0001 – with respect to AIIMS and SGPGIMS; **p<0.001 – ANOVA; NS – Not significant compared to NIN; Figures in parentheses indicate number of observations statistically significant ($p<0.0001$) lower mean haemoglobin level compared to the other three centres. No significant correlation of haemoglobin with BMD at any of the three sites viz. hip, forearm and spine was observed in either males or females.

The mean serum albumin of males and females was 4.7 ± 0.6 and 4.5 ± 0.6 g/dl respectively. The mean serum albumin level of males and females in NIRRH were found to be significantly lower ($p<0.0001$) compared to other centres. Statistically significant negative correlations of serum albumin with BMD at hip in males ($r = -0.252$, $p<0.0001$) and in females ($r = -0.108$, $p<0.03$) were observed. An ANOVA done with different groups of serum albumin further confirmed that increasing levels of serum albumin were associated with significant decrease in BMD at hip in both males ($p<0.03$) and females ($p<0.0001$) (Table 4c). However, the present data in both males and females had shown significant negative correlations between serum albumin and serum vitamin D ($r=-0.229$, $p<0.0001$; $r=-0.168$, $p<0.001$) and significant positive correlations between serum albumin and serum PTH ($r=0.296$, $p<0.0001$; $r=0.293$, $p<0.0001$). There were however, no significant centre wise differences noted with regard to serum albumin and BMD at hip.

The mean serum calcium in males and females were observed to be 9.7 ± 0.8 and 9.6 ± 0.7 mg/dl respectively. The mean serum calcium levels in both males and females in NIRRH were significantly lower ($p<0.001$) compared to other centres. However, serum calcium level did not show significant correlation with BMD at any of the three sites in any centre.

The mean serum phosphorus in males was 4.1 ± 0.7 mg/dl and in females it was 4.1 ± 0.8 mg/dl. In NIN a significantly high value ($p<0.0001$) and in AIIMS a significantly low value ($p<0.0001$) of mean phosphorus level was observed compared to other centres in males. In females, however, AIIMS reported significantly higher ($p<0.0001$) mean serum phosphorus level compared to SGPGIMS. A significantly positive correlation of $r = 0.223$ with $p<0.0001$ was observed between serum phosphorus level and BMD at forearm in males. An ANOVA further confirmed centre wise significant differences ($p<0.02$) in BMD at forearm with increasing level of serum phosphorus in males.

The mean serum alkaline phosphatase level in males and females was 172.7 ± 80.4 and 166.7 ± 71.1 iu respectively. However, the mean serum alkaline phosphatase levels in both males (119.1 ± 65.2 iu) and females (136.7 ± 62.5 iu) in AIIMS were significantly low ($p<0.0001$) compared to other centres. Significantly positive correlations were observed between serum alkaline phosphatase level and BMD at forearm in males ($r=0.106$, $p<0.04$) and females ($r=0.131$, $p<0.02$). An ANOVA done with different groups of serum alkaline phosphatase further confirmed that increasing levels of serum alkaline phosphatase were associated with significant increase in BMD at forearm in both males ($p<0.004$) and females ($p<0.01$) (Table 4d). Further, there were centre wise significant differences observed in BMD at forearm with increasing level of serum alkaline phosphatase in both males ($p<0.0002$) and females ($p<0.0002$).

The mean serum vitamin D levels in males and females were observed to be 16.9 ± 10.5 and 13.6 ± 10.2 ng/ml respectively. Statistically significant differences ($P<0.0001$) were observed in mean serum vitamin D levels amongst all the centres in males. In females, however, statistically significant differences ($P<0.0001$) were observed in mean serum vitamin D levels amongst NIN, AIIMS and

Table 4c. Effect of serum albumin on BMD at hip

Serum albumin (g/dl)	Mean BMD at hip ± SD (gm/cm²)	
	Male*	Female***
≤4.5	1.006±0.120 (164)	0.921±0.116 (234)
4.6-5.5	0.973±0.134 (198)	0.873±0.096 (153)
≥5.6	0.967±0.101 (26)	0.828±0.114 (7)
Total	0.986±0.127 (388)	0.901±0.111 (394)

*p<0.03; ***p<0.0001; Figures in parentheses indicate number of observations

Table 4d. Effect of serum phosphatase on BMD at forearm

Serum phosphatase (iu)	Mean BMD at forearm ± SD (gm/cm²)	
	Male***	Female**
≤120	0.597±0.044 (103)	0.525±0.047 (92)
121-220	0.611±0.050 (190)	0.540±0.043 (235)
221-320	0.623±0.058 (77)	0.543±0.043 (44)
≥321	0.600±0.044 (19)	0.547±0.032 (15)
Total	0.609±0.050 (389)	0.537±0.044 (386)

***p<0.004; **p<0.01; Figures in parentheses indicate number of observations

SGPGIMS. The mean serum vitamin D level in females in NIRRH was significantly high (P<0.0001) compared to that in AIIMS and SGPGIMS. Interestingly, the mean serum vitamin D levels in both males and females in NIN and NIRRH were substantially high compared to the other two centres. A significantly high correlation (r=0.204, p<0.0001) was observed between serum vitamin D level and BMD at hip in females. This was further corroborated by an ANOVA which showed that higher levels of serum vitamin D were significantly associated (p<0.0001) with higher levels of BMD at hip in females (Table 4e).

The mean parathyroid hormone (PTH) levels in males and females were observed to be 56.8±62.8 and 64.8±63.6 pg respectively. The mean PTH level of females in SGPGIMS was substantially high, being 114.7±100.7 pg. Since the distribution of observations on PTH was skewed to the right, all the observations were log-transformed and then the means were compared by carrying out an ANOVA on the log-transformed observations. Statistically significant differences (P<0.0001) were observed in mean log PTH levels amongst all the centres except between NIN and NIRRH in males. In females also, statistically significant differences (P<0.003) were observed in mean log PTH levels amongst all the centres except between NIN and NIRRH. A statistically significant negative correlation was observed between log PTH levels and BMD at hip in females (r=-0.283, p<0.0001). A one way ANOVA further

Table 4e. Effect of serum vitamin D on BMD at hip

Serum vitamin D (ng/ml)	Mean BMD at hip ± SD (gm/cm²)	
	Male	Female***
<8	0.941±0.105 (67)	0.861± 0.098 (122)
8.0-8.9	1.024±0.115 (11)	0.847±0.127 (22)
9.0-9.9	0.988±0.131 (19)	0.873±0.092 (21)
10.0-10.9	1.048±0.134 (10)	0.862±0.103 (20)
11.0-11.9	0.967±0.127 (22)	0.897±0.091 (17)
12.0-12.9	0.973±0.150 (22)	0.856±0.112 (13)
13.0-13.9	0.963±0.073 (9)	0.932±0.116 (21)
14.0-14.9	0.991±0.120 (11)	0.952±0.063 (20)
15.0-15.9	0.996±0.132 (23)	0.964±0.120 (21)
16.0-16.9	1.021±0.154 (23)	0.987±0.090 (16)
17.0-17.9	1.023±0.171 (14)	0.902±0.116 (13)
18.0-18.9	1.021±0.119 (17)	0.964±0.084 (11)
19.0-19.9	1.015±0.207 (16)	0.957±0.060 (9)
≥20	0.992±0.109 (116)	0.941±0.117 (65)
Total	0.988±0.127 (380)	0.901±0.111 (391)

***p<0.0001; Figures in parentheses indicate number of observations confirmed that increasing levels of PTH were significantly associated ($p<0.0001$) with decreasing values of BMD at hip in females.

Parathyroid Hormone (PTH) is important for the regulation of calcium and Vitamin D in the body. PTH also plays a pivotal role in bone remodeling. In view of the significantly negative correlations observed between serum vitamin D and log PTH levels in both males ($r=-0.335$, $p<0.0001$) and females ($r=-0.409$, $p<0.0001$) and also in view of the significant correlations of BMD at hip with both serum vitamin D and serum log PTH levels as reported above in females, a 2-way ANOVA was attempted with different classes of dietary calcium, serum vitamin D and serum log PTH levels. The classes considered were 400-800, 800-1199 and ≥1200 mg/day for dietary calcium; <10, 10.0-19.9 and ≥20 ng/ml for serum vitamin D; <3.0, 3.0-3.9, 4.0-4.9 and ≥5.0 for log PTH. The results of this analysis are as described below:

Keeping dietary calcium below 800 mg/day and increasing vitamin D, a significant rise in BMD ($p<0.009$) at hip in females was observed (Table 6b) indicating that sufficient intake of vitamin D might ensure rise in BMD at hip even at low levels of dietary Ca. Further, keeping dietary Ca between 800-1200 mg/day and increasing vitamin D also yielded significant rise in BMD at hip ($p<0.0001$) in females as also a statistically non-significant but consistent rise in males (Table 6a). A statistically non-significant but consistent rise in BMD at forearm was observed at dietary Ca levels of ≥800 mg/day if vitamin D was increased in females. However, in males, if dietary Ca was kept at ≥1200 mg/day and vitamin D was increased, a statistically non-significant but consistent fall in BMD at spine was noted. Similarly, keeping dietary Ca at ≥ 1200 mg/day and increasing serum PTH led to statistically non-significant but consistent fall in BMD at hip in both males and females. Furthermore, with dietary Ca at ≥1200 mg/day, increasing serum PTH also led to statistically non-significant but consistent fall in BMD at spine in males. If vitamin D varied between 10.0-19.9 ng/ml and serum PTH was increased, a significant fall in BMD at hip ($p<0.02$) was noted in males. Similarly in females, if vitamin D was ≥20 ng/ml and serum PTH was increased, a statistically non-significant but consistent decline in BMD at hip was noted. Statistically non-significant but consistent increase in BMD at forearm was noted when vitamin D was kept at ≥20 ng/ml and serum PTH was increased in males. Thus it is clear from the above analysis that an optimum combination of dietary Ca, serum vitamin D and serum

Table 6a. Mean BMD at hip ± SD (gm/cm²) at various levels of dietary Ca and serum vitamin D in males

Serum vitamin D (ng/ml)	Mean BMD at hip ± SD (gm/cm²) Dietary Ca (mg/day) <800	800-1199	≥1200	Total
<10	0.972±0.130	0.938±0.094	0.964±0.117	0.959±0.113
10-19.9	1.028±0.123	0.979±0.134	1.000±0.159	0.998±0.143
≥20	1.006±0.102	1.006±0.096	0.966±0.127	0.993±0.109
Total	1.010±0.117	0.980±0.116	0.979±0.139	0.987±0.127

Table 6b. Mean BMD at hip ± SD (gm/cm²) at various levels of dietary Ca and serum vitamin D in females

Serum vitamin D (ng/ml)	Mean BMD at hip ± SD (gm/cm²) Dietary Ca (mg/day) <800	800-1199	≥1200	Total
<10	0.878±0.104	0.872±0.102	0.844±0.093	0.864±0.100
10-19.9	0.927±0.118	0.940±0.102	0.926±0.081	0.932±0.104
≥20	0.972±0.104	0.965±0.131	0.894±0.105	0.948±0.118
Total	0.919±0.115	0.914±0.113	0.880±0.098	0.906±0.111

PTH for achieving significant rise in BMD at any of the three sites is a researchable issue.[19] The mean log serum PTH at different levels of dietary Ca and serum vitamin D for males and females is also depicted in figures 2a & 2b respectively.

The mean urinary fluoride levels in males and females were 0.9±0.6 and 0.8±0.6 ppm respectively. Statistically significant differences ($P<0.003$) were observed in mean urinary fluoride levels amongst all the centres in males except between NIN and NIRRH. In females, however, the mean urinary fluoride level was found to be highest (1.2±0.7 ppm) in SGPGIMS, which was found to be statistically significant ($P<0.001$) compared to other centres. No significant impact of urinary fluoride on BMD at any site was observed either in males or in females.

3.5 Multiple regression analysis

A multiple stepwise linear regression analysis was also carried out separately for males and females to identify and isolate the effect (effect adjusted for other factors) of possible predictors of BMD at the three sites. The dependent variables being BMD at the three sites and independent variables were the factors contributing significantly in BMD as found in the preceding sub-sections. The results are as described below:

Males

With BMD at total hip as the dependent variable, weight ($b=0.005$, $p<0.0001$) and serum albumin ($b=-0.030$, $p<0.02$) and log PTH ($b=-0.020$, $P<0.05$) turned out to be the main predictors explaining only 11.5% ($R^2=0.115$) of the variation. Further, with BMD at total lumbar spine as the dependent variable, weight ($b=0.005$, $p<0.0001$) and brisk walking and equivalent activities ($b=0.024$, $p<0.04$) turned out to be the significant contributors explaining only 14.3% of the variation. Finally with BMD at forearm as the dependent variable, weight ($b=0.001$, $p<0.0001$), serum phosphorus ($b=0.011$, $p<0.009$), brisk walking and equivalent activities ($b=0.017$, $p<0.003$), serum vitamin D ($b=0.001$, $p<0.03$) and doing vigorous bone loading activities ($b=0.019$, $p<0.03$) came out to be the significant contributors explaining 12.4% of the variation. Thus weight consistently emerged as a strong predictor of BMD at all the three sites followed by brisk walking and equivalent activities at total lumbar spine and forearm.

Females

Likewise, with BMD at total hip as the dependent variable, weight ($b=0.005$, $p<0.0001$), log PTH ($b=-0.020$, $p<0.004$) and being married ($b=-0.032$, $p<0.008$) and serum albumin ($b=-0.019$, $p<0.05$) were found to be the significant predictors explaining about 25% of the variation. Further, with BMD at total lumbar spine as the dependent variable, only weight ($b=0.005$, $p<0.0001$) emerged as the sole predictor with the model explaining only 13.6% of the variation. Finally with BMD at forearm as the dependent variable, weight ($b=0.002$, $p<0.0001$), being married ($b=0.013$, $p<0.01$) and BMI ($b=-0.003$, $p<0.05$) turned out to be the significant contributors explaining only 12.7% of the variation. Thus weight again emerged as a strong predictor of BMD at all the three sites followed by being married at hip and forearm.

The results of regression analysis had reconfirmed the role of quite a few of the factors as identified in the previous sub-sections. But certain well known factors such as height, BMI and doing bone loading activities did not turn out to be contributing in BMD significantly in spite of having significant positive correlations with BMD. The fact that a large part of the variation in BMD still remained unexplained could be a possible explanation for this phenomenon. A large epidemiological study covering people of much wider age-range and representing all strata of the community might explain the role of various factors affecting the BMD.

4. Conclusions and Recommendations

From the above results it was clear that the pooled estimates of BMD of healthy normal males and females as well as their Peak Bone Mineral Density at the three sites namely, hip, forearm and spine were found to be significantly lower than the corresponding NHANES III and Hologic normative values.

The demographic parameters such as religion, father's occupation during childhood, self occupation, caste and dental mottling had no impact on BMD at any of the three sites. The nature of activity performed significantly affected BMD at forearm and spine both in males and females regardless of the centres. Among the physical and clinical parameters, age, age at menarche and age at first birth in females had no impact on BMD at any site.

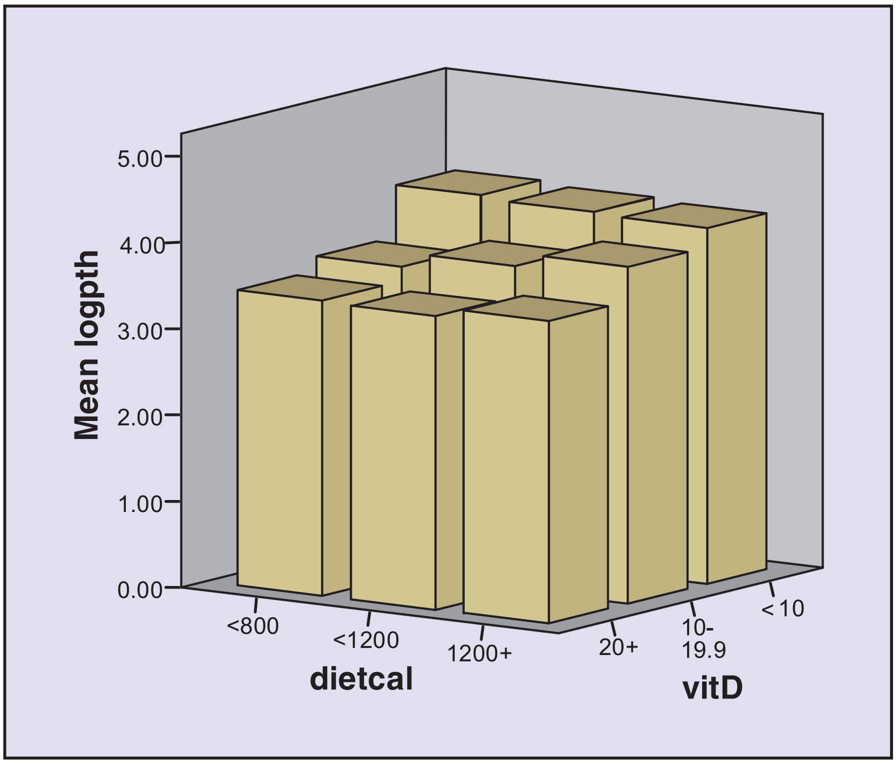

Figure 2a. Mean log Serum PTH at different levels of Dietary Calcium & Vit D in males

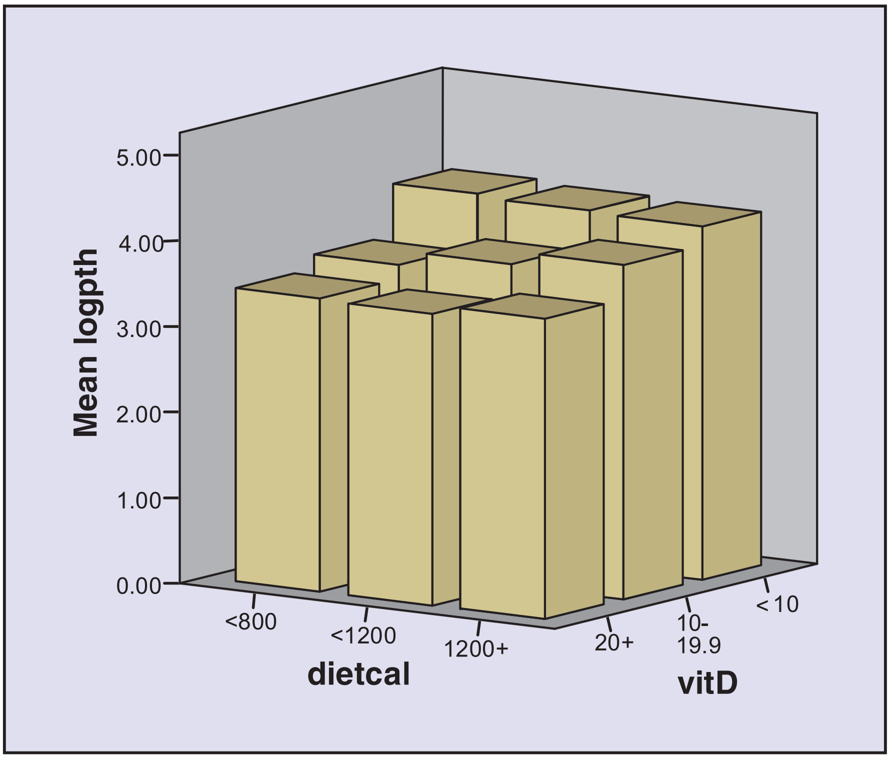

Figure 2b. Mean log Serum PTH at different levels of Dietary Calcium & Vit D in females

The height, weight and BMI all showed significantly high and positive correlations with BMD at all the three sites in both males and females. However, weight alone showed much higher positive impact on BMD at all the three sites. In females, number of births had negative impact on BMD at hip. Among the biochemical parameters, hemoglobin and urinary fluoride showed no impact on BMD at any of the three sites. Serum albumin had a negative impact on BMD at hip in both males and females. Serum alkaline phosphatase positively affected BMD at forearm in both males and females and serum phosphorus positively affected BMD at forearm in males only. Serum vitamin D showed positive impact on BMD at hip in females whereas serum PTH showed negative impact on BMD at hip in females.

An insight in to the data on BMD showed significant centre-wise differences thereby making the task of preparing common BMD reference standards for the whole population a difficult exercise. However, in view of the homogeneity of variances of BMD across all the four centres and also in view of the meager absolute relative differences in BMD (0-4.5 %) at each centre at the three sites namely, hip, forearm and spine from the corresponding pooled means of BMD, the pooled means of BMD at the three sites were finally proposed as the Indian reference standards. These are as given below:

Sex	n	Mean BMD±SD (gm/cm^2)		
		Hip	Forearm	Spine
Male	404	0.988±0.131	0.611±0.052	0.976±0.105
Female	404	0.901±0.111	0.538±0.044	0.954±0.095

Subsequently, the cut-off values for the Indian population for diagnosing osteoporosis as per the WHO guidelines[20] (Mean-2.5 SD) in males at hip, forearm and spine were found to be 0.661, 0.481 and 0.714 gm/cm^2 respectively and those in females were found to be 0.624, 0.428 and 0.717 gm/cm^2 respectively.

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COLLABORATING CENTRES

	INSTITUTION	STATE	RESEARCH TEAM
1.	National Institute of Nutrition Hyderabad	Andhra Pradesh	Dr. Veena Shatrugna, Deputy Director Dr. T. Longvah, Deputy Director Dr. A.L. Khandare, Assistant Director Dr. J.J. Babu, Assistant Director Dr. N. Balkakrishna, Assistant Director Dr. Bharati Kulkarni, Sr. Res. Officer Ms. Prabhavati Paranjape, Sr. Tech. Officer Mr.G. Amarender Reddy, Tech Officer Mr. P. Ajay Kumar, Tech. Assistant Ms. K. Usha Rani, Technician Mr. Chenna Krishna Reddy, Tech. Assistant Ms. A. Padma Sirromani, Health Visitor Mr. G.V. Narasimha Rao, Tech. Assistant Ms. P. Suvarna, Health Visitor Mr. R.S. Venkateswara Rao, Tech. Assistant Mr. R. Sambasiva Rao, Tech. Assistant Dr. P. Thilak Ravindra Reddy, Medical Officer Mr. S. Venu, Sr. Tech. Assistant Mr. V. Chandrababu, Research Assistant Ms. P. Kavita, Research Assistant (Nutritionist) Ms. S. Shobha Rani, Technician Mr. B. Neelkanta, Lab. Attendant
2.	All India Institute of Medical Sciences, New Delhi	Delhi	Dr. A.C. Ammini, Professor Dr. Nikhil Tandon, Professor Dr. Nandita Gupta, Additional Professor Dr. Ravinder Goswami, Associate Professor Dr. Namit Gupta, Research Officer Mr. Dinesh Kumar, Social Worker Ms. Neena Seth, Social Worker Mr. Rustam Kumar, Data Entry Operator Ms. Shelly Khurana, Dietician

INSTITUTION	STATE	RESEARCH TEAM
3. Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow	Uttar Pradesh	Dr. Eesh Bhatia, Professor Dr. Madan Godbole, Professor Dr. Vijaylakshmi Bhatia, Professor Dr. Sushil Gupta, Additional Professor Dr. Preeti Dabadghao, Associate Professor Dr. Subhash Yadav, Associate Professor Dr. Preeti Gupta, Research Associate Dr. Poonam Sharma, Sr. Research Fellow Dr. Genitta George, Sr. Research Fellow Ms. Diwa Pandey, Dietician Ms. Nirupama Singh, Dietician Mr. Himanshu Pandey, Social Worker Dr. Anil Kumar, Social Worker Mr. Manoj Singh, Social Worker Mr. Sanjeev Pandey, Technician Mr. Intsar Ahmed, Technician Mr. Arjun Singh, Lab. Attendent
4. National Institute of Research in Reproductive Health, Mumbai	Maharashtra	Dr. Rashmi Shah, Deputy Director (Sr. Grade) Dr. Lalita Saverdekar, Assistant Director Dr. Rajlakshmi Srinivasan, Research Associate Dr. Himali Dalvie, Research Associate Dr. Ikram Khatkhatey, Sr. Research Officer Mr. Sushant Chavan, Tecnician Mrs. Reshma Sathe, Technician Mrs. Kirti Bodke, Technician Mrs. Prerna Gade, Social Worker Mrs. Madhu Singh, Social Worker Mrs. Kirti Bodke, Social Worker Mrs. Pratibha Sonawane, Social Worker Ms. Payal Batra, Dietician Mr. Kiran Chavan Laboratory Atttendent
5. Indian Council of Medical Research Division of RHN	Delhi	Dr. Rakesh Mittal, Deputy Director General (Sr. Grade) Dr. Ajit Mukherjee, Deputy Director General (Sr. Grade) Mr. Ajit Mathur, Deputy Director General Dr. Neeta Kumar, Research Officer

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