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RESEARCH PUBLICATIONS


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 phytases were documented by diet history. Serum calcium, phosphorous 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 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.\(^5\)

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.\(^6\) 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.\(^7\) Subsequently other reports ensued.\(^8\) 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-hydroxycholecalciferol 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°N and Longitude [Long] 79.57°E), Peddaithuppadremudram [PTM] (Lat.13.43°N, Long. 78.13°E), Sandramatula palli [S Palli] (Lat.13.40°N, Long. 78.14°E), Adharam [A] (Lat.13.37°N, Long. 79.47°E) and Kaoludri [K] (Lat.13.36°N, Long. 79.47°E) villages around Tirupati, belonging to Chittoor 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 are
In all the above locations, the average duration of sunlight is around 8 to 10 hours per day throughout the year. Winters are short with minimum and maximum temperatures ranging from 17°C to 30°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 to 10 hours a day. Clothes 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. 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°C and stored at -20°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 phosphorous levels were estimated by titrimetric method and by Fiske Subba Row method 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-asay (at 12.75 ng/ml) and inter-asay (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 (version 10). 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 1. Dietary Pattern, serum calcium and 25 (OH) D statuses of Tirupati Rural and Urban population

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

Values represent mean ± SEM; *P <0.0001; 25(OH)D – for conversion from ng/ml to mmol/l – multiply by 2.5.
Table 2. Dietary pattern, serum calcium and 25(OH)D status of Tirupati urban and rural population – categories based on 25 (OH) D levels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>25(OH)D &lt; 10 ng/ml (Group – 1)</th>
<th>25(OH)D 10 – 20 ng/ml (Group – 2)</th>
<th>25(OH)D &gt; 20 ng/ml (Group – 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D deficiency</td>
<td>Vitamin D insufficiency</td>
<td>Normal vitamin D levels</td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>Urban</td>
<td>Whole Group</td>
<td>Rural</td>
</tr>
<tr>
<td>N (%)</td>
<td>5</td>
<td>44</td>
<td>49 (15%)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>40±2.4</td>
<td>46±1.8</td>
<td>46±1.7</td>
</tr>
<tr>
<td>S.Ca</td>
<td>10.0±0.25*</td>
<td>9.76±0.08</td>
<td>9.79±0.07</td>
</tr>
<tr>
<td>25 (OH) D ng/ml</td>
<td>9.04±0.78*</td>
<td>7.36±0.3</td>
<td>7.53±0.28</td>
</tr>
<tr>
<td>Dietary Ca mg/day</td>
<td>227±12*</td>
<td>355±9.1</td>
<td>342±10</td>
</tr>
<tr>
<td>Dietary Plan</td>
<td>416±21</td>
<td>730±16</td>
<td>699±20</td>
</tr>
<tr>
<td>Phy./Calc. Ratio</td>
<td>0.87±0.03*</td>
<td>0.65±0.01</td>
<td>0.63±0.01</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM; *P<0.001/J compared to urban group. \# P<0.001 compared to urban group.

Percentage of total energy intake

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 J normal vitamin D [25(OH)D levels >20ng/ml]. Based on this classification only 31% (N=97) of the sample population had normal vitamin D levels. About 32% (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 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. 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 (<500 mg/day) in patients with osteomalacia.24-26 Besides this, it has been shown in the studies by Parmar et al.27 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 rural 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.

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 inadequate dietary calcium intake and low 25(OH)D concentrations mobilizes mineral and matrix from the skeleton. This increases the risk of fractures, especially in postmenopausal 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 recommends 400 IU for vitamin D for those aged under 50 and 800 IU 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.

Acknowledgements (contributions of each author). There is no conflict of interest by any of the authors.

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High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians1–2

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ABSTRACT

Background: Data on the vitamin D status of the population in a tropical country such as India have seldom been documented. Vitamin D deficiency is presumed to be rare.

Objective: The objective was to document the dietary habits and concentrations of serum calcium, 25-hydroxyvitamin D (25(OH)D), and parathyroid hormone of Indian urban and rural populations.

Method: Healthy urban (n = 943) and rural (n = 205) subjects were studied for their dietary patterns and concentrations of serum calcium, phosphorus, alkaline phosphatase, 25(OH)D, and non-suppressed parathyroid hormone.

Results: The daily dietary calcium intake of both the urban and rural populations was low compared with the recommended dietary allowances issued by the Indian Council of Medical Research. Dietary calcium and phosphorus were significantly lower in rural adults than in urban adults (P < 0.0001). The dietary phytase-to-calcium ratio was higher in rural subjects than in urban subjects (P < 0.0001). The 25(OH)D concentrations of the rural subjects were higher than those of urban subjects (P < 0.001), and men and women in the rural subjects, 25(OH)D-deficient (<20 ng/ml), insufficient (20–30 ng/ml), and sufficient (>30 ng/ml) states were observed in 44%, 39.3%, and 16.5% of the men and 70%, 29%, and 1% of the women, respectively. In urban subjects, 25(OH)D-deficient, insufficient, and sufficient states were observed in 62%, 26.9%, and 12% of the men and 75%, 19%, and 6% of the women, respectively.

Conclusion: Low dietary calcium intake and 25(OH)D concentrations were associated with deleterious effects 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 Dietary calcium, phytate consumption, vitamin D insufficiency, bone mineral density, Indians, high prevalence

INTRODUCTION

Nutritional factors play a vital role in the bone homeostasis. During infancy, childhood, and adolescence, increasing dietary calcium intake favors bone mineral accrual (1). Adequate calcium intake along with vitamin D helps to maintain bone mineral mass attained at the end of the growth period (i.e., the peak bone mass). Serum 25-hydroxyvitamin D (25(OH)D) concentration is the most reliable indicator of vitamin D adequacy (2). The production of 25(OH)D is not regulated, and the serum concentration thus reflects both cutaneous synthesis and absorption from diet. Although vitamin D deficiency [25(OH)D concentrations <20 ng/ml] is associated with bone changes (rickets or osteomalacia), vitamin D insufficiency [25(OH)D concentrations between 20 and 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 causes, mobilizes mineral and matrix from skeleton and leads to an enhanced bone loss and a high risk of fracture (3–6). Vitamin D deficiency 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 immigrants who migrate away from the equator (7–10). This was attributed to the poor cutaneous synthesis of vitamin D resulting from pigmentation and inadequate sunlight exposure along with a low dietary calcium intake. 25(OH)D deficiency was presumed to be rare in tropical countries such as India, and also the data on the vitamin D status of this population has seldom been documented.

Previously, we reported the prevalence of low 25(OH)D concentrations in India in a group of healthy subjects and in patients with primary hyperparathyroidism (11). Later, other reports ensued (12–14). It is surprising to find low concentrations of 25(OH)D in healthy subjects in a country with abundant sunshine. So far, no large population-based study has documented the dietary habits and serum concentrations of calcium, 25(OH)D, and parathyroid hormone of the Indian population. We studied these aspects in subjects residing at Tirupati and the surrounding villages.

SUBJECTS AND METHODS

The study was conducted in 943 urban and 205 rural healthy subjects of Tirupati, southern Andhra Pradesh, India (lat 13.4°N, long 79.2°E). In the urban and rural locations, the average duration of cloud-free sunshine is 4–6 h/d throughout the year with

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Received July 17, 2006.
Accepted for publication November 20, 2006.

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DIETARY CALCIUM AND 25(OH)D STATUS OF SOUTH INDIANS

the solar zenith angle of 9.9° in summer and 38.2° in winter. The UV index at the above-mentioned latitudes during those periods is 7–12. Winter is short with lowest temperatures of 17°C (night) and 25°C (day) with memory rainfalls. Most often, there is a little seasonal variation of the peak intensity of sunlight.

Medical and paramedical personnel and their relatives and postmenopausal women and their relatives constituted the urban population. The rural population included men and women who were included after a demographic survey. Patients with hepatic, renal, or dermatological disorders; alcoholics; and pregnant women were excluded from the study.

The dietary assessment of total energy, calcium, phosphorus, and phyate were documented by recalling the diet consumed in the previous 5–7 d. The documentation of dietary pattern was by a single observer. The validity and reproducibility of the documentation was checked at random by one of us over the period of the study. From the raw weights, the intakes of total energy, calcium, phosphorus, and phyate were calculated with the use of a published food composition table, detailing the nutritive values of Indian foods (15). Because the ratio of dietary calcium to phyate is more predictive of the severity of interference of calcium absorption than is dietary calcium alone, the phyate-to-calcium ratio was calculated (12). For all patients, venous blood samples were collected in the fasting state without applying a tourniquet for the estimation of serum calcium, phosphorus, alkaline phosphatase (SAP), creatinine, and albumin, and samples for 25(OH)D and immunoreactive parathyroid hormone (N-tact PTH) were placed on ice. The serum was separated in a refrigerated centrifuge at 700 × g at 4°C for 10 min and stored at -20°C until the analysis for determining 25(OH)D and N-tact PTH. The blood samples collected from the rural population were transported in cool packs until they were separated and stored in the laboratory.

The serum concentrations for calcium, phosphorus, alkaline phosphatase, creatinine, and albumin were determined by an automated analyzer (C8; Beckman, Brea, CA) with the use of commercial kits. The normal laboratory range for serum calcium is 8.5–10.5 mg/dL, serum phosphorus is 2.5–4.5 mg/dL, and SAP is <95 JU/L.

The 25(OH)D concentrations were measured by competitive radioimmunoassay after acetonitrile extraction (DiaSorin, Stillwater, MN; catalog no. 681060E). The minimal detectable limit of the 25(OH)D assay is 1.5 ng/mL. N-tact PTH was measured by immunoradiometric assay (DiaSorin; catalog no. 26106). The minimal detectable limit of the N-tact PTH assay is 0.7 pg/mL. The subjects were classified as vitamin D—deficient, —insufficient, or —sufficient on the basis of 25(OH)D concentrations of <20 ng/mL, 20–30 ng/mL, and >30 ng/mL, respectively, according to recent consensus (16–18).

Descriptive results are presented as means ± 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 values < 0.05 were considered significant. Analysis of variance was used to estimate the main effects and interactions. Tukey’s test was used to identify the groups that are homogenous with respect to mean. Analysis was performed with the use of SPSS (version 11.5; SPSS Inc, Chicago, IL).

RESULTS

A total of 1148 subjects were evaluated during the study. The mean age was 46 ± 0.43 y for urban subjects and 43 ± 1.01 y for rural subjects. Urban subjects were fully dressed with only the face and forearm exposed to sunlight with a white-collar job (working indoors between 1000 and 1700). Those subjects not in a job are indoors most of the time. The rural subjects are agricultural workers starting their day at 0800 and working outdoors until 1700 with their face, chest, back, legs, arms, and forearms exposed to sunlight.

The diet of urban subjects constituted ≈2200 kcal/d. Carbohydrates contributed 55% of the total energy intake, proteins 10%, and fat 10%. Vegetables contributed 10% of the total energy intake, and milk and milk products contributed 15%. The carbohydrate source was primarily from cereals with rice providing 50% of total carbohydrates, wheat 25%, and Ragi (Eleusine coracana) 25%. Vegetables 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/d. Carbohydrates contributed 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%; Ragi: 40%). Vegetable sources were drumstick leaves, brinjals, tomatoes, and so forth. Animal sources of protein were consumed once fortnightly. No other source of calcium or any other mineral was consumed in both groups.

The daily dietary calcium intake of both rural and urban subjects was low (Table 1) when compared with that of the recommended dietary allowance (RDA) of 400 mg/d for adults (both sexes) issued by the Indian Council of Medical Research (ICMR).

| TABLE 1 | Comparison of dietary intake of urban and rural groups† |
|---|---|---|---|
| Men | Women | Men | Women |
| Urban | Rural | Urban | Rural |
| Dietary calcium (mg/d) | 322 ± 2 (307, 340) | 271 ± 2 (265, 280) | 300 ± 2 (282, 310) | 282 ± 2 (233, 271) |
| Dietary phosphorus (mg/d) | 674 ± 2 (646, 707) | 452 ± 9 (475, 511) | 651 ± 9 (643, 660) | 481 ± 10 (462, 501) |
| Phosphate-to-calcium ratio | 0.3 ± 0.02 (0.34, 0.34) | 0.76 ± 0.49 (0.73, 0.78) | 0.31 ± 0.01 (0.35, 0.32) | 0.76 ± 0.01 (0.74, 0.78) |

†All values are ± SEM; 95% CI is in parentheses. Recommended dietary allowance of calcium is 400 mg/d in adults. There was no significant interaction between sex and location (urban and rural). The main effects of sex and dietary calcium were significant, P < 0.001. Significant location (urban and rural) × dietary calcium, location (urban and rural) × dietary phosphorus, and location (urban and rural) × phosphate-to-calcium ratio interactions were observed, P < 0.0001.
for the Indian population (Table 1). Dietary intake of calcium and phosphorus were significantly lower (P < 0.0001) in the rural subjects than in the urban subjects. The dietary phytate-to-calcium ratio was significantly (P < 0.0001) higher in the rural subjects (Table 1).

Dietary phytate correlated positively with dietary calcium in the urban subjects (r = 0.55, P < 0.0001) and rural subjects (r = 0.36, P < 0.0001) (Figure 1). Dietary calcium intake correlated negatively with the phytate-to-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). The phytate-to-calcium ratio correlated positively with N-tact PTH (r = 0.2, P < 0.01) and SAP (r = 0.3, P < 0.0001).

Thus, the diet of both the rural and urban subjects was far below the RDA of calcium recommended by ICMR. The diet in rural subjects had a high phytate-to-calcium ratio, thus retarding the absorption of already low intakes of dietary calcium. The main effects of sex and dietary calcium are significant (P < 0.012). Significant interactions for location (urban and rural) × dietary calcium, location (urban and rural) × dietary phosphorus, and location (urban and rural) × phytate-to-calcium ratio were observed (P < 0.0001).

The serum calcium concentration of the urban and rural subjects was within the normal range (Table 2). The serum concentrations of phosphorus and SAP were the normal range in both the urban and rural subjects. The 25(OH)D concentrations of rural subjects were significantly higher (P < 0.001) than that of urban subjects in both the male and female groups (Table 2). A significant interaction of sex × location (urban and rural) was observed for SAP (P = 0.032). The main effect of sex is significant for 25(OH)D (P < 0.0001). The main effect of location (urban and rural) is significant on all indicators except N-tact PTH (P < 0.0001 for each).

### Table 2
Comparison of biochemical and hormonal profiles of urban and rural groups

<table>
<thead>
<tr>
<th></th>
<th>Urban</th>
<th>Rural</th>
<th>Urban</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.48 ± 0.02 (9.64, 9.73)</td>
<td>9.68 ± 0.05 (9.95, 10.2)</td>
<td>9.48 ± 0.02 (9.64, 9.73)</td>
<td>9.98 ± 0.06 (9.87, 10.15)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH (IU/L)</td>
<td>84.80 ± 3.87 (78.85, 90.97)</td>
<td>55.67 ± 2.07 (49, 61)</td>
<td>80.4 ± 3.07 (78, 90.17)</td>
<td>63.7 ± 3.41 (56, 68.4)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>23.71 ± 0.8 (22, 25)</td>
<td>37.5 ± 0.3 (14.9, 16)</td>
<td>19 ± 0.99 (17.54, 21)</td>
<td>&lt;0.0017</td>
</tr>
<tr>
<td>N-tact PTH (pg/mL)</td>
<td>28.3 ± 1.6 (26, 32.35)</td>
<td>29.24 ± 1.6 (26, 32.35)</td>
<td>28.3 ± 1.6 (26, 32.35)</td>
<td>29.21 ± 1.7 (25.75, 32.7)</td>
</tr>
</tbody>
</table>

1 All values are ± SE; 95% CIs in parentheses; a = urban, SAP, serum albumin phosphorus; 25(OH)D, 25-hydroxyvitamin D; N-tact-PTH, intact circulating parathyroid hormone. To convert 25(OH)D from ng/mL to nmol/L, multiply by 2.5.
2 Main effect of sex (urban and rural).
3 Interaction between sex × location (urban and rural).
4 Main effect of sex.
In the rural subjects, vitamin D--deficient, --insufficient, and --sufficient states were observed in 64%, 35.5%, and 16.5% of the men and 70%, 29%, and 1% of the women, respectively. In the urban subjects, vitamin D--deficient, --insufficient, and --sufficient states were observed in 62%, 26%, and 12% of the men and 75%, 19%, and 6% of the women, respectively.

N-tct PTH concentrations were 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) (Figure 2). No significant difference was observed in the r values between rural and urban subjects. In rural subjects, the N-tct PTH concentrations 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 the urban subjects. The r value for correlation between N-tct PTH and serum phosphorus was significantly higher in urban subjects than in rural subjects (P < 0.001).

DISCUSSION

Reports were recently made of a high prevalence of suboptimal dietary calcium intake and 25(OH)D insufficiency in south Indian populations (19, 20). A few reports available from north India are from a group of healthy subjects (12) of urban and semirural children (21). The study reporting on a small group of healthy adult subjects is limited for interpretation because of the critical values of 25(OH)D concentrations used for the definitions of vitamin D deficiency and vitamin D insufficiency (22, 23). In India, metabolic bone disease secondary to dietary calcium insufficiency and 25(OH)D deficiency is prevalent. There are no reports of large population-based studies from other parts of the country.

The dietary intake of calcium of first-generation healthy Asian Indian immigrants in the United States (24) was found to be less than two thirds of the dietary reference intake recommended for a healthy person in the United States. Recently, the RDA was revised and redefined as the dietary reference intake, which is a result of collaborative effort between the United States and Canada (25). The RDA for calcium in India as recommended by the ICMR is lower than the recently revised dietary reference intake (Table 3) (15, 25–28). Milk is not fortified with calcium or vitamin D in India.

The dietary calcium intake by both the rural and urban subjects (Table 1) was much lower than the RDA of calcium recommended by the ICMR guideline (15). Intake of a diet rich in phytate (inoculated hexaphate) retards the absorption of calcium from the gut. Inoculated hexaphate forms chelates with divalent cations of calcium and reduces the absorption of calcium from the gut. Studies by Pawar et al (29) have shown that the calculated values for all nutrients are significantly higher than the analytic values. Hence, a patient with a calculated low intake of

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Recommended dietary allowance of calcium in India and the United States</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>India*</td>
</tr>
<tr>
<td>Men</td>
<td>400</td>
</tr>
<tr>
<td>Women</td>
<td>400</td>
</tr>
<tr>
<td>Pregnancy and lactating mothers</td>
<td>1000</td>
</tr>
</tbody>
</table>

*Recommendation from food composition table (15).

*Recommendation from National Academy consensus (25).
calcium with the background of a diet that contains foods high in phytates, as in the current study, may be more calcium deficient than calculated from dietary intake data. The quality of the diet in rural subjects was low in calcium and high in the phytate-to-calcium ratio compared with the urban diet; hence, the rural subjects are more affected. Although for rural subjects more body surface area is exposed to sunlight for longer durations by virtue of their occupations, the poor quality of diet impedes the bone homeostasis significantly.

The calcium absorptive performance of the gut is a function of a person's 25(OH)D status (30, 31). When the 25(OH)D concentrations are low, the effective calcium absorption from the gut is reduced (30, 31). It was shown that low dietary calcium can reverse the 25(OH)D to polar metabolites in the liver and leads to secondary 25(OH)D deficiency (32). 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)D to 1,25-dihydroxyvitamin D which, in turn, stimulates the intestinal calcium absorption. In addition, 1,25-dihydroxyvitamin D induces its own destruction by increasing 24-hydroxylase. This is the likely explanation for the low 25(OH)D concentrations in persons on a high-phytate or low-calcium diet. In the present study, low prevalence of 25(OH)D deficiency was seen in rural male subjects compared with that of the urban male subjects. The same observation was made for the women. This is probably due to occupation, dress code, and duration of exposure to sunlight of the rural subjects, who are agricultural laborers working for 8–10 h in sunlight. In the region of this study season has little impact on cutaneous synthesis of vitamin D.

There are reports of low dietary intakes of calcium (<300 mg/d) from the Indian subcontinent causing osteomalacia (33, 34) and in postmenopausal women (19, 35), children (21), and pregnant women and their offspring (36).

The work in baboons has shown the effect of low dietary calcium intake on the development of rickets among vitamin D–deficient animals (37, 38). The studies in rat models (37, 38) have shown that a low-calcium diet or a high-phytate diet resulted in increased catabolism of 25(OH)D concentrations, leading to the formation of inactive metabolites with resultant reduction in 25(OH)D concentrations. It was also proposed that the pathogenesis of rickets in the Asian community in the United Kingdom is attributable to a high-calcium, low-calcium diet which induces mild hyperparathyroidism (39). Thus, the role of low dietary calcium intake in the pathogenesis of 25(OH)D deficiency is probably greater than originally recognized.

To the best of our knowledge, the current study is the first to investigate and compare the relation among the dietary calcium intake, biochemical indicators of bone and mineral metabolism, and vitamin D status in rural and urban subjects. There are methodologic limitations in this study such as the urban subjects are a sample of convenience. More subjects in all age groups in both access in urban and rural locations in different parts of the country should be studied in the 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 subjects to sunlight. This could have a deleterious effect on bone mineral homeostasis and peak bone mass achieved, and it subsequently reflects as a low bone mineral density of the Indian population (14). Low 25(OH)D concentrations were associated with a diminutive 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.

CIV designed and supervised the study; conducted the survey; supervised the dietary survey, sample collection, and quality control of biochemical assay; conducted the literature survey; performed the statistical analysis; and wrote and revised the manuscript. KVS helped in the statistical analysis. TR was involved in the dietary survey. UVP, DS, and GITVK helped in verification of the dietary survey and performed the 25(OH)D and N-ace PTH assays. PVLNS supervised the biochemical estimations. None of the authors had a personal or financial conflict of interest.

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JOURNAL OF
BONE AND MINERAL
RESEARCH
VOL. 19, SUPPL. 1, OCTOBER 2004, P. S31-S503

2004 Abstracts

Twenty-Sixth Annual Meeting
of the American Society for
Bone and Mineral Research

Washington State Convention & Trade Center
Seattle, Washington, USA
October 1-5, 2004

JBMR

To study the bone mineral markers in serum and urine in south Indian postmenopausal women.

Postmenopausal women (n=437) were evaluated for their dietary intake patterns, bone mineral markers in serum and urine.

The mean ± standard error of mean (SEM) of age (yrs), weight (kg), BMI of the 437 patients were 52±0.4; 62±0.4; 27±0.3 respectively. The serum albumin (gm/dl), creatinine (mg/dl), LH and FSH(U/l) were 3.96±0.02; 0.9±0.01; 26±0.8; 65±1.8 respectively. The dietary calcium, phosphorous (mg/day) and phytocalcium ratio were 322±67; 0.56±0.01. The whole group was subclassified based on 25(OH)D levels into: Group - 1-severe deficiency(<5 ng/ml); Group - 2 moderate deficiency(5-10 ng/ml); Group - 3-mild deficiency(10-20 ng/ml) and Group - 4-vitamin D sufficiency(>20 ng/ml). The bone mineral marker parameters of the respective group are depicted in the table below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group - 1</th>
<th>Group - 2</th>
<th>Group - 3</th>
<th>Group - 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.Calcium(mg/dl)</td>
<td>9.63±0.13</td>
<td>9.67±0.05</td>
<td>9.68±0.04</td>
<td>9.76±0.07</td>
</tr>
<tr>
<td>S.Phosphorous(mg/dl)</td>
<td>3.4±0.2</td>
<td>3.4±0.05</td>
<td>3.45±0.04</td>
<td>3.57±0.06</td>
</tr>
<tr>
<td>S.Alk.Phos(1U/l)</td>
<td>106±15</td>
<td>117±6</td>
<td>106±3.5</td>
<td>107±7.3</td>
</tr>
<tr>
<td>S.TRAP(1U/l)</td>
<td>6.5±1.5(4)</td>
<td>5.9±0.4(27)</td>
<td>5.7±0.3(49)</td>
<td>7±0.7(9)</td>
</tr>
<tr>
<td>25(OH)D(nM/ml)</td>
<td>4.15±0.2</td>
<td>7.88±0.14</td>
<td>14.86±0.2</td>
<td>26±0.65</td>
</tr>
<tr>
<td>PTH ntact (pg/ml)</td>
<td>39±5</td>
<td>25.5±1.7</td>
<td>25±1.2</td>
<td>25±1.5</td>
</tr>
<tr>
<td>Ca/Cr ratio</td>
<td>0.16±0.02</td>
<td>0.17±0.01</td>
<td>0.18±0.01</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>PEI (U/l)</td>
<td>0.03±0.03</td>
<td>0.005±0.008</td>
<td>-0.02±0.02</td>
<td>-0.01±0.008</td>
</tr>
<tr>
<td>Urinary DPC(nM/mmol of Cr)</td>
<td>15.6±6(4)</td>
<td>6.35±0.9(27)</td>
<td>7.9±0.4(51)</td>
<td>8.7±1.9(10)</td>
</tr>
<tr>
<td>Urinary OH Proline</td>
<td>4.8±0.09(4)</td>
<td>8.43±1.3(24)</td>
<td>6.6±0.71(47)</td>
<td>5.4±2.3(10)</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; Parenthesis - patient number

There was no significant difference in the dietary pattern in the subgroups. The dietary calcium intake of the whole group is inadequate compared to the Recommended Daily Dietary allowances (RDA) of national guidelines. About 80% of the study group had varying degrees of 25(OH)D deficiency [severe deficiency 4% (19); moderate deficiency 24%(104) and mild deficiency 52% (226)]. Only 20% of the population have normal 25(OH)D levels. Serum PTH negatively correlated with 25(OH)D levels (r -0.2; p <0.01) and positively with SAP (r 0.8; p <0.01). A significant positive correlation (p<0.01) was observed between dietary phytates with SAP (r 0.6); dietary calcium with phytates (r0.75) and SAP (r 0.65).

In all postmenopausal women diet should be enriched with calcium and vitamin D supplemented while considering HRT. The dietary calcium insufficiency and 25(OH)D deficiency could reflect fallaciously as osteopenia in BMD measurements.

Disclosures: C.V. Harinarayan, None.
2005 Abstracts

Twenty-Seventh Annual Meeting
of the American Society for
Bone and Mineral Research

Gaylord Opryland Resort and
Convention Center
Nashville, Tennessee, USA
September 23–27, 2005
Vitamin D Insufficiency in South Indian Rural and Urban Population. C. V.Harinarayan1, U. V.Prasad1, T. Ramalakshmi*1, E. G.T.V.Kumar*1, P. V.L.N.Srinivasarao2, P. R.Parthasarathy*3, C. R.Surendranath*1, M. M.Suchitra*2. 1Endocrinology and Metabolism, Sri Venkateswara Institute of Medical Sciences, Tirupati, India, 2Biochemistry, Sri Venkateswara Institute of Medical Sciences, Tirupati, India, 3Biochemistry, SV University, Tirupati, India.

To study the prevalence of dietary calcium intake and vitamin D status of south Indian rural and urban population. Urban (n=943) and rural (n=205) population were studied for their dietary intake of calcium(DC), phosphorous and phytate/calcium ratio. Serum calcium (SC), phosphorous(SP), alkaline phosphatase (SAP) levels, 25(OH)D and parathormone(PTH) levels were measured. The dietary calcium, phosphorous (mg/day) and phytate/calcium ratio of urban population was 307±51; 653±104; 0.5±0.1 and rural population was 267±30; 488±68; 0.8±0.1 respectively. The SC, SP (mg/dl), SAP (IU/L), 25(OH)D (ng/ml) and parathormone levels (pmol/l) of the urban population are 9.7±0.5; 3.6±0.7; 81±31; 16.7±8.4; 28±17 and that of rural population was 10±0.7; 2.9±0.5; 82±6; 21.4±7.1; 29±10 respectively. The rural population had significantly (p<0.001) higher 25(OH)D levels, SC and lower SAP, DC than the urban population. The distribution of varying degree of 25(OH)D levels in the population are shown in table:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age yrs</th>
<th>n</th>
<th>Percentage of population with 25(OH)D levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;10 ng/ml</td>
</tr>
<tr>
<td>Urban WG</td>
<td>46±13</td>
<td>943</td>
<td>21</td>
</tr>
<tr>
<td>Rural WG</td>
<td>43±14</td>
<td>205</td>
<td>2.5</td>
</tr>
<tr>
<td>Males WG</td>
<td>46±16</td>
<td>243</td>
<td>3</td>
</tr>
<tr>
<td>Females WG</td>
<td>46±13</td>
<td>905</td>
<td>21.4</td>
</tr>
<tr>
<td>Urban Males</td>
<td>47±17</td>
<td>134</td>
<td>6</td>
</tr>
<tr>
<td>Urban Females</td>
<td>46±13</td>
<td>809</td>
<td>23.5</td>
</tr>
<tr>
<td>Rural Males</td>
<td>45±15</td>
<td>109</td>
<td>NIL</td>
</tr>
<tr>
<td>Rural Females</td>
<td>41±14</td>
<td>96</td>
<td>5.2</td>
</tr>
</tbody>
</table>

WG - whole group

In urban population, PTH correlated negatively with 25(OH)D levels (r=-0.13;p<0.001), SC (r=0.13;p<0.001) and SP (r=0.01;p<0.01). 25(OH)D levels correlated positively with SC (r=0.13;p<0.001). In rural population, PTH correlated negatively with 25(OH)D (r=-0.24;p<0.002), DC (r=0.4;p<0.001) and positively with SAP (r=0.3;p<0.001). 25(OH)D levels correlated positively with DC (r=0.3;p<0.001) and negatively with SAP (r=0.22;p<0.002). The DC intake of the whole group is inadequate compared to the

Recommended Daily allowances of national guidelines. Around 30% of urban population and 50% of rural males have normal 25(OH)D levels. About 50% of the urban and rural population had 25(OH)D insufficiency. 21% of urban women and 5% of rural women had 25(OH)D deficiency. 25(OH)D deficiency and insufficiency is more common in urban female. The dietary calcium and 25(OH)D insufficiency could reflect fallaciously as osteopenia in BMD measurements. There is an urgent need to enrich the diet with calcium and supplement vitamin D with monitored food fortification programs in the country.

Disclosures: C. V.Harinarayan, None.