Original Article

High prevalence of low dietary calcium and low vitamin D status in healthy south Indians

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Calcium and vitamin D under nutrition can adversely affect the bone mineral metabolism. There is no population-based study from India documenting dietary habits, serum calcium and vitamin D levels. Our study investigated the dietary habits of rural and urban societies in and around Tirupati and their relationship with serum calcium, phosphorous and vitamin D [25(OH)D] levels. Four hundred and seven subjects from 5 villages around Tirupati, (rural population) and 125 asymptomatic staff of our hospital (urban population) were studied. Dietary intakes of calcium, phosphorous and phytates were documented by diet history. Serum calcium, phosphorus and 25 (OH) D levels were estimated in 191 rural subjects and 125 urban subjects. Compared to urban subjects, rural subjects had a significantly lower intake of dietary calcium (P <0.0001) and a significantly higher dietary phytate/calcium ratio and serum calcium and 25 (OH) D levels (P <0.0001). Dietary calcium intake was inadequate in both rural and urban subjects compared to the recommended daily allowances (RDA) for our country. About 31% of the population had normal vitamin D levels, 54% had vitamin D insufficiency and 15% vitamin D deficiency. About two-thirds of the population had low levels of vitamin D. Inadequate dietary calcium intake associated with high phytate/calcium ratio reduces the bioavailable calcium in the gut. Hence, there is a need to fortify food with calcium and to propose new guidelines for 25 (OH) D in Indian subjects. Multicentric studies with large sample populations are required to generate normal standards and nationally relevant guidelines.

Key Words: diet, calcium, serum 25 (OH) D, vitamin D, fortification of foods, RDA, ICMR, rural, urban, South India

Introduction

Nutritional factors play a vital role in bone homeostasis during adulthood. During infancy, childhood and adolescence, increasing dietary calcium intake favours bone mineral accrual. Adequate calcium intake along with vitamin D helps to maintain bone mineral mass attained at the end of growth period i.e. peak bone mass. Serum 25-hydroxyvitamin D [25(OH)D] is the most reliable indicator of vitamin D levels of an individual. Vitamin D insufficiency [25(OH)D levels between 10 – 20 ng/ml] is associated with secondary hyperparathyroidism (SHPT). 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 a high risk of fracture.[1-5]

Vitamin D deficiency and/or poor dietary calcium intake can 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 equator.[6-9] This was attributed to the poor cutaneous synthesis of vitamin D due to pigmentation and inadequate sunlight exposure along with an inadequate dietary calcium intake. Vitamin D deficiency [25 (OH) D levels <10 ng/ml] was presumed to be rare in tropical countries like India. Previously, we reported the prevalence of low vitamin D levels in India in a group of normal subjects and patients with primary hyperparathyroidism.[10] Subsequently other reports ensued.[11-14] It is indeed surprising to find low vitamin D in healthy subjects in India, a country with abundant sunshine. So far, there is no large study documenting the dietary habits, serum calcium and vitamin D levels of an Indian population. We studied the dietary habits, and its relationship with serum calcium, and vitamin D [25-hydroxy cholecalciferol 25 (OH) D] in patients residing in Tirupati and surrounding villages.

Materials and methods

Between January 2000 and July 2003, 407 apparently healthy, asymptomatic subjects from Sathyavedu [SY] (Latitude [Lat] 13.26^{0}N and Longitude [Long] 79.57^{0}E), Peddathippasamudram [PTM] (Lat.13.43^{0}N,Long. 78.13 ^{0}E), Sandramakula palli [S. Palli] (Lat.13.40^{0}N, Long. 78.14^{0}E), Adharam [A] (Lat.13.37^{0}N, Long. 79.47^0 E) and Kandluru [K] (Lat.13.36^{0}N, Long. 79.47^{0}E) villages around Tirupati, belonging to Chitoor district, Andhra Pradesh were included in the study. They constituted the Tirupati rural population. Students and staff of the Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati

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Table 1. Dietary Pattern, serum calcium and 25 (OH) D statuses of Tirupati Urban and Rural population

Parameter	Tpt - Rural	Tpt – Urban
N	191	125
Age (Yrs)	44 ± 1.03	45.5 ± 0.95
S. Calcium (mg/dl)	10 ± 0.05*	9.71 ± 0.06
S. Phosphorous (mg/dl)	3 ± 0.04*	3.28 ± 0.53
S. 25(OH)D (ng/ml)	21 ± 0.46*	13.52 ± 0.59
Dietary Calcium (mg/day)	264 ± 1.94*	356 ± 5.0
Dietary Phosphorous (mg/day)	490 ± 4.98*	721 ± 10.2
Dietary Phytates (mg/day)	200 ± 1.9	207 ± 4.7
Phytates/Calcium Ratio	1 ± 0.01*	0.58 ± 0.01

Values represent mean± SEM; **P* <0.0001; 25(OH)D – for conversion from ng/ml to nmol/l – multiply by 2.5

[TPT] (Lat.13.40 ^{0} N, Long. 77.2 ^{0} E), Chitoor district, Andhra Pradesh and their asymptomatic relatives (*N*=125) were studied. They constituted Tirupati urban population.

In all the above locations, the average duration of sunlight is around 8 to10 hours per day throughout the year. Winters are short with minimum and maximum temperatures ranging from 17^{0}C to 30^{0}C with poor rainfall. Most often, there is little seasonal variation of the peak sunlight. The visual skin complexion of the subjects studied is wheatish to dark in color. Most of the rural subjects are agricultural workers who are exposed to sunlight for a period of 8 to10 hours a day. Cloths or veils did not restrict the exposure to sunlight.

In all the villages, a prior visit was undertaken to study the pattern of living and dietary. The subjects were asked to remain fasting on the day of collection of blood sample. The dietary intake of calcium, phosphorous and phytates were documented by recalling the diet consumed in the previous 5 to 7 days. The documentation of dietary pattern was by a single observer. The validity and repeatability of the documentation was rechecked at random by one of us (authors) over the period of the study. There was no significant error in the documentation of dietary history. From the raw weights, the calcium and phosphorous intakes were calculated using the published food composition table detailing the nutritive value of Indian foods.[15]

For all patients fasting venous blood samples were collected from the most accessible peripheral vein between 0800 to 0900 hours in the fasting state without applying tourniquet for the estimation of serum calcium, phosphorus and on ice for 25(OH)D. The serum was separated in refrigerated centrifuge at 4^{0}C and stored at – 20^{0}C until the analysis for the estimation of 25(OH)D. The blood samples collected from village populations were transported under cool packs until they were separated and stored for further analysis. The 25 (OH)D levels were estimated in 191 rural subjects and 125 urban subjects. The serum calcium and phosphorus levels were estimated by titrimetric method[16] and by Fiske Subba Row method[17] respectively. 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 assay is 1.5 ng/ml [reference range 9 to 37.6 ng/ml]. The kit manufacturer to monitor assay performance provided two quality control sera (control A and B). The control A (range: 11.6 to 24.4 ng/ml) and the control B (range: 34.7 to 73.5 ng/ml) values for 25 (OH)D assay were 17.8 ng/ml and 57.3 ng/ml respectively. The intra- assay (at 12.75 ng/ml) and inter-assay (at 11.0 ng/ml) variations for 25 (OH) D were 0.9% and 3.95% respectively.

Statistical methods

A statistical analysis was performed using SPSS package (version10). Descriptive results are presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to estimate the differences between the study groups. If a significant difference was found, a multiple comparison test was performed using LSD post hoc test to analyze the differences between the study groups. Probability value of *P*<0.05 was considered significant.

Results

The diet in rural subjects consisted of 1700 KJ/day approximately. Carbohydrates provided 75% of the total energy intake, proteins 10%, fat 5%, vegetables 5%, and milk and milk products 5%. The carbohydrate source was from cereals [Rice – 60% and Ragi *(Eleusine Coracana)* – 40%]. Vegetable sources included drumstick leaves, brinjals and tomatoes. Animal sources of protein were consumed once fortnightly. The diet in urban subjects consisted of 2200 KJ/day approximately. Carbohydrates provided 55% of the total energy intake, proteins 10% and fat 10%. Vegetables contributed 10% to total energy intake and milk and milk products contributed 15% (Fig. 1). The carbohydrate source was primarily from cereals with rice providing 50% of total carbohydrates, wheat 25% and ragi 25%. Vegetable sources included amaranth leaves, cauliflower, carrots, ladies fingers, other seasonal vegetables and tubers. Animal sources of protein were consumed once a week. There was no other source of calcium or any other mineral in both groups. Milk is not fortified with calcium or vitamin D in India.

The mean ± SEM of dietary calcium, phosphorous, phytates and dietary phytate/calcium ratio, serum calcium, phosphorous and 25 (OH) D levels of the Tirupati rural and urban population is described in Table 1. The age groups of Tirupati rural and urban populations were comparably similar. The daily dietary calcium intake by both Tirupati rural and urban populations were low (mean ± SEM: rural 264 ± 1.94; urban 354 ± 5 mg/day) compared to that of Recommended Daily/Dietary Allowance (RDA) issued by the Indian Council of Medical Research (ICMR) for the Indian population. Dietary calcium, phosphorous and serum phosphorous were significantly lower (*P*<0.0001) in the rural subjects compared to the urban subjects. Though the dietary phytates were comparable in both the rural and urban groups, the dietary phytate/calcium ratio was significantly (*P* <0.0001) higher in rural subjects (Table 1). The serum calcium and 25 (OH) D levels were significantly higher *P* <0.0001) in the rural subjects compared to the urban subjects.

Table 2. Dietary pattern, serum calcium and 25(OH)D status of Tirupati urban and rural population – categories based on 25 (OH) D levels

Parameter	25(OH)D < 10 ng/ml *(Group – 1)* Vitamin D deficiency			25(OH)D 10 – 20 ng/ml *(Group – 2)* Vitamin D insufficiency			25(OH)D > 20 ng/ml *(Group – 3)* Normal vitamin D levels		
	Rural	*Urban*	*Whole Group*	*Rural*	*Urban*	*Whole Group*	*Rural*	*Urban*	*Whole Group*
N (%)	5	44	49 (15%)	107	63	170 (54%)	79	18	97 (31%)
Age (yrs)	40±7.4	46±1.8	46 ±1.7	42±1.4	44±1.28	43±1.0	47±1.5	48±2	47±1.3
S.Ca mg/dl	10±0.25*	9.76±0.08	9.79±0.07	10±0.07*	9.61±0.1	9.87±0.06	10±0.07*	9.9±0.18	10±0.07
25 (OH) D ng/ml	9.04±0.7$^{@}$	7.36±0.3	7.53±0.28	16.7± 0.26$^{@}$	14.3±0..34	15.83±0.23	27±0.5$^{@}$	25.8±0.95	26.92±0.44
Dietary Ca mg/day	227±12*	355±9.1	342±10	264±3*	360±7.4	299±5	266±2.15*	344±10	281±4.15
Dietary Phos mg/day	416±21	730±16	699±20	481±7	724±16	481±7	506±7	694±23	544±10
Phyt./ Calc. Ratio	0.87±0.03*	0.6±0.01	0.63±0.01	0.79±0.01*	0.58± 0.01	0.71± 0.01	0.72±0.01*	0.54±0.02	0.68±0.01

Values represent mean ± SEM; **P=<0.0001* compared to urban group; $^{@}$ *P* <0.001 compared to urban group

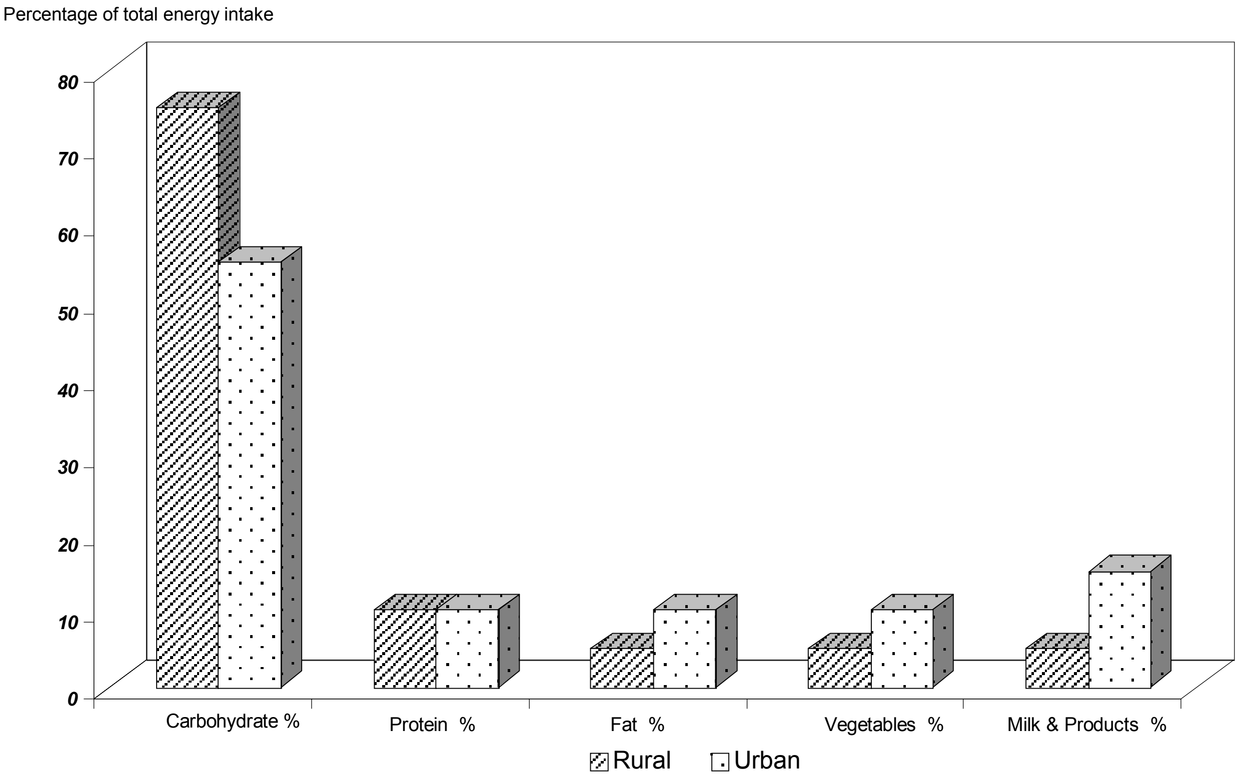

Figure 1. Dietary pattern (percentage of total energy intake) of rural and urban subjects

The 25(OH)D levels of the sample (rural and urban groups) were classified into: *group 1* vitamin D deficiency [25(OH)D levels <10ng/ml]; *group 2* vitamin D insufficiency or marginal intake [25(OH)D levels 10-20 ng/ml] and *group 3* normal vitamin D [25(OH)D levels >20ng/ ml].[18] Based on this classification only 31% (*N*=97) of the sample population had normal vitamin D levels. About 50% (*N* =170) had vitamin D insufficiency and 15% (*N*=49) had vitamin D deficiency (Table 2). Severe vitamin D deficiency (25(OH)D levels <5ng/ml) was found in three subjects (1% of the whole population). The 25(OH)D levels ranged from zero to 4.05 ng/ml in the severe vitamin D deficiency group. They did not have any other secondary cause attributable to vitamin D deficiencies. All of them were urban subjects.

The village and urban subjects were sub-classified based on 25 (OH) D levels into three groups (Table 2). The rural population had significantly (*P* <0.001) higher 25(OH)D levels compared to the urban group in all the three sub-categories. One way ANOVA amongst the three groups (between rural and urban subjects) revealed significantly (*P* <0.0001) lower dietary calcium, higher phytate/calcium ratio, and higher serum calcium in the vitamin D deficiency group compared to the vitamin D insufficient group and the group with normal vitamin D levels in rural subjects (Table 2).

Discussion

The dietary intake of calcium in first generation normal Asian Indian immigrants in USA[19] was found to be less than two-thirds of the dietary reference intake recommended for a normal person as per the guidelines of the USA. Recently the RDA has been revised and redefined as the Dietary Reference Intake (DRI), which is a collaborative effort between USA and Canada.[20] The RDA for calcium in India recommended by the Indian Council of Medical Research (ICMR) is lower than the recently revised recommendations by the USA and Canada (Table 3).[21–23] There is neither a recommendation for dietary intake of vitamin D nor a monitored food fortification program for the intake of calcium or vitamin D by ICMR.

The dietary intake in the urban group was high in calories, milk, milk products and vegetables. The major cereal consumed was rice, rather than ragi and wheat, which has lower phytate levels. Even the carbohydrate portion was occasionally replaced by sweets containing milk and its products. The dietary calcium intake by the Tirupati rural population is less than that of the urban population. Intake of Ragi (rich in phytates) by the rural population retards the absorption of calcium from the gut. The daily consumption of milk and milk products was only 5% of their total energy intake. The other source of calcium was from leafy vegetables (especially drumstick leaves). There is no other source of vitamin D in the diets of the sample population. Nevertheless, the dietary calcium intakes by both the rural and the urban samples were much lower than the RDA for calcium as per the ICMR guidelines (Table 3). These data highlight the high prevalence of inadequate dietary calcium intake across the population compared to the RDA. To the best of our knowledge, there are no population-based studies from India comparing rural and urban populations with their dietary habits and 25 (OH) D levels. There are reports of very low dietary intakes of calcium (<300 mg/day) in patients with osteomalacia.[24,25] Besides this, it has been shown in the studies by Panwar *et al.,*[26] 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 diet containing foods high in phytates, as in our study, may be more calcium deficient than calculated from dietary intake data. The inadequate dietary calcium intake is significant when viewed in the background of high phytate/calcium ratio associated with low 25 (OH)D levels.

Phytate in the diet retards the absorption of calcium in the gut. Though the 25(OH)D levels were high in rural subjects in all the three groups, the dietary calcium intake was inadequate with high phytate/calcium ratio compared to the urban subjects (Table 2). The high phytate/calcium ratio in the rural subjects retards calcium absorption. In the present study, all the subjects had adequate sunlight exposure and the dress code did not affect the exposure to sunlight. About two-thirds (69%) of the population have low levels of vitamin D. About 15% of the population had vitamin D deficiency and 54% had vitamin D insufficiency. In our study, all patients with severe 25 (OH)D deficiency (<5ng/ml) were from the urban sample. The significantly higher levels of 25 (OH) D in the rural population compared to the urban population can be partly explained by the former group having greater exposure to sunlight as a result of their agricultural occupation.

Table 3. Recommended Dietary Allowances of calcium in India and USA

Category	India[14,15]	USA[16]
Units	mg/day	mg/day
Infants		
Infants 0–6 months	500	500
Infants 6– 12 months	500	750
Children Boys & Girls		
1 – 9 yrs	400	800
10 – 15 yrs	500	1200 -1300
16 – 18 yrs	500	1200 -1300
Men	400	800 -1000
Women	400	800 - 1000
Pregnant & Lactating mothers	1000	1200 - 1300

Vitamin D insufficiency is associated with secondary hyperparathyroidism (SHPT), which is further amplified by inadequate calcium intake. Thus, in the background of low vitamin D levels and inadequate dietary calcium intakes, when an individual is exposed to the additional insult of an environmental toxin like fluoride, the clinical expression of the disease is altered. Various studies have shown that the effect of an environmental toxin like fluoride on bone mineral metabolism is severe and more complex in children with poor dietary calcium intake when compared to the children with adequate dietary calcium intake.[27–29]

It has also been shown that calcium absorptive performance of the gut is a function of 25(OH)D status of an individual.[30,31] When there are low 25 (OH) D concentrations, the effective calcium absorption from the gut is reduced.[31,32] This is further amplified by the low dietary calcium intake. The SHPT consequent to inade-quate dietary calcium intake and low 25(OH)D concentrations mobilizes mineral and matrix from the skeleton. This increases he risk of fractures, especially in post-menopausal women and elderly patients. These are further amplified by age related changes with calcium supplementation.[33] High phytate/calcium ratio amplifies the inadequate dietary calcium intake.

There are several studies documenting that vitamin D and calcium supplements have synergistic effects in preventing proximal femoral fractures in postmenopausal and older patients.[34-36] The shortcomings of the inadequate dietary calcium intake associated with the reduced bioavailable calcium in the gut due to phytates and age related calcium conservation in the gut can be overcome by up-revising the RDA for calcium, restricting phytates in food and recommending new guidelines for vitamin D. Monitored food fortification programs are to be implemented at national level so that the existing diets are supplemented with food that are rich (or have been enriched) with calcium.

In summary, two-thirds of the study population (rural and urban groups) had low vitamin D levels. There is no RDA proposed by ICMR for vitamin D for the Indian population. The revised DRI of the USA and Canada[20] recommends 400 IU for vitamin D for those aged under 50 and 800IU for those aged 50 plus. The dietary calcium intake by the urban and rural populations did not meet the existing RDA by ICMR.

The present study has certain methodological limitations. The urban sample was a sample of convenience because of logistic and operational reasons. However, even after taking these limitations into account, our observations argue strongly for the revision of the RDA for calcium and new recommendations for the 25(OH)D for the Indian population. Multicentric studies with a large sample size are required to generate normal standards for the purpose of nationally relevant guidelines.

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References

1. 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.
2. Villareal DT, Civitelli R, Chines A, Avioli LV. Subclinical vitamin D deficiency in postmenopausal women with low vertebral bone mass. J Clin Endocrinol Metab 1991; 72: 628-34.
3. 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.
4. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the Elderly: Consequences for bone loss and fractures and Therapeutic implications. Endocrine Reviews 2001; 22: 477-501.
5. Harris SS, Soteriades E, Stina Coolidge JA, Mudgal S and Hughes BD. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. J Clin Endocrinol Metab 2001; 85: 4125-30.
6. Preece MA, Ford JA, MacIntosh WB, Dunnigan MG, Tomlinson S, O'Riordan JLH. Vitamin D deficiency among Asian immigrants to Britain. Lancet 1973;1:907–10.
7. Dent CE, Rowe DJF, Round JM, Stamp TCR. Effect of chapattis and ultraviolet irradiation on nutritional rickets in Indian immigrants. Lancet 1973; 1: 1282–84.
8. Awumey EMK, Mitra DA, Hollis BW, Rajiv kumar and Bell NH. Vitamin D metabolism is altered in Asian Indians in the southern United Status: A clinical research center study. J Clin Endocrinol Met 1998;83:169–73.
9. Gibson RS, Bindra GS, Nizan P, Draper HH. The vitamin D status of East Indian Punjabi immigrants to Canada. Br Jr Nutr 1987; 58 (1): 23-9.
10. Harinarayan CV, Gupta N, Kochupillai N. Vitamin D status in primary hyperparathyroidism in India. Clin Endocrinol (Oxf). 1995; 43: 351-8.
11. 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.
12. 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 (2):111-3.
13. Arya V, Bhambari R, Godbole MM, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporosis Int 2004; 1: 56-61.
14. Tandon N, Marwaha RK, Kalra S, Gupta N, Dudha A, Kochupillai N. Bone mineral parameters in healthy young Indian adults with optimal vitamin D availability. Nat Med J India 2003; 16: 298-302.
15. Gopalan C, Ramasastri BB, Balasubramanyam SC. Nutritive value of Indian Food. Indian Council of Medical research, National Institute of Nutrition, Hyderabad 1998.
16. Raghuramulu N, Madhavan Nair K and Kalyanasundaram S. A Manual of Laboratory Techniques. Indian council of Medical Research, National Institute of Nutrition, Hyderabad 1983;136-7.
17. Raghuramulu N, Madhavan Nair K and Kalyanasundaram S. A Manual of Laboratory Techniques, Indian Council of Medical Research, National Institute of Nutrition, Hyderabad 1983; 140-1.
18. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the Elderly: Consequences for bone loss and fractures and Therapeutic implications. Endocrine Reviews 2001; 22: 477-501.
19. 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.
20. RDA – Recommended Dietary Allowance of nutritional elements. Available at URL: http://www.anyvitamins.com/rda.htm.
21. Food composition table. In: Gopalan C, Sastri BVR, Balasubramanyam SC, eds. Nutritive value of Indian foods. Hyderabad, India: National Institute of Nutrition ICMR 1996; Appendix 1: 92– 4.
22. Swaminathan M. Recommended Dietary Intake of Nutrients. Indian Council of Medical Research, 1981.
23. Recommended Dietary Allowances. In: Essentials of Food & Nutrition. Fundamental aspects. Place of publication: Bappco Pub 1997; 1:508–21.
24. Standing Committee on Scientific evaluation of Dietary Reference Intakes. Calcium. In: Dietary Reference Intakes for calcium, Food and Nutrition Board, National Academy Press 1999; 71–145.
25. 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.
26. 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.
27. Panwar B, Punia D Analysis of composite diets of rural pregnant women and comparison with calculated values. Nutr Health 2000; 14: 217-23.
28. Krishnaramachari KAVR. Skeletal fluorosis in humans. A review of recent progress in understanding of the disease. Prog Food Nutr Sci 1986; 10: 279-314.
29. Teotia M, Teotia SP, Singh KP. Endemic chronic fluoride toxicity and dietary calcium deficiency interaction syndromes of metabolic bone disease and deformities in India: year 2000. Indian J Pediatr 1998; 65: 371-81.
30. Mithal A, Trivedi N, Gupta SK, Kumar S, Gupta RK. Radiological spectrum of endemic fluorosis: relationship with calcium intake. Skeletal Radiol 1993; 22: 257-61.
31. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, Nebiker M, Theiler R, Pfeifer M, Begerow B, Lew RA Conzelmann M. Effects of vitamin D and calcium supplementation on falls: A randomized controlled Trial. J Bone Min Res 2003; 18: 343 –51.

32. Heaney RP. Vitamin D depletion and effective calcium absorption. A letter to the editor. J Bone Min Res 2003; 18: 1342.
33. 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.
34. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes of calcium, magnesium, phosphorous, vitamin D and fluorides. Washington DC: National Academy Press, 1997.
35. 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.
36. Brazier M, Kamel S, Maamer M, Agbomson F, Elesper I, Garabedian M, Desmet G, Sebert JL The markers of bone remodeling in the elderly subjects: effects of vitamin D insufficiency and its correction. J Bone Miner Res 1995; 10: 1753-61.
37. Sahota O, Gaynor K, Harwood RH, Hosking DJ. Hypovitaminosis D and 'functional hypoparathyroidism'- the NoNoF (Nottingham Neck of Femur) study. Age Ageing 2001; 30: 467-72.