Original Articles

Bone mineral parameters in healthy young Indian adults with optimal vitamin D availability

N. TANDON*, R. K. MARWAHA*, S. KALRA, NANDITA GUPTA, A. DUDHA, N. KOCHUPILLAI

ABSTRACT

Background. Several recent studies indicate a marked prevalence of vitamin D deficiency in asymptomatic, apparently healthy urban subjects from different socioeconomic groups in north India.

Methods. To further examine this trend, we studied 40 men and 50 women, 20–30 years of age, from the Indian paramilitary forces. These individuals consume a nutritious, high-protein diet, have optimal exposure to sunlight and undertake strenuous outdoor physical exercise.

Results. The mean serum calcium, phosphorus and alkaline phosphatase levels were normal in both men and women. The mean (SD) serum intact parathyroid hormone and 25-hydroxy-vitamin D_3 levels were 19.3 (8.2) pg/ml and 18.4 (5.3) ng/ml in men, and 11.9 (6.6) pg/ml and 25.3 (7.4) ng/ml in women. Bone mineral density estimated in 20 men and 22 women revealed that in comparison with white Caucasians, 35%–50% of men and 14%–32% of women were osteopenic at different sites, while an additional 10% of men had osteoporosis of the lumbar spine.

Conclusion. We found that with optimal nutrition, good sunlight exposure and regular physical exercise, healthy young individuals have normal bone and mineral biochemical values. The reasons for the abnormalities detected in bone mineral density in them needs further study. The impact of childhood nutrition on accumulation of peak bone mass may contribute to our findings. There is a need for establishing normative bone mineral density data for Indians.

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INTRODUCTION

Ethnicity and race are well known determinants of skeletal health.[1] The frequent occurrence of metabolic bone disease in Indians has been documented not only within India,[2,3] but also in Indian immigrants living abroad.[4] Subclinical bone disease, with abnormal biochemical values of bone and mineral metabolism, has been reported previously.[5–7]

All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India
N. TANDON, R. K. MARWAHA, S. KALRA, NANDITA GUPTA, A. DUDHA, N. KOCHUPILLAI
Department of Endocrinology
*N. Tandon and R. K. Marwaha have equal contributions to the study and are joint first authors

Correspondence to N. KOCHUPILLAI; *kochupillai@hotmail.com*

Interethnic differences in hip fracture rates, which is a surrogate marker for bone mineral density (BMD), have been reported.[8,9] There are reports suggesting that Indian women have low bone density,[10,11] but large population-based studies to examine whether Indians have a lower bone mass than Caucasians have not been reported.

Various factors including diet, exercise, exposure to sunlight and serum levels of vitamin D and parathyroid hormone (PTH) are recognized determinants of BMD. While the effect of dietary nutrient intake on BMD has been demonstrated in Caucasians[12] and the Chinese,[13] no study has been done among Indians.

All the studies reported so far in Indians have been done on randomly selected, clinically 'normal' subjects/hospital staff and students. No BMD studies are reported on well-characterized subjects with normal status of vitamin D, and other nutritional and environmental factors known to influence bone mineral metabolism. We selected two groups of healthy young adults with optimal sunlight exposure, nutrition and physical activity, and evaluated biochemical values of bone mineral metabolism and BMD.

METHODS

Two groups of normal healthy subjects from the Indian paramilitary forces were studied after obtaining ethical clearance and informed consent. Forty healthy men, 20–30 years of age, who had a normal dietary intake, good exposure to sunlight and physical activity were selected randomly from one subunit of a regiment, which had been stationed continuously for the past 2 years in one location. The subjects consumed food from a common kitchen. They were studied during the months of January and February (winter). Fifty healthy policewomen, 20–30 years of age, were studied during the months of May and June (summer). All the subjects were in service for a minimum of 5 years, ensuring adequate environmental exposure to sunlight for at least this duration.

The exclusion criteria included chronic liver disease, malabsorption, history of ingestion of corticosteroids, use of drugs affecting the metabolism of vitamin D, as well as recent intake of calcium or vitamin D. Women with a history of pregnancy and/or lactation within 1 year or ³3 pregnancies were excluded.

A detailed history was taken and thorough clinical examination carried out. An outdoor score using a modification of the method of Hodkinson *et al.*[14] quantified the average exposure to sunlight. This scoring system measures the time spent outdoors in direct sunlight between 0900 and 1600 hours during a typical day. Evaluation of dietary intake of calories, protein, fibre, fat, phytate (phytin-P) and calcium was done by 24-hour dietary recall. Since all the subjects ate food cooked in a common institutional kitchen,

the dietary evaluation was reconfirmed by cross-checking the quantity of rations allocated to each subject from the inventory. Calculations were based on published food tables detailing the nutritive value of Indian foods.[15] Dairy products and food fats are not fortified with vitamin D in India.

Fasting venous samples for the study were collected between 0800 and 0900 hours, without venostasis, for estimation of serum calcium, phosphorus, alkaline phosphatase, intact PTH, and 25-hydroxyvitamin D_3 concentration.

Serum calcium, phosphate and alkaline phosphatase were measured using methods reported previously.[6] The normal values, standardized in our laboratory, are as follows: serum calcium 8.5–10.5 mg/dl, serum phosphorus 2.5–4.5 mg/dl; serum alkaline phosphatase 6–15 KAU/dl. Serum 25-hydroxyvitamin D_3 (reference range 9–37.6 ng/ml) and serum intact PTH (13–54 ng/L) estimation were done by radioimmunoassay and immunoradiometric assay, respectively (Incstar Corporation, Stillwater, MN, USA).

Twenty men and 22 women, chosen at random, were subject to bone densitometry using dual energy X-ray absorptiometry (DEXA) (Hologic QDR 4500 A densitometer). BMD was measured at three sites: lumbar spine (L1–L4) using lateral and antero-posterior views, left radius and left hip. For the purpose of analysis, the various regions of interest (ROI) of the hip were analysed separately: neck of femur, trochanter, intertrochanteric region and Ward triangle. The data obtained were compared with normative data for the Caucasian population.[16] The degree of osteopenia/osteoporosis was reported according to WHO standards. A T score >–1 was considered normal, between –1 and –2.5 was diagnostic of osteopenia, and a T score <–2.5 was diagnosed as osteoporosis. The reference group used for calculation of T and Z scores was also provided by the manufacturers (Hologic Inc.) as a software incorporated within the machine.

Statistical analysis

Statistical analysis was performed using standard descriptive statistical tests, including unpaired Student *t* test and Pearson correlation coefficient.

RESULTS

The physical characteristics and sunlight exposure scores are given in Table I. All the women had normal menstrual periods and were nulliparous. The total calories, protein, fat, fibre and calcium intake was adequate in both the groups as judged by the Indian Council of Medical Research (ICMR) recommended daily allowances (Table I).

TABLE I. Physical characteristics and sunlight exposure score

Characteristics	Men (*n*=40)	Women (*n*=50)	p value
Age (years)	22.7 (2.8)	23.4 (3.1)	ns
Weight (kg)	61.68 (5.56)	52.48 (6.11)	<0.001
Height (cm)	171.3 (4.29)	160.0 (4)	<0.001
Body mass index (kg/m^2)	21.0 (1.48)	20.43 (2.37)	ns
Sun exposure score	12 (0)	12 (0)	ns
Energy intake (calories)*	3040 (812)	2068 (447)	<0.001
Protein (g)*	104.3 (37.9)	72.8 (27.1)	<0.001
Fibre (g)*	11.2 (4.2)	5.67 (2.8)	<0.001
Calcium (mg)*	1041.7 (452.6)	764.8 (440.2)	<0.01
Phosphorus (mg)	2655.1 (690.5)	1539.7 (452.0)	<0.001

* The intake of energy, protein and calcium met the recommended daily allowance for moderately active individuals prescribed by the Indian Council of Medical Research. Values in parentheses are standard deviations

TABLE II. Mean values of biochemical tests in men and women subjects

Analyte	Men (*n*=40)	Women (*n*=50)	p value
Serum calcium (mg/dl)	9.4 (0.7)	9.5 (0.3)	ns
Serum phosphorus (mg/dl)	3.9 (0.5)	4.7 (0.4)	<0.001
Alkaline phosphatase (KAU)	12.2 (5.3)	7.9 (1.8)	<0.001
Urinary calcium (mg/dl)	9.8 (5.8)	8.2 (3.2)	ns
Urinary phosphorus (mg/dl)	41.1 (31.4)	58.0 (36.6)	<0.05
Urinary creatinine (mg/dl)	92.7 (53.0)	142.9 (68.9)	<0.001
Ca:creatinine ratio	0.13 (0.08)	0.07 (0.06)	<0.001
PTH intact (pg/ml)	19.3 (8.2)	11.9 (6.5)	<0.001
25(OH) vitamin D_3 (ng/ml)	18.4 (5.3)	25.3 (7.4)	<0.00

PTH parathyroid hormone Values in parentheses are standard deviations

The mean (SD) serum calcium was within the normal range and similar in both men (9.4 [0.7] mg/dl) and women (9.5 [0.3] mg/dl; Table II). However, the serum phosphorus was significantly lower in men (3.9 [0.5] mg/dl) than in women (4.7 [0.4] mg/dl; p<0.001). There was also a significant difference between the alkaline phosphatase level in men (12.2 [5.3] KAU/dl) and women (7.9 [1.8] KAU/dl); (p<0.001). The serum alkaline phosphatase was >15 KAU/dl in 9 men (22.5%), 5 of whom (12.5%) had a level >20 KAU/dl.

All the subjects had vitamin D_3 and intact PTH values in the normal range (Table II). However, women had a significantly higher mean 25-hydroxyvitamin D_3 level and lower PTH than men. The serum intact PTH was 19.3 (8.2) pg/ml in men as compared to 11.9 (6.5) pg/ml in women (p<0.001). The 25-hydroxyvitamin D_3 level was 18.4 (5.3) ng/ml in men while it was 25.3 (7.4) ng/ml in women (p<0.001). It should be noted that the sample collection was done in winter for men and in summer for women. There was no correlation between the PTH and vitamin D_3 levels, or calcium and vitamin D_3 levels, in either men or women.

The BMD data of 20 men are provided in Table III. On comparing the mean BMD data of men with the normative data for white Caucasians,[16] the BMD at each site in men was lower. However, this difference was statistically significant only at the lumbar spine, with the mean BMD in men (0.947 [0.086] g/cm^2) being less than that of Caucasian controls (1.091 [0.110] g/cm^2; p= 0.0004). The BMD data of women is provided in Table IV. The BMD at each site was only marginally lower than that reported in age-matched white Caucasians. This difference was less marked than that observed between men in our study and white Caucasian men.

Comparing individual BMD data with peak bone mass in white Caucasians revealed that 35%–50% of men and 14%–32% of women were osteopenic at different sites, while an additional 10% of men were osteoporotic at the lumbar spine (Table V).

TABLE III. Comparison of the mean (SD) bone mineral density of Indian and Caucasian men

Site	Bone mineral density (g/cm^2)	
	25-year-old Caucasian men	Indian men
Lumbar spine	1.091 (0.110)	0.947 (0.086)*
Neck of femur	0.979 (0.110)	0.911 (0.129)
Trochanter	0.797 (0.110)	0.740 (0.117)
Ward triangle	0.832 (0.120)	0.798 (0.146)
Inter-trochanter	1.243 (0.150)	1.157 (0.159)
Hip	1.072 (0.130)	1.016 (0.133)
Forearm	0.802 (0.060)	0.619 (0.072)

* p<0.0004; all other values were not significantly different

TABLE IV. Comparison of the mean (SD) bone mineral density of Indian and Caucasian women

Site	Bone mineral density (g/cm²)	
	25-year-old Caucasian women	Indian women
Lumbar spine	1.040 (0.110)	0.981 (0.092)
Neck of femur	0.895 (0.110)	0.850 (0.101)
Trochanter	0.707 (0.090)	0.707 (0.096)
Ward triangle	0.796 (0.110)	0.769 (0.121)
Inter-trochanter	1.134 (0.140)	1.137 (0.122)
Hip	0.975 (0.120)	0.957 (0.103)
Forearm	0.667 (0.060)	0.541 (0.034)

No statistically significant difference at any of the sites

TABLE V. Prevalence of osteopenia and osteoporosis

Site	Men (*n*=20)			Women (*n*=22)		
	Osteo-penia	Osteo-porosis	Total	Osteo-penia	Osteo-porosis	Total
Lumbar spine	10	2	12 (60)	7	0	7 (32)
Hip	7	0	7 (35)	3	0	3 (14)
Neck of femur	8	1	9 (45)	7	0	7 (32)
Trochanter	8	0	8 (40)	6	0	6 (27)
Inter-trochanter	4	0	4 (20)	4	0	4 (19)
Ward triangle	7	0	7 (35)	4	0	4 (19)
Forearm	10	0	10 (50)	5	0	5 (23)

Values in parentheses are percentages

Osteopenia was most prevalent at the lumbar spine in both groups, followed by the forearm in men and neck of the femur in women. Osteoporosis was reported, according to Caucasian normative data, in 2 men (both at the lumbar spine).

The biochemical results of osteopenic/osteoporotic patients were compared with their counterparts who exhibited normal BMD according to normal Caucasian data. There was no significant difference between osteopenic and non-osteopenic individuals with respect to serum calcium, phosphate, alkaline phosphatase, PTH, and 25-hydroxyvitamin D_3. Serum vitamin D_3 and PTH in osteopenic and non-osteopenic individuals is shown in Table VI.

BMD was compared with all the anthropological, dietary and biochemical parameters to assess if there was any determinant of BMD (Table VII). Women showed a strong correlation between BMD and weight at most sites. Lumbar spine BMD showed a negative correlation with dietary fibre and a positive correlation with ingestion of dairy products in men. No other correlation, either positive or negative, was identified in either group.

TABLE VI. Mean (SD) vitamin D and parathyroid hormone (PTH) levels in subjects with and without osteopenia

Site	Men		Women	
	Serum 25 (OH) vitamin D	Serum PTH	Serum 25 (OH) vitamin D	Serum PTH
Lumbar spine				
Osteopenic	19.78 (5.03)	17.33 (4.50)	23.60 (6.12)	12.86 (6.28)
Non-osteopenic	18.87 (5.49)	20.37 (5.83)	25.90 (6.32)	11.33 (6.01)
Hip				
Osteopenic	20.33 (4.97)	17.71 (4.20)	24.14 (6.00)	12.71 (6.13)
Non-osteopenic	18.97 (5.13)	19.30 (6.18)	25.96 (6.32)	11.33 (5.86)
Forearm				
Osteopenic	21.08 (5.68)	17.70 (3.80)	24.85 (5.78)	12.76 (6.51)
Non-osteopenic	19.10 (3.62)	19.30 (6.31)	25.82 (6.12)	11.50 (5.98)

TABLE VII. Positive correlates of bone mineral density

Group	Site	Anthropological and dietary variable	R	p value
Men	Lumbar AP	Fibre	–0.442	<0.05
Men	Lumbar AP	Dairy calcium	+0.533	<0.05
Women	Lumbar AP	Weight	+0.493	0.05
Women	Hip	Weight	+0.457	0.05

AP anteroposterior view

DISCUSSION

We did this study to assess the biochemical parameters of bone and mineral metabolism and bone density in young, healthy Indian adult men and women consuming a nutritious diet, exposed to optimal sunlight and performing regular physical activity. We did not attempt to establish normative data. However, the observations can be considered to reflect the bone mineral health of Indians who are exposed to optimal environmental conditions.

Several studies, both in India and in Indian migrants to the UK, have shown that privational vitamin D deficiency is more common in this population than in white Caucasians. It was first reported in India in the early part of the twentieth century[2,3] and later confirmed by hospital-based studies in the 1960s and 1970s, and in Asian migrants to the UK.[5,4,17–21] The proposed reasons for the high prevalence include limited exposure to sunlight,[22] skin pigmentation,[23] genetic factors,[4] vegetarian diet,[21,24–27] and excess of phytic acid in the diet.[28,29] However, some studies have suggested that dietary factors possibly play only a minor role in the causation of hypovitaminosis D.[30,31] Lo *et al.*[32] have suggested that Indian immigrants have the same capacity to produce vitamin D in response to ultraviolet light, though longer exposure to sunlight is required to achieve a similar response. We, therefore, studied the vitamin D and BMD status in a selected population of healthy young adults with optimal sunlight exposure, nutrition and physical activity to exclude possible environmental factors responsible for hypovitaminosis D.

The mean energy, protein and calcium intake of both men and women were adequate in reference to the recommended dietary allowance prescribed by the ICMR. Exposure to sunlight was optimal in all subjects.

Before analysing the biochemical results it is pertinent to point out that men were studied during winter, and women were studied during summer. That seasonality has an impact on the vitamin D status has been well established in the literature.[33–37] This has also been confirmed in an earlier study conducted by us in Delhi (latitude 28 ºN), which showed significantly higher vitamin D levels in summer.[7]

The mean serum calcium, phosphorus and alkaline phosphatase were normal in both men and women. These data are consistent with the results reported by Marya *et al.*[38] and Hodgkin *et al.*[39] These two studies were conducted in the states of Punjab and Haryana. However, 9 men had elevated serum alkaline phosphatase. Clinically, none of them had evidence of hepatic dysfunction or metabolic bone disease. We did not estimate bone and liver-specific alkaline phosphatase activity separately in them. However, there was no statistically significant difference in vitamin D and PTH levels of subjects with and without elevated serum alkaline phosphatase. In view of the normal serum calcium, phosphate, vitamin D and PTH, and absent clinical features of metabolic bone disorders, the increased alkaline phosphatase level is unlikely to be of bone origin.

Estimation of 25-hydroxyvitamin D_3 and intact PTH concentration revealed that all the subjects studied had values within the

normal range. However, women studied in summer had a significantly higher mean 25-hydroxyvitamin D_3 (25.3 [7.4] ng/ml) compared with men studied in winter (18.4 [5.3] ng/ml; $p<0.001$). Correspondingly, intact PTH values were significantly higher in men (19.3 [8.2] pg/ml) compared with women (11.9 [6.5] pg/ml; $p<0.001$). It is relevant to compare these values with two previous studies conducted by us. Harinarayan *et al.*[6] estimated 25-hydroxyvitamin D_3 levels in 10 healthy volunteers and found that the mean value was 8.3 (2.5) ng/ml. In a later study, Goswami *et al.*[7] studied vitamin D and intact PTH levels in 19 doctors and nurses in both summer and winter. (It is prudent to comment that this group remains indoors for most of the working day.) The mean 25-hydroxyvitamin D_3 value in winter was 3.2 ng/ml (3.96 [1.72] ng/ml in men; 2.4 [0.48] ng/ml in women) and in summer it was 7.9 ng/ml (9.6 [4.7] ng/ml in men; 6.1 [2.4] ng/ml in women). Serum intact PTH, which was only measured in samples collected in winter was 38.8 (18.2) pg/ml. The mean intact PTH for men and women was 26.5 (9.7) pg/ml and 40.4 (18.3) pg/ml, respectively. If we compare vitamin D_3 and intact PTH values of men collected in winter in the study by Goswami *et al.*,[7] with the corresponding values of men in our study, the difference is statistically significant ($p<0.001$ for vitamin D3; $p<0.05$ for intact PTH). A similar comparison of vitamin D_3 values between women in our study with samples collected in summer in the study by Goswami *et al.*[7] also reveals a statistically significant difference ($p<0.001$). Since the dietary energy, protein and calcium intake of the subjects in both studies met the RDA prescribed by ICMR, the difference in vitamin D levels, discussed above, can be attributed to differences in sunlight exposure.

We evaluated BMD by DEXA and compared the values with normative data for white Caucasians provided by the manufacturer. The mean BMD of both the lumbar spine and hip for women, and hip for men was not significantly different from Caucasian norms (Tables III and IV). The mean BMD of the lumbar spine of the men in our study was significantly lower than that of western Caucasian controls (Table III). Ahuja,[40] in a necropsy-based study, showed that the apparent density and ash content per millilitre of the bones of the Indian population studied was generally comparable to those reported for Caucasians in north America and western Europe, but were lower than those reported for American blacks.

In our study, individual values for different sites were lower than those of the reference population. Among men, osteopenia was found in 50% at the lumbar spine, 35% at the hip and 50% at the forearm. In addition, 10% of men were osteoporotic at the lumbar spine. Among women, osteopenia was found in 32% at the lumbar spine, 14% at the hip and 23% at the forearm. Little is known about BMD, as measured by DEXA, in ethnic Indians as compared with white Caucasians. Recently, using DEXA, Nangia *et al.*[11] and Arya *et al.*[10] have shown that both spine and hip BMD in normal Indian women is significantly less compared with the reference American population.

We evaluated the possible causative factors for the low BMD. In both groups, there was no statistically significant difference in vitamin D and PTH levels between subjects with and without osteopenia/osteoporosis (Table VI). Among other anthropometric, dietary and biochemical variables, in men, positive correlation of the lumbar spine BMD was observed with dairy calcium ingestion, and a negative correlation with fibre intake (Table VII). Among women, weight correlated positively with BMD at the lumbar spine and hip (Table VII). The correlation of BMD with weight has been reported earlier.[41–43] The negative correlation with dietary fibre could result from reduction in the availability of calcium due to fibre. Indian diets are traditionally high in fibre and phytates. The latter also bind calcium as insoluble complexes in the gut, reducing calcium availability. Correlation with dietary calcium has not been consistently reported. Hu *et al.*[13] and Neville *et al.*[43] reported findings similar to ours, while Heaney[44] found no such correlation.

Other proposed risk factors for low BMD include race, familial prevalence, increased alcohol and caffeine intake, increased consumption of animal proteins, smoking, low physical activity and oestrogen deficiency. Among these factors, there was no history to suggest excess consumption of alcohol, caffeine or cigarettes. All the women had regular menstrual cycles and had never been pregnant or lactated. Physical activity has been shown to be positively correlated with BMD.[45] Both groups were involved in regular, strenuous, physical activity, both weight-bearing and aerobic. Hence, low physical activity cannot be implicated as a cause for the low BMD in our subjects.

It has been estimated that approximately 40%–50% of the total skeletal mass at maturity is accumulated during childhood and adolescence.[46] Most of this increase occurs between 10 and 15 years of age, corresponding to the period of the growth spurt. It is reasonable to speculate that nutritional deficiency during this period would have a considerable adverse impact on the peak bone mass attained. The subjects in our study had been recruited in the paramilitary forces at the age of 18 years or later. We can be sure that optimum nutrition was provided to the subjects after their entry to the service. However, their nutritional status during childhood and adolescence is not known to us. Hence, the osteopenia in 35%–50% of men and 14%–32% of women, despite current exposure to ideal environmental and dietary conditions, could possibly relate in part to inadequate nutritional intake during their childhood and adolescence. However, considering the fact that recruits to the paramilitary forces are selected on the basis of an above average level of physical fitness, this may not be a valid argument.

In conclusion, Indians who lead a physically active life, with adequate sunlight exposure and have a nutritious diet, have normal biochemical parameters of calcium, vitamin D, and PTH. The significant proportion of healthy young adults with low BMD at different sites, as per the western norms, is difficult to explain on the basis of known determinants of low BMD. However, factors that operate in childhood to help achieve ideal peak bone mass remain inadequately understood. Our study also suggests that there could be normal variation in BMD between ethnic groups, emphasizing the need for establishing ethnically appropriate BMD norms.

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Erratum

In the Letter from North America, Controversies of cola beverages (*Natl Med J India* 2003; **16:**277–9), the name of the second author SCOTT TUORTO has appeared in italics suggesting that SCOTT TUORTO is part of the affiliation of the first author PRASAD S. ADUSUMILLI. We apologize for the error

—Editor