High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians \(^1\)-\(^2\)

Chittari V Harinarayan, Tirupati Ramalakshmi, Upadrashta V Prasad, Desineni Sudhakar, Pemmaraju VLN Srinivasarao, Kadainti VS Sarma, and Ethamakula G Tiruvenkata Kumar

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.

**Design:** Healthy urban (\(n=943\)) and rural (\(n=205\)) subjects were studied for their dietary pattern and concentrations of serum calcium, phosphorus, alkaline phosphatase, 25(OH)D, and immunoreactive 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 phosphorous were significantly lower in rural subjects than in urban adults (\(P<0.0001\)). The dietary phytate-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 (\(P<0.0001\)), both 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%, 39.5%, and 16.5% of the men and 70%, 29%, and 1% of the women, respectively. In the urban subjects, 25(OH)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.

**Conclusions:** 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 (ie, 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 osseous 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 ensues, 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 immigrant Indians 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 \(\approx8-10\) h/d throughout the year with...
the solar zenith angle of 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 lowest temperature of 17 °C (night) and 28 °C (day) with scanty rainfall. 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 phytates 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 repeatability of the documentation was rechecked at random by one of us over the period of the study. From the raw weights, the intakes of total energy, calcium, phosphorus, and phytates were calculated with the use of a published food composition table, detailing the nutritive value of Indian foods (15). Because the ratio of dietary phytates to calcium is more predictive of the severity of interference of calcium absorption than is dietary calcium alone, the phytate-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 for further analysis.

The serum concentrations for calcium, phosphorus, alkaline phosphatase (SAP), creatinine, and albumin were determined by an automated analyzer (CX 9; Beckman, Brea, CA) with the use of commercial kits. The normal laboratory range for serum calcium –20 °C until the analysis for determining 25(OH)D and 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 for further analysis.

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The serum concentrations for calcium, phosphorus, alkaline phosphatase (SAP), creatinine, and albumin were determined by an automated analyzer (CX 9; Beckman, Brea, CA) with the use of commercial kits. The normal laboratory range for serum calcium was 8.5–10.5 mg/dL, serum phosphorus was 2.5–4.5 mg/dL, and SAP was <95 IU/L.

The 25(OH)D concentrations were measured by competitive radioimmunoassay after acetonitril extraction (DiaSorin, Stillwater, MN; catalog no. 68100E). The minimal detectable limit of the N-tact PTH assay is 0.7 pg/mL. The 25(OH)D assay is 1.5 ng/mL. N-tact PTH was measured by immunoradiometric assay (DiaSorin; catalog no. 26100). 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. 26100). 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 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 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 ± 0.11 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%. 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/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>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Comparison of dietary intake of urban and rural groups</strong></td>
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<tr>
<td><strong>Men</strong></td>
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<tr>
<td><strong>Urban</strong>&lt;br&gt;(n = 32)</td>
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<tr>
<td>Dietary calcium (mg/d)</td>
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<td>Dietary phosphorus (mg/d)</td>
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<td>Phytate-to-calcium ratio</td>
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1 All values are x ± SEM; 95% CIs in parentheses. Recommended dietary allowance of calcium in diet recommended by the Indian Council of Medical Research 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 < 0.012. Significant location (urban and rural) × dietary calcium, location (urban and rural) × dietary phosphorus, and location (urban and rural) × phytate-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) $\times$ dietary phosphorus, and location (urban and rural) $\times$ 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 in 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 $\times$ 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$^1$

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<tr>
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<th>Urban</th>
<th>Rural</th>
<th>Urban</th>
<th>Rural</th>
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<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.74 ± 0.06 (9.63, 9.85)</td>
<td>10.06 ± 0.06 (9.95, 10.2)</td>
<td>9.68 ± 0.02 (9.64, 9.73)</td>
<td>9.98 ± 0.06 (9.87, 10.15)</td>
<td>&lt; 0.001$^2$</td>
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<td>Serum phosphorus (mg/dL)</td>
<td>3.50 ± 0.07 (3.37, 3.64)</td>
<td>2.84 ± 0.07 (2.27, 2.97)</td>
<td>3.64 ± 0.03 (3.59, 3.69)</td>
<td>2.74 ± 0.07 (2.79, 3.09)</td>
<td>&lt; 0.001$^2$</td>
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<tr>
<td>SAP (IU/L)</td>
<td>84.87 ± 3.87 (78.85, 90.9)</td>
<td>55.67 ± 2.07 (49, 61)</td>
<td>80.4 ± 3.07 (78, 90.17)</td>
<td>62.7 ± 3.41 (56, 69.4)</td>
<td>&lt; 0.001$^2$</td>
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<td>$25(OH)D$ (ng/mL)</td>
<td>18.54 ± 0.8 (17, 20)</td>
<td>23.73 ± 0.8 (22, 25)</td>
<td>15.5 ± 0.3 (14.9, 16)</td>
<td>19 ± 0.89† (17.54, 21)</td>
<td>&lt; 0.001$^2$</td>
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<tr>
<td>N-tact PTH (pg/mL)</td>
<td>27 ± 1.6 (23.9, 30)</td>
<td>29.24 ± 1.6 (26, 32.35)</td>
<td>28.35 ± 0.6 (27, 29.5)</td>
<td>29.21 ± 1.7 (25.75, 32.7)</td>
<td>&lt; 0.01$^4$</td>
</tr>
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</table>

$^1$ All values are $\bar{x}$ ± SEM; 95% CIs in parentheses; $n$ in brackets. SAP, serum alkaline phosphatase; $25(OH)D$, 25-hydroxyvitamin D; N-tacts-PTH, immunoreactive parathyroid hormone. To convert $25(OH)D$ from ng/mL to nmol/L, multiply by 2.5.

$^2$ Main effect of location (urban and rural).

$^3$ Interaction between sex $\times$ location (urban and rural).

$^4$ Main effect of sex.
In the rural subjects, vitamin D–deficient, –insufficient, and –sufficient states were observed in 44%, 39.5%, 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-tact 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-tact 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-tact 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 calcium intake and 25(OH)D insufficiencies in south Indian populations (19, 20). A few reports available from north India are from a group of healthy subjects (12) of urban and semisemurban children (21). The study reporting on a small group of healthy adult subjects is limited for interpretation because of critical values of 25(OH)D concentrations used for the definition 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 guidelines (15). Intake of a diet rich in phytate (inositol hexaphosphate) retards the absorption of calcium from the gut. Inositol hexaphosphate forms chelates with divalent cations of calcium and reduces the absorption of calcium from the gut. Studies by Panwar 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

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<th>TABLE 3</th>
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<td>Recommended dietary allowances of calcium in India and the United States</td>
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<th></th>
<th>India⁷</th>
<th>United States⁸</th>
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<tbody>
<tr>
<td>Men</td>
<td>400</td>
<td>800–1000</td>
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<tr>
<td>Women</td>
<td>400</td>
<td>800–1000</td>
</tr>
<tr>
<td>Pregnant and lactating mothers</td>
<td>1000</td>
<td>1200–1300</td>
</tr>
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³ Recommendation from food composition table (15).
⁴ Recommendation from Sallamander concepts (25).

FIGURE 2. Relation between serum concentrations of immunoreactive parathyroid hormone (N-tact PTH) and 25-hydroxyvitamin D [25(OH)D] by category in the urban group (● and dashed line, n = 943) and the rural group (▴ and solid line, n = 205). The categories of 25(OH)D concentration were ≤10 ng/mL (urban, n = 197; rural, n = 5), 10.1–20 ng/mL (urban, n = 490; rural, n = 110), 20.1–30 ng/mL (urban, n = 189; rural, n = 71), and >30 ng/mL (urban, n = 67; rural, n = 19). Significant parabolic trend is observed between 25(OH)D concentrations and N-tact PTH (model shown in figure). The relation between N-tact PTH and 25(OH)D is well modeled by a second-degree curve with a downward slope for urban (R² = 0.016; P < 0.0001) and rural (R² = 0.06; P < 0.0001) locations. A steep decrease in N-tact PTH is observed in rural subjects with relation to 25(OH)D when compared with urban subjects. The main effects of sex and location (urban and rural) are significant (P < 0.05). The effect of 25(OH)D concentrations × N-tact PTH is significant (P < 0.0001). The effect of location (urban and rural) on 25(OH)D is significant (P < 0.001). The effect of sex on 25(OH)D is significant (P < 0.001). The effect of 25(OH)D on N-tact PTH is significant in rural men (P < 0.05), rural women (P < 0.03), urban men (P < 0.0001), and urban women (P < 0.0001). No significant sex × location (urban and rural) interaction was observed on 25(OH)D concentrations. Serum N-tact PTH in the <10 ng/mL group was significantly different from other groups (Tukey’s test).
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 occupation, the poor quality of diet impedes the bone homeostasis significantly.

The calcemic 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 converts 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 a 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 h/d 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-cereal, 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 intakes, 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 sexes 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 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.

CVH designed and supervised the study; conducted the survey; supervised the dietary survey, sample collection, and quality control of hormonal assay; conducted the literature survey; performed the statistical analysis; and wrote and edited the manuscript. KVSS helped in the statistical analysis. TR was involved in the dietary survey. UVP, DS, and EGTVK helped in verification and edited the manuscript. TRLNS supervised the biochemical estimations. None of the authors had a personal or financial conflict of interest.

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