

Vitamin D status in Andhra Pradesh : A population based study[†]

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Background & objectives: Data on the vitamin D status of the population in a tropical country like India have seldom been documented. Vitamin D deficiency is presumed to be rare. We carried out this study to document the dietary habits, serum calcium, 25(OH)vitamin D [25(OH)D], and parathyroid hormone levels of urban and rural population in a State in south India.

Methods: A total of 943 healthy urban and 205 rural adult subjects and 76 urban and 70 rural healthy children were studied for their dietary pattern, serum calcium, phosphorus, alkaline phosphatase, 25(OH)D, and N-tact parathyroid hormone levels (N-tact PTH).

Results: The daily dietary calcium intake of both the urban and rural population was low compared to that of recommended daily/dietary allowances (RDA) issued by Indian Council of Medical Research (ICMR). Dietary calcium and phosphorus were significantly lower ($P<0.0001$) in both the rural adult and children compared to that of the urban adult and children. The dietary phytate to calcium ratio was significantly ($P<0.0001$) higher in rural adult and children compared to that of urban adult and children. N-tact PTH levels negatively correlated with 25(OH)D in rural ($r=-0.24$; $P<0.002$), in urban adult subjects ($r=-0.12$; $P<0.0001$) and in rural and urban children ($r=-0.2$; $P<0.05$). The 25(OH)D levels of rural adult subjects were significantly higher ($P<0.001$) than that of urban adult subjects in both males and female groups. The 25(OH)D levels of both the urban and rural children were low.

Interpretation & conclusions: Low dietary calcium intake and 25(OH)D levels were associated with deleterious effect on bone mineral homeostasis. Prospective longitudinal studies are required to assess the effect on bone mineral density, a surrogate marker for fracture risk and fracture rates.

Key words Bone mineral density - dietary calcium - high prevalence - Indians - phytate consumption - vitamin D insufficiency

Nutritional factors play a vital role in the bone homeostasis. Adequate calcium intake along with vitamin D helps to maintain bone mineral mass attained

at the end of growth period (peak bone mass). During infancy, childhood and adolescence, increasing dietary calcium intake favours bone mineral accrual¹. Adequate

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nutrition and sufficient activity provide mechanical impetus for bone development which may be critical in attaining bone growth potential. Vitamin D and calcium status correlate with increased bone mineral density and have the potential to increase the peak bone mass²⁻⁴. Increasing bone mineral content during periods of rapid growth (childhood and adolescence) increases "peak bone mass" and may effectively prevent osteoporosis at later age.

Serum 25-hydroxyvitamin D [25(OH)D] is the most reliable indicator of vitamin D adequacy of an individual². The production of 25(OH)D is not regulated and the serum concentration thus reflects both cutaneous synthesis and absorption from diet. While vitamin D deficiency [25(OH)D levels <20ng/ml] is associated with osseous changes (rickets/osteomalacia), vitamin D insufficiency [25(OH)D levels between 20 to 30 ng/ml] is associated with secondary hyperparathyroidism (SHPT) and negative skeletal consequences. Low dietary calcium intake further amplifies the parathyroid response to vitamin D insufficiency. The SHPT, which ensues, mobilizes mineral and matrix from skeleton, leads to an enhanced bone loss and to a high risk of fracture⁵⁻⁸ and, low peak bone mass in children²⁻⁴.

Vitamin D deficiency and/or poor dietary calcium intake can together lead to a defect in mineralization of bone (rickets in children; osteomalacia in adults). Rickets and osteomalacia are known to develop in immigrant Indians who migrate away from the equator⁹⁻¹². This was attributed to the poor cutaneous synthesis of vitamin D due to pigmentation and inadequate sunlight exposure along with a low dietary calcium intake. 25(OH)D deficiency was presumed to be rare in tropical country like India with abundant sunshine^{13,14} and also the data on vitamin D status of Indian population has seldom been documented.

Previously, we reported the prevalence of low 25(OH)D levels in India in a group of normal subjects and in patients with primary hyperparathyroidism¹³. Later other reports ensued¹⁴⁻¹⁷. Recent studies have highlighted associations between maternal nutritional status during pregnancy and bone mass in the offspring¹⁸.

So far, there is no large population based study documenting the dietary habits, serum calcium, 25(OH)D and parathyroid hormone levels in adults and children of rural and urban Indian population. We studied these aspects in subjects residing at Tirupati and the surrounding villages.

Material & Methods

The study was conducted in 943 urban and 205 rural healthy adult subjects, and 76 urban and 70 rural healthy children of Tirupati (located at 13.4 °N and 79.2 °E) southern Andhra Pradesh, India in the past 7 years. Medical and paramedical personnel working in the hospital, their relatives and their children; asymptomatic post-menopausal women and their relatives constituted the urban population. The rural population included men; women and children included after a demographic survey. Patients with hepatic, renal, dermatological disorders, alcoholics and pregnant women were excluded from the study. In the urban and the rural locations, the average duration of cloud free sunshine is around 8 to 10 h per day throughout the year with the solar zenith angle 9.92° in summer and 38.2° in winter. The UV index at the above said latitude during those periods is 7-12. Winter is short with a low 17°C and 28°C with scanty rainfall. There is a little seasonal variation of the peak intensity of sunlight.

The dietary assessment of total energy, calcium, phosphorus and phytates were documented by recalling the diet consumed in the previous 5 to 7 days. From the raw weights, the total energy, calcium, phosphorus and phytate intakes were calculated using a published food composition table, detailing the nutritive value of Indian foods¹⁹. Since the ratio of dietary phytates to calcium is more predictive of the severity of interference of calcium absorption than dietary calcium alone, phytate to calcium ratio was calculated¹⁵.

For all patients, peripheral venous blood samples (10 ml) were collected in the fasting state without applying tourniquet for the estimation of serum calcium, phosphorus, alkaline phosphatase (SAP), creatinine, and albumin, and samples kept on ice for 25(OH)D and N-tact PTH. The serum was separated in refrigerated centrifuge at 4°C for 20 min at 700 g and stored at -20°C until the analysis for the estimation of 25(OH)D and N-tact PTH. The blood samples collected from rural populations were transported in cool packs until they were separated and stored for further analysis. The serum calcium, phosphorus, alkaline phosphatase, creatinine and albumin levels were estimated by Beckman automated analyzer (CX 9, CA, USA) using commercial kits. The detailed methodology is described in our previous publication²⁰. The normal laboratory range for serum calcium is 8.5 to 10.5 mg/dl, serum phosphorus 2.5 to 4.5 mg/dl and for SAP is less than 95 IU/l in adults and less than 390 IU/l in children.

The 25(OH)D concentrations were measured by competitive radioimmunoassay after acetonitril extraction (DiaSorin, Stillwater, MN, USA, catalogue No. 68100E). The minimal detectable limit of 25(OH)D assays is 1.5 ng/ml. N-tact PTH was measured by immunoradiometric assay (IRMA) (DiaSorin, Stillwater, MN, USA, catalogue No. 26100). The minimal detectable limit of N-tact PTH assay is 0.7 pg/ml. The subjects were classified as vitamin D – deficient, – insufficient and – sufficient on the basis of 25(OH)D concentrations of < 20, 20 to 30 and >30 ng/ml respectively according to recent consensus²¹⁻²³.

Statistical analysis: Data are presented as mean \pm standard error of mean (SEM). Student's 't' test was used to compare the differences between the urban and the rural subjects. Pearson's coefficient was calculated for the correlation. $P < 0.05$ was considered significant. Analysis of variance (ANOVA) was used to estimate the main effects and interactions. Tukey test was used to identify the groups that are homogenous with respect to mean. Analysis was performed using SPSS (version 11.5, SPSS Inc, Chicago, IL).

Results

A total of 1148 adult subjects and 146 children were evaluated during the study. The mean \pm SEM (95% CI) age of adult urban subjects and rural subjects were 46 ± 0.43 (45 to 47) and 43 ± 1.01 (41 to 45) years respectively. The mean \pm SEM (95% CI) age of urban and rural children were 12.50 ± 0.56 (11 to 13.6) and 12.57 ± 0.42 (11.6 to 13.3) years respectively.

Urban subjects were fully dressed with only the face and forearm exposed to sunlight with a white collar job (working indoors between 1000 to 1700 h). Those not in a job were indoors most of the time. The rural subjects were agricultural workers starting their day from 0800 h working outdoors till 1700 h with their face, chest, back, legs, and arm and forearms exposed to sunlight. Urban children were those at home and as well as school going, fully dressed with only the face and forearms exposed to sunlight. Among the rural children, some were school going while the adolescent children (especially the boys) helped the family in agriculture (from 0800 h working outdoors till 1700 h). Both urban and rural children (boys and girls) had their face, legs, and arms and forearms exposed to sunlight in their dress code. In addition, the rural children (adolescent males) helping the family in agriculture had their torso and back exposed to sunlight.

The diet of urban subjects constituted of 2200 kcal/day approximately. Carbohydrates contributed 55 per cent of the total energy intake, proteins 10 per cent and fat 10 per cent. Vegetables contributed 10 per cent of the total energy intake and milk and milk products contributed to 15 per cent. The carbohydrate source was primarily from cereals with rice providing 50 per cent of total carbohydrates, wheat 25 per cent and *Ragi* (*Eleusine coracana*) 25 per cent. Vegetable sources included amaranth leaves, cauliflower, carrots, ladies fingers, other seasonal vegetables and tubers. Animal sources of protein were consumed once a week. The diet of rural subjects consisted of 1700 kcal/day approximately. Carbohydrates contributed 75 per cent of the total energy intake, proteins 10 per cent, fat 5 per cent, vegetables 5 per cent, and milk and milk products 5 per cent. The carbohydrate source was from cereals (rice - 60% and *Ragi* - 40%). Vegetable sources were drumstick leaves, brinjals and tomatoes, *etc.* Animal sources of protein were consumed once fortnightly. There was no other source of calcium or any other mineral in both groups.

Adults: Dietary calcium and phosphorus were significantly lower ($P < 0.0001$) in the rural subjects compared to that of the urban subjects. The dietary phytate/calcium ratio was significantly ($P < 0.0001$) lower in urban subjects compared to that of rural subjects (Table I). Dietary phytate correlated positively with dietary calcium in the urban subjects ($r = 0.55$; $P < 0.001$) and rural subjects ($r = 0.36$; $P < 0.0001$). Dietary calcium intake correlated negatively with phytate/calcium ratio in urban subjects ($r = -0.28$; $P < 0.0001$) and in rural subjects ($r = -0.43$; $P < 0.0001$). The r values are significantly different from each other ($P = 0.039$).

Children: Dietary calcium and phosphorus were significantly lower ($P < 0.0001$) in the rural children compared to that of the urban children. The dietary phytate and phytate/calcium ratio was significantly ($P < 0.0001$) lower in urban children compared to that of the rural children (Table I). The dietary calcium correlated negatively with phytate/calcium ratio in the urban ($r = -0.31$; $P = 0.01$) and in the rural ($r = -0.61$; $P < 0.001$) children. The r value was significantly different between urban and rural locations ($P < 0.02$). In the urban children, the dietary calcium correlated positively with dietary phytate ($r = 0.60$; $P < 0.0001$) and dietary phosphorus ($r = 0.66$; $P < 0.0001$). In the rural children, dietary calcium correlated positively with dietary phosphorus ($r = 0.46$; $P < 0.0001$).

Table I. Comparison of dietary intake of urban and rural groups - adults and children

Parameter	Adults males		Adults females		Children males		Children females	
	Urban (n=32)	Rural (n=109)	Urban (n=476)	Rural (n=96)	Urban (n=28)	Rural (n=34)	Urban (n=43)	Rural (n=36)
Dietary calcium (mg/day)	323 ± 8* (307 - 340)	271 ± 3 (263 - 280)	306 ± 2* (302 - 310)	262 ± 3 (253 - 271)	293 ± 6 (280 - 305)	277 ± 6 (265 - 289)	317 ± 9* (298 - 336)	270 ± 7 (254 - 285)
Dietary phosphorus (mg/day)	674 ± 17* (640 - 707)	493 ± 9 (475 - 511)	651 ± 9* (643 - 660)	481 ± 10 (462 - 501)	632 ± 18* (595 - 670)	483 ± 15 (451 - 514)	667 ± 15* (637 - 698)	489 ± 16 (458 - 521)
Phytate/calcium ratio	0.5 ± 0.02* (0.47 - 0.54)	0.76 ± 0.01 (0.74 - 0.78)	0.51 ± 0.01* (0.50 - 0.52)	0.76 ± 0.01 (0.74 - 0.78)	0.49 ± 0.02* (0.45 - 0.53)	0.75 ± 0.02 (0.70 - 0.80)	0.52 ± 0.01* (0.49 - 0.55)	0.72 ± 0.03 (0.66 - 0.77)

Values expressed as mean ± SEM (95% Confidence Intervals) (all such values). n= sample size; * $P < 0.0001$ compared to rural

Table II. Comparison of biochemical and hormonal profile of urban and rural groups - adults and children

Parameter	Adult males		Adult females		Children males		Children females	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Serum calcium (mg/dl)	9.74 ± 0.06 (9.63 to 9.85) (n=100)	10.06 ± 0.06* (9.95 to 10.2) (n=109)	9.68 ± 0.02 (9.64 to 9.73) (n=678)	9.98 ± 0.06* (9.87 to 10.15) (n=96)	9.67 ± 0.17 (9.33 to 10) (n=28)	10.28 ± 0.09* (10.09 to 10.48) (n=34)	9.78 ± 0.09 (9.59 to 9.97) (n=36)	10.03 ± 0.11 (9.80 to 10.27) (n=36)
Serum phosphorous (mg/dl)	3.50 ± 0.07 (3.37 to 3.64) (n=99)	2.84 ± 0.07* (2.27 to 2.97) (n=109)	3.64 ± 0.03 (3.59 to 3.69) (n=679)	2.74 ± 0.07* (2.79 to 3.09) (n=96)	4.0 ± 0.22 (3.55 to 4.45) (n=27)	3.15 ± 0.12* (2.92 to 3.39) (n=33)	4.08 ± 0.16 (3.76 to 4.41) (n=36)	3.31 ± 0.13* (3.05 to 3.56) (n=35)
SAP (IU/l)	84.87 ± 3.87 (78.85 to 90.9) (n=98)	55.67 ± 2.07* (49 to 61) (n=109)	80.4 ± 3.07 (78 to 90.17) (n=683)	62.7 ± 3.41* (56 to 69.4) (n=96)	161.55 ± 17.5 (125.7 to 197) (n=28)	90.7 ± 11.9* (66.5 to 115) (n=34)	132 ± 16 (99 to 165) (n=36)	87.6 ± 11.75** (63.8 to 111.5) (n=36)
25-(OH)D (ng/ml)	18.54 ± 0.8 (17 to 20) (n=134)	23.73 ± 0.8* (22 to 25) (n=109)	15.5 ± 0.3 (14.9 to 16) (n=807)	19 ± 0.89* (17.54-21) (n=96)	15.57 ± 1.21 (13 to 18) (n=30)	17 ± 1.3 (14 to 20) (n=34)	18.5 ± 1.66 (15 to 22) (n=39)	19 ± 1.59 (16 to 22) (n=36)
N-tact PTH (pg/ml)	27 ± 1.6 (23.9 to 30) (n=135)	29.24 ± 1.6 (26 to 32.35) (n=109)	28.35 ± 0.6 (27 to 29.5) (n=803)	29.21 ± 1.7 (25.75 to 32.7) (n=96)	36.7 ± 10 (16 to 58) (n=28)	26.51 ± 1.6 (23 to 30) (n=34)	21 ± 1.5 (18 to 24) (n=41)	25.28 ± 1.8 (22 to 29) (n=36)

Values as mean ± SEM (95% Confidence Intervals) (all such values); n= sample size; * $P < 0.0001$; ** $P < 0.05$ compared to urban; SAP-serum alkaline phosphates; 25-(OH)D-25-hydroxy vitamin D; N-tact-PTH, immunoreactive parathyroid hormone. Conversion of 25(OH)D from ng/ml to nmol/l multiply by factor 2.5

Adapted with permission from the American Journal of Clinical Nutrition (Ref. 20).

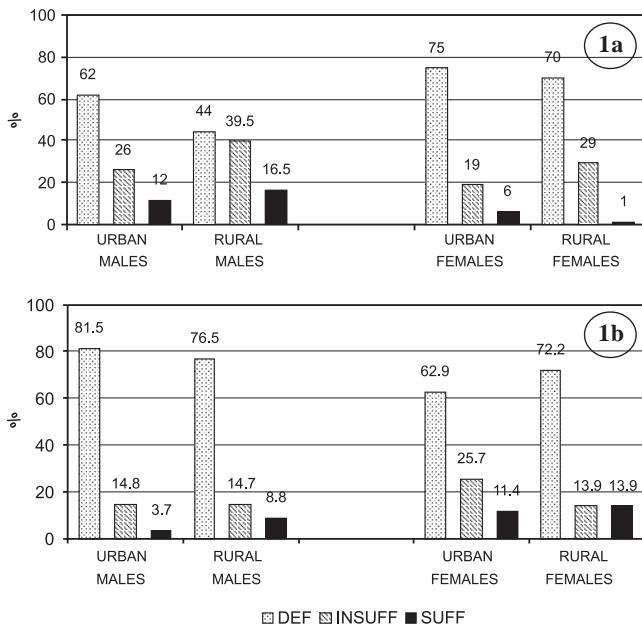


Fig. 1a&1b. Distribution of 25(OH)D levels in urban and rural adults (1a) and children (1b) DEF-25(OH)D deficiency- 25(OH)D levels <20 ng/ml; INSUFF -25(OH)D insufficiency - 25(OH)D levels 20-30 ng/ml; SUFF -25(OH)D sufficiency - 25(OH)D levels >30 ng/ml.

Biochemical and hormonal parameters

Adults - The serum calcium level of the urban and the rural subjects was within the normal range (Table II). The serum phosphorus and SAP were in the normal range in both urban and rural subjects. The 25(OH)D levels of rural subjects were significantly higher ($P<0.001$) than that of urban subjects in both males and female groups. The vitamin D – deficient, – insufficient and – sufficient states of rural subjects are depicted in Fig. 1a.

N-tact PTH levels negatively correlated with 25(OH)D in rural subjects ($r = -0.24$; $P<0.002$), and in urban subjects ($r = -0.12$; $P<0.0001$) (Fig. 2). There was no significant difference in the r values between rural and urban subjects. In rural subjects, N-tact PTH levels correlated negatively with serum phosphorus ($r = -0.3$; $P<0.001$) and positively with SAP ($r = 0.3$; $P<0.0001$). Similar correlation was seen in urban subjects. The r value for correlation between N-tact PTH and serum phosphorus were significantly higher in urban subjects compared to rural subjects ($P<0.001$).

Children - The serum calcium, phosphorous and SAP levels of the urban and the rural children were

within the normal range (Table II). Compared to urban children, the serum calcium was significantly high ($P<0.01$), serum phosphorous and SAP low ($P<0.01$) in rural children. The effect of location (urban and rural) was significant for serum calcium, phosphorus and SAP ($P<0.0001$).

The 25 (OH)D levels of both the urban and rural children were low. The vitamin D – deficient, – insufficient and – sufficient (replete) states of rural and urban children are shown in Fig. 1b. There is no significant association between location (urban and rural) and 25(OH)D levels in both boys and girls by chi square test ($P=NS$). There was no significant association between the urban and rural locations based on vitamin D – deficient, – insufficient and – sufficient states ($P=NS$).

N-tact PTH correlated negatively with 25(OH)D ($r = -0.2$; $P<0.05$). Serum calcium correlated negatively with N-tact PTH in rural subjects ($r = -0.24$; $P<0.05$) and in urban children ($r = -0.58$; $P<0.001$). The difference in the r values between both the locations is significant ($P<0.02$). The SAP declined with age ($r = -0.2$; $P<0.02$).

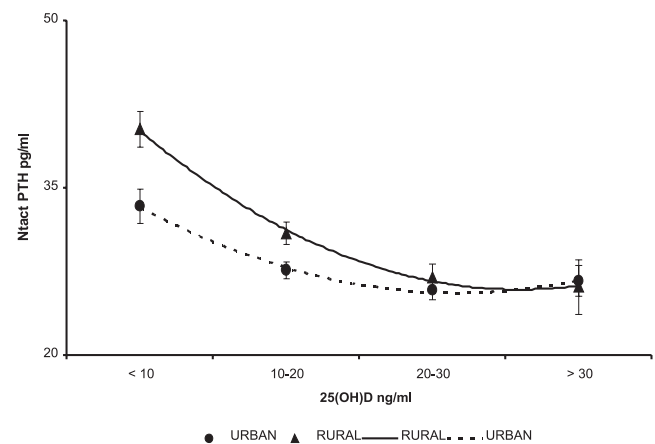


Fig. 2. Relation between serum immunoreactive parathyroid hormone (N tact PTH) and 25-hydroxyvitamin D [25(OH)D] concentrations by category in urban and rural group. Significant parabolic trend is observed between 25(OH)D levels and N tact PTH. (Model shown in figure). The relationship between N tact PTH and 25(OH)D is well modeled by a second degree curve with a downward slope for urban and rural locations. $R^2_{urban} = 0.016$; $P<0.0001$ and $R^2_{rural} = 0.06$; $P<0.0001$. A steep decrease in N tact PTH is observed in rural subjects with relation to 25(OH)D when compared to urban subjects. (Adapted with permission from *Am J Clin Nutr*).

Table III. Recommended dietary allowances (RDA in mg/day) of calcium in India and USA

Category	India ^{19,26,27}	USA ²⁵
<i>Infants:</i>		
Infants 0-6 months	500	500
Infants 6-12 months	500	750
<i>Children boys & girls:</i>		
1 - 9 yr	400	800
10 - 15 yr	500	1200 - 1300
16 - 18 yr	500	1200 - 1300
Men	400	800 - 1000
Women	400	800 - 1000
Pregnant & lactating mothers	1000	1200 - 1300

Discussion

The dietary intake of calcium of first generation healthy Asian Indian immigrants in USA²⁴ was found to be less than two-thirds of the dietary reference intake recommended for a normal person as per the guidelines of the USA²⁵. Recently the recommended daily/dietary allowance (RDA) has been revised and redefined as the dietary reference intake (DRI), which is a result of collaborative effort between USA and Canada²⁵. The RDA for calcium in India recommended by the Indian Council of Medical Research (ICMR), New Delhi, India is lower than the recently revised DRI (Table III)^{19,26-28}. Milk is not fortified with calcium or vitamin D in India.

The daily dietary calcium intake of both rural and urban adults and children were far below that of RDA of 400 mg/day for adults and 400 to 500 mg/day for children issued by the ICMR for the Indian population of both genders and age groups. The diet in rural subjects was high in phytate/calcium ratio thus retarding the absorption of already low dietary calcium. Intake of diet, rich in phytate (inositol hexaphosphate) retards the absorption of calcium from the gut. Inositol hexaphosphate forms chelates with divalent cations calcium, and reduce the absorption of calcium from the gut. Panwar and Punia²⁹ have shown that the calculated values for all nutrients are significantly higher than the analytical values. Hence, a patient with a calculated low intake of calcium with a background of diet containing foods high in phytates, as in the current study, may be more calcium deficient than calculated from dietary intake data.

The quality of diet in the rural subjects is low in calcium and high in phytate/calcium ratio compared to that of urban diet. Hence the rural subjects are more affected. Though in rural subjects more body surface

area is exposed to sunlight for longer duration by virtue of their occupation, the poor quality of diet impedes the bone homeostasis significantly.

The calcium absorptive performance of the gut is a function of 25(OH)D status of an individual^{30,31}. When the 25(OH)D concentrations are low, the effective calcium absorption from the gut is reduced^{30,31}. In children where the calcium requirements are high, in the background of low dietary calcium and 25(OH)D levels, the peak bone mass achieved is low, which in turn leads to high risk of fractures in old age group at a later date.

It has been shown that low dietary calcium converts the 25(OH)D to polar metabolites in the liver and leads to secondary 25(OH)D deficiency³². The SHPT that ensues increases the risk of fractures, especially in postmenopausal women and elderly patients.

Also, low calcium intake increases PTH which increases conversion of 25(OH)D₂ to 1,25-dihydroxy vitamin D [1,25(OH)₂D] which in turn stimulates the intestinal calcium absorption. In addition, 1,25(OH)₂D induces its own destruction by increasing the 24-hydroxylase. This probably explains the low 25(OH)D levels in individuals on high phytate or low calcium diet.

In the present study, low prevalence of 25(OH)D deficiency is seen in rural male subjects compared to that of the urban male subjects. The same observation is made for females. This is probably due to occupation, dress code and duration of exposure to sunlight of the rural subjects, who are agricultural laborers working for about 8 h a day in sunlight. In the region where this study was conducted, season has little impact on cutaneous synthesis of vitamin D. There are reports from Indian subcontinent of very low dietary intakes of calcium (<300 mg/day) causing osteomalacia^{33,34}, low dietary calcium and 25(OH)D status in postmenopausal women^{14,35,36}, children³⁷ and pregnant women and their offsprings³⁸.

Low dietary calcium intake results in the development of rickets amongst vitamin D deficient baboons^{39,40}. In rats^{39,40} low calcium diet or high phytate diet resulted in increased catabolism of 25(OH)D concentrations leading to formation of inactive metabolites with resultant reduction in 25(OH)D concentrations. The pathogenesis of rickets in Asian community in United Kingdom has been attributed to high-cereal, low-calcium diet which induces mild

hyperparathyroidism⁴¹. The role of low dietary calcium intake in the pathogenesis of 25(OH)D deficiency is probably greater than that is originally recognized.

In conclusion, we compared the relationship among the dietary calcium intakes, biochemical parameters of bone and mineral metabolism and vitamin D status in rural and urban subjects, both adults and children from India. There are methodological limitations in this study like the urban subjects were a sample of convenience. More subjects in all age groups in both genders in urban and rural subjects in different parts of the country should be studied in future. Still, this study clearly brings forth the low dietary calcium intake of both the urban and rural subjects, high phytate content of the rural diet and the limited exposure of the urban adults and children to sunlight. This could have a deleterious effect on bone mineral homeostasis; peak bone mass achieved and subsequently reflects as a low bone mineral density of Indian population¹⁷. Low 25(OH)D levels were associated with a deleterious effect on bone mineral homeostasis. Prospective longitudinal studies are required to assess the effect on bone mineral density, a surrogate marker for fracture risk or fracture rates.

References

- Parfitt AM, Gallagher JC, Heaney RP, Johnston CC, Neer R, Whedon GD. Vitamin D, and bone health in the elderly. *Am J Clin Nutr* 1982; 35 : 1014-31.
- Jones G, Dwyer T. Bone mass in prepubertal children: gender differences and the role of physical activity and sunlight exposure. *J Clin Endocrinol Metab* 1998; 83 : 4274-9.
- Johnston CC Jr, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, *et al*. Calcium supplementation and increases in bone mineral density in children. *N Engl J Med* 1992; 327 : 82-7.
- Slemenda CW, Peacock M, Hui S, Zhou L, Johnston CC. Reduced rates of skeletal remodeling are associated with increased bone mineral density during the development of peak skeletal mass. *J Bone Miner Res* 1997; 12 : 676-82.
- Villareal DT, Civitelli R, Chines A, Avioli LV. Sub clinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. *J Clin Endocrinol Metab* 1991; 72 : 628-34.
- Mezquita-Raya P, Munoz-Torres M, Luna JDD, Luna V, Lopez-Rodriguez F, Torres-Vela F, *et al*. Relationship between vitamin D insufficiency, bone density and bone metabolism in healthy postmenopausal women. *J Bone Miner Res* 2001; 16 : 1408-15.
- Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes* 2000; 9 : 87-98.
- Szulc P, Meunier PJ. Synergistic effect of vitamin D and calcium in preventing proximal femoral fractures in older patients. *Joint Bone Spine* 2003; 70 : 157-60.
- Preece MA, Ford JA, MacIntosh WB, Dunnigan MG, Tomlinson S, O'Riordan JLH. Vitamin D deficiency among Asian immigrants to Britain. *Lancet* 1973; i : 907-10.
- Dent CE, Rowe DJF, Round JM, Stamp TCR. Effect of chapattis and ultraviolet irradiation on nutritional rickets in Indian immigrants. *Lancet* 1973; i : 1282-4.
- Awumey EMK, Mitra DA, Hollis BW, Rajiv kumar, Bell NH. Vitamin D metabolism is altered in Asian Indians in the southern United States: A clinical research center study. *J Clin Endocrinol Metab* 1998; 83 : 169-73.
- Gibson RS, Bindra GS, Nizan P, Draper HH. The vitamin D status of East Indian Punjabi immigrants to Canada. *Br J Nutr* 1987; 58 : 23-9.
- Harinarayan CV, Gupta N, Kochupillai N. Vitamin D status in primary hyperparathyroidism in India. *Clin Endocrinol (Oxf)* 1995; 43 : 351-8.
- Harinarayan CV. Prevalence of vitamin D insufficiency in postmenopausal south Indian women. *Osteoporos Int* 2005; 16 : 397-402.
- Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N. Prevalence and significance of low 25-hydroxy D concentrations in healthy subjects in Delhi. *Am J Clin Nutr* 2000; 72 : 472-5.
- Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Marwer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. *Arch Dis Child* 2002; 87 : 111-3.
- Arya V, Bhambari R, Godbole MM, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. *Osteoporos Int* 2004; 1 : 56-61.
- Ganpule A, Yajnik CS, Fall CH, Rao S, Fisher DJ, Kanade A, *et al*. Bone mass in Indian children-relationships to maternal nutritional status and diet during pregnancy: the Pune Maternal Nutrition Study. *J Clin Endocrinol Metab* 2006; 91 : 2994-3001.
- Food composition table. In: Gopalan C, Sastri BVR, Balasubramanyam SC, editors. *Nutritive value of Indian foods*. Hyderabad, India: National Institute of Nutrition (ICMR) 1996; Appendix 1 : 92-4.
- Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PVLN, Sarma KVS, *et al*. High prevalence of low-dietary calcium, high-phytate consumption, and vitamin D deficiency in healthy south Indians. *Am J Clin Nutr* 2007; 85 : 1062-5.
- Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005; 16 : 713-6.
- Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. *Altern Med Rev* 2005; 10 : 94-111.
- Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005; 135 : 317-22.
- Jonnalagadda SS, Diwan S. Nutrient intake of first generation Gujarati Asian Indian immigrants in the U.S. *J Am Coll Nutr* 2002; 21 : 372-80.

25. RDA - Recommended Dietary Allowance of nutritional elements. Available at : <http://www.anyvitamins.com/rda.htm> (accessed on January 16, 2006).
26. Swaminathan M. *Recommended Dietary Intake of Nutrients*. Indian Council of Medical Research; 1981.
27. Recommended Dietary Allowances. In: *Essentials of Food and Nutrition. Fundamental aspects*. Bappco Pub 1997; 1 : 508-21.
28. Standing Committee on Scientific Evaluation of Dietary Reference Intakes. Calcium. In: *Dietary Reference Intakes for calcium*, Food and Nutrition Board, National Academy Press 1999; p. 71-145.
29. Panwar B, Punia D. Analysis of composite diets of rural pregnant women and comparison with calculated values. *Nutr Health* 2000; 14 : 217-23.
30. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003; 22 : 142-6.
31. Heaney RP. Vitamin D depletion and effective calcium absorption. *J Bone Miner Res* 2003; 18 : 1342.
32. Clements MR, Johnson L, Fraser DR. A new mechanism for induced vitamin D deficiency in calcium deprivation. *Nature* 1987; 325 : 62-5.
33. Rajeswari J, Balasubramanian K, Bhatia V, Sharma VP, Agarwal AK. Aetiology and clinical profile of osteomalacia in adolescent girls in northern India. *Natl Med J India* 2003; 16 : 139-42.
34. Mathew JT, Seshadri MS, Thomas K, Krishnaswami H, Cherian AM. Osteomalacia - Fifty-five patients seen in a teaching institution over a 4-year period. *J Assoc Physicians India* 1994; 42 : 692-4.
35. Harinarayan CV, Ramalakshmi T, Venkataprasad V. High prevalence of low dietary calcium and low vitamin D status in healthy south Indians. *Asia Pac J Clin Nutr* 2004; 13 : 359-65.
36. Shatrugna V, Kulkarni B, Kumar PA, Rani KU, Balakrishna N. Bone status of Indian women from low income group and its relationship to the nutritional status. *Osteoporos Int* 2005; 16 : 1827-35.
37. Marwaha RK, Tandon N, Reddy DRHK, Aggarwal R, Singh R, Sawhney RC, *et al*. Vitamin D and bone mineral density status of healthy school children in northern India. *Am J Clin Nutr* 2005; 82 : 477-82.
38. Sachan A, Gupta R, Das V, Agarwal A, Awasthi PK, Bhatia V. High prevalence of vitamin D deficiency among pregnant women and their new borns in north India. *Am J Clin Nutr* 2005; 81 : 1060-4.
39. Mellanby E. An experimental investigation on rickets. *Lancet* 1919; i : 407-12.
40. Sly MR, van der Walt WH, Du Bruyn D, Pettifor JM, Marie PJ. Exacerbation of rickets and osteomalacia by maize: a study of bone histomorphometry and composition in young baboons. *Calcif Tissue Int* 1984; 36 : 370-9.
41. Clements MR. The problem of rickets in UK Asians. *J Hum Nutr Diet* 1989; 2 : 105-16.

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