



OPEN Associations of bone health biomarkers and bone mineral density with dietary intakes in vitamin D deficient women: a cross-sectional study

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Vitamin D, an essential nutrient for bone health, is widely deficient in India despite abundant sunlight. The prevalence ranges between 70 and 90% in adult women. We studied the prevalence of vitamin D deficiency/insufficiency and the association between serum 25-hydroxyvitamin D, parathyroid hormone, osteocalcin, bone-specific alkaline phosphatase, bone mineral density, and dietary intakes in young urban women. Among 220 women screened, 84.7% of participants had suboptimal vitamin D (64.5% deficient 20.2% insufficient). In 108 vitamin D deficient women, 75% reported below 15 min of daily sun exposure. Dietary calcium intake was 480.6 ± 117.7 mg/day. Serum 25(OH)D was negatively correlated with PTH ($\rho = -0.364$, $p < 0.001$) and osteocalcin ($\rho = -0.342$, $p < 0.001$). Osteocalcin was positively correlated with dietary calcium ($\rho = 0.228$, $p < 0.01$), Ca: P ($\rho = 0.240$, $p < 0.01$) and milk intake ($\rho = 0.226$, $p < 0.01$). Positive associations were observed between osteocalcin and Ca: P ratio [$\beta = 23.58$, 95% CI: -0.614 to 47.771 , $p = 0.056$] and with milk intake [$\beta = 0.03$, 95% CI: -0.001 to 0.065 , $p = 0.058$]. The coexistence of vitamin D deficiency with inadequate calcium intake, and limited sun exposure, underscores the importance of strategies to promote calcium-rich diets and adequate sunlight practices to support bone health.

Keywords Vitamin D Deficiency, Bone Biomarkers, Bone Mineral Density, Osteocalcin, Calcium, Sun Exposure

Abbreviations

25(OH)D	25-hydroxyvitamin D
AM	Age matched
BSAP	Bone Specific Alkaline Phosphatase
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BTM	Bone Turnover Marker
Ca	P: Calcium-to-Phosphorus ratio
CV	Coefficient of variation

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DEXA	Dual-Energy X-ray Absorptiometry
EAR	Estimated Average Requirement
HCES	Household consumption expenditure survey
IEC	Institutional Ethics Committee
IFCT	Indian Food Composition Tables
IOM	Institute of Medicine (now National Academy of Medicine)
OC	Osteocalcin
PTH	Parathyroid hormone
SD	Standard Deviation
SHPT	Secondary Hyperparathyroidism
SPF	Sun protection factor
UV-B	Ultraviolet-B
WHO	World Health Organization
YA	Young adult

Vitamin D deficiency is a global public health concern, affecting an estimated one billion individuals across all ages and ethnicities, with significant geographical variation¹. The prevalence of vitamin D deficiency has been reported to be 5.9%, 7.4% and 13.0% in the United States, Canada and Europe, respectively². Despite abundant sunlight, India has a high burden of vitamin D deficiency, with a pooled prevalence of about 67% among all age groups including women³. This is probably driven by limited sun exposure due to indoor lifestyles, high pollution levels, darker skin tone, cultural clothing practices and low dietary intake, compounded further by high-phytate diets that inhibit calcium absorption and vitamin D utilization⁴.

Vitamin D is a unique micronutrient that enters the human body through two primary pathways: cutaneous synthesis and dietary intake. The predominant source is the cutaneous production of vitamin D₃ (cholecalciferol) obtained upon exposure to ultraviolet-B (UV-B) radiation from sunlight, which accounts for approximately 80–90% of the body's total vitamin D requirement⁵. The remaining 10–20% is derived from dietary sources, primarily as D₃ from animal-based foods such as fatty fish, fish liver oil and egg yolk and as D₂ from fortified foods^{6,7}.

Once vitamin D is absorbed or synthesized, it binds to vitamin D-binding protein (DBP) in the bloodstream and is transported to the liver, where it is hydroxylated to 25-hydroxyvitamin D (25(OH)D or calcidiol), the main circulating form and most reliable biomarker of vitamin D status due to its long half-life (~ 3 weeks)⁸. In the kidneys, 25(OH)D is further hydroxylated to 1,25-dihydroxyvitamin D (1,25(OH)₂D or calcitriol)^{9,10}. Vitamin D is a fat-soluble micronutrient and is often regarded as a prohormone, playing a pivotal role in many physiological functions¹¹. In addition to its role in immune regulation, cell differentiation and endocrine function, it is best known for its critical involvement in calcium homeostasis and bone health^{12,13}. The active form of vitamin D facilitates calcium absorption in the intestine, supports bone mineralization and regulates calcium and phosphorus levels in response to physiological demands, which are regulated by parathyroid hormone (PTH)¹⁴. With vitamin D deficiency, elevated PTH levels lead to increased bone turnover and calcium loss from bone, eventually contributing to osteoporosis and a heightened risk of fractures. Therefore, a deficiency of vitamin D coupled with low intake of calcium can result in hypocalcemia and impaired skeletal integrity progressing to rickets in children and osteomalacia in adults if left untreated¹⁵.

The vitamin D (25(OH)D) status cut-off varies across guidelines: the US National Academy of Medicine (formerly, Institute of Medicine, IOM) defines deficiency as a 25(OH)D concentration < 30 nmol/L (< 12 ng/mL) in the serum as deficient, between 30 and 50 nmol/L (12–20 ng/mL) as insufficient and ≥ 50 nmol/L (≥ 20 ng/mL) as sufficient, whereas the Endocrine Society defines deficiency at a higher threshold of ≤ 50 nmol/L (≤ 20 ng/mL)¹⁶.

Bone, a metabolically active tissue, undergoes continuous remodelling through the coordinated processes of resorption by osteoclasts (cells that breakdown bone) and formation by osteoblasts (cells that build new bone)^{17,18}. Biomarkers of bone formation, measured in serum or plasma, include bone-specific alkaline phosphatase (BSAP), osteocalcin and procollagen type 1 N-terminal propeptide (P1NP). BSAP is often elevated in states of high bone turnover and has been shown to decrease with the correction of vitamin D deficiency, reflecting its role in bone metabolism¹⁹. Serum osteocalcin, a biomarker of bone remodelling, serves as a valuable indicator for assessing osteoporosis and predicting fracture risk²⁰. Given the role of vitamin D in regulating calcium balance and bone mineralization, combining bone turnover marker (BTM) assessment with serum 25(OH)D levels can enhance the understanding of skeletal health. Dual-energy X-ray absorptiometry (DEXA) remains the gold standard for assessing bone mineral density (BMD)¹⁹.

However, most available evidence linking vitamin D status with bone biomarkers and BMD originates from studies in postmenopausal women and elderly populations. Few studies have examined these relationships in young women, particularly in India, where peak bone mass accrual during late adolescence and early adulthood is critical for long-term skeletal health. Understanding these associations in this group is essential for informing early preventive strategies. Therefore, this study aimed to (1) determine the prevalence of vitamin D deficiency and insufficiency among young urban Indian women and (2) examine associations of serum 25(OH)D with PTH, osteocalcin, BSAP, BMD, and dietary calcium and phosphorus intake. We hypothesized that lower 25(OH)D concentrations would be associated with higher PTH and bone turnover markers and with reduced BMD. This study is among the first to investigate these relationships in this population, highlighting the need for early screening and nutrition-based interventions.

Methods

Study design and setting

This cross-sectional study is a part of a double-blind, randomized clinical trial conducted at St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India. Ethical approval from the Institutional Ethics Committee, St. John's Medical College and Hospital (IEC Ref. No: 370/2018) was obtained and was registered prospectively with the Clinical Trials Registry – India (CTRI) under the registration number: (CTRI/2020/03/024395; registered on 31/03/2020). Written informed consent was obtained from all participants. All research procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee.

Sample size

Sample size was calculated using serum 25(OH)D concentration as the primary outcome. Considering the prevalence of vitamin D deficiency as 80% in young adult women^{4,21}, with 10% relative precision and 95% confidence interval, the number required was 97. Accounting for 10% attrition, the total number required was 108 study participants.

Study population

Screening was conducted in two phases. Phase 1 took place between November 2020 and April 2021 (post monsoon to summer), during which 176 women aged 18–35 years were initially screened. Owing to COVID-19-related lockdowns and restrictions on movement and campus access, a second round of screening (Phase 2) was carried out between August and December 2021 (monsoon to early winter). In the 2nd phase, 145 women were screened, including 44 newly recruited participants and 101 participants who returned from Phase 1 for rescreening. In total, 220 (phase 1–75 and Phase 2–145) participants were screened across both phases, of whom 203 underwent blood screening for serum 25(OH)D concentrations to assess vitamin D status. Eligible participants were women aged 18–35 years, with a BMI between 18.5 and 24.9 kg/m² and serum 25(OH)D concentrations < 20 ng/mL (deficient or insufficient). Hence 17 participants were excluded as their BMI were above the required cutoff of the study.

Exclusion criteria included use of vitamin D or calcium supplements within the past month; a history of diabetes, liver or kidney disease, cardiovascular disease or gastrointestinal conditions affecting nutrient absorption; had hypo- or hyperparathyroidism; or were on medications for any other illness. Among the 203 participants, 108 women with vitamin D deficiency or insufficiency (serum 25(OH)D < 20 ng/L) and a BMI between 18.5 and 24.9 kg/m² were enrolled in the vitamin D dose-response trial, and their baseline data were used for analysis in this study.

Anthropometric measurements

Anthropometric measurements were obtained during screening in both Phase 1 and Phase 2 by trained research staff following standardized protocols. All participants were instructed to report to the Nutrition and Lifestyle Clinic at St. John's Hospital on the morning of their scheduled assessment, following an overnight fast of 8–10 h. Body weight was measured to the nearest 0.1 kg via a calibrated digital weighing scale (ESSAE DS-415), and height was recorded to the nearest 0.1 cm via a mobile stadiometer (Standard Steel-HM01, Medifit). BMI was calculated using the following formula: [weight (kg)/height (m²)] and classified according to the World Health Organization (WHO) criteria: underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5–24.9 kg/m²), overweight (BMI 25.0–29.9 kg/m²) and obese (BMI ≥ 30.0 kg/m²)²².

Biochemical measurements

Venous blood samples (6.0 mL) were collected in serum vacutainers (Becton Dickinson, BD, USA) by trained phlebotomists under aseptic conditions. The serum was aliquoted post 30 min of collection into labelled Eppendorf tubes and stored at -80°C until biochemical analyses. Serum 25(OH)D was measured for all participants screened, while the remaining biochemical measurements, PTH, osteocalcin and BSAP, were analysed for those enrolled in the vitamin D dose-response trial. Serum 25(OH)D, PTH and osteocalcin were measured using an electrochemiluminescence immunoassay on a Roche E411 automated analyser (Roche Diagnostics, Switzerland). Instrument specific, two-level quality controls were used for quality assurance for each day of analysis. BSAP was measured using an immunoenzymatic assay (Ostase BAP EIA kit, Cat# AC-20; Immunodiagnostic Systems, USA). The concentration of BSAP in the samples was measured at 405 nm using a microplate reader (AM 2100, Alere Reader, USA). All samples were analysed in batches, with quality controls run in duplicate and with kit-specific controls used as quality checks. The precision of the intra- and inter-day assays for all levels of quality control was < 5% across all biochemical assays. Serum 25(OH)D levels were classified according to the IOM guidelines: deficiency < 12 ng/mL; insufficiency 12–20 ng/mL; and sufficiency ≥ 20 ng/mL²³. Other biochemical parameters, including PTH, osteocalcin and BSAP, were analysed as continuous variables. Reference ranges used are as follows: 16–87 pg/mL for PTH, 9–40 ng/mL for osteocalcin (adults) and 0–14 µg/L for BSAP (premenopausal women)²⁴.

Dietary assessment

Dietary intake was assessed during screening using three consecutive 24-hour dietary recalls (two weekdays and one weekend day) interviewer-administered within a one-week period for each participant. All nutritionists were trained to collect the dietary data in a standard manner. The participant first listed the foods and beverages consumed during the previous 24 h, including the vitamin and mineral supplements taken, followed by reporting of portion sizes using portion size aids and then probing for forgotten foods. The food portion sizes were estimated using standardized measuring cups and spoons. Nutrient and food group intakes were calculated

using a standardized food nutrient database²⁵. For the dietary assessment, the usual macronutrient intakes of energy (kcal), protein (g), fat (g) and carbohydrate (g) and the micronutrient intakes of calcium (mg), phosphorus (mg) and vitamin D (μg) were considered. The estimated average requirements (EAR)²⁶ were used to determine the inadequacy of calcium, phosphorus and vitamin D intake. Nonvegetarian intake (g/day) was computed by aggregating the reported usual intakes of individual items meat, poultry, fish and eggs into a consolidated value.

Sun exposure assessment

Sun exposure was assessed through a structured questionnaire adapted to the Indian context^{27,28} and designed to capture individual sun exposure habits over the preceding month. The questionnaire included details on the daily duration of direct sunlight exposure, the timing of sun exposure and typical clothing coverage (sleeve and bottom length). The participants were also asked about their use of sunscreen (including the sun protection factor level) and sun-protective accessories such as scarves and sunglasses.

Bone mineral density assessment

DEXA was used to assess BMD using a whole-body scanner (Lunar Prodigy Advanced PA + 301969, with software version 12.30, GE Medical Systems, USA). Scans were performed with participants wearing minimal, clothing and no metal accessories worn to minimize interference and ensure accurate measurements. Bone health was evaluated using Total body BMD (g/cm^2), young adult (YA) T scores, age-matched (AM) Z scores and bone mineral content (BMC) (g). T scores were categorized based on WHO criteria, where a T score of -1.0 or greater is classified as normal, between -1.0 and -2.5 as osteopenia and -2.5 or lower as osteoporosis and severe osteoporosis is defined as -2.5 or lower with a fragility fracture²⁹. Since T scores compare BMD to a young adult reference population and are typically used in postmenopausal women or older adults, Z scores were more appropriate for our younger participants (< 20 years). A Z score < -2.0 is considered below the expected range for age, indicating low bone density relative to age-matched norms. Daily quality checks for BMD were performed using phantom scans, confirming measurement stability with an interday precision of 4.79%.

Statistical analysis

Descriptive statistics were used to summarize the anthropometric and biochemical characteristics of the study population. Continuous variables are expressed as the means \pm standard deviations (SDs), whereas categorical variables are presented as frequencies and percentages. Spearman's rank correlation was used to assess the correlation between bone biochemical markers (25(OH)D, PTH, osteocalcin and BSAP) and dietary intake of calcium, phosphorus, calcium-to-phosphorus ratio and vitamin D as well as BMD measurements. Simple and multiple linear regression analyses were conducted to examine the associations between dietary intake (e.g., calcium, phosphorus and the calcium-to-phosphorus ratio) and biochemical markers Serum 25(OH)D and Osteocalcin. All the markers that showed significant associations with dietary intake in the preliminary analyses were included in the adjusted models. The multiple linear regression model was adjusted for age and energy intake to account for potential confounding effects. Adjusted beta coefficients with 95% confidence interval were reported. The usual intake of nutrients was estimated via the National Cancer Institute (NCI) method, where the intake was modelled using gamma distribution and the frequency of intake was modelled using beta binomial distribution. This method accounts for within-person variability in dietary intake. Inadequacy of intake of calcium and phosphorus were calculated using the EAR cut-point method. A p value < 0.05 was considered statistically significant. All the statistical analyses were performed via SPSS software version 29.0 and R software version 4.4.1.

Results

Participant characteristics and biochemical findings

Screening ($n=203$) and baseline data ($n=108$) from participants enrolled in a vitamin D dose-response trial ($n=108$) were analysed to examine associations between bone health indicators and nutrient intake in vitamin D-deficient and vitamin D-insufficient women. The participant characteristics are summarized in Table 1. Among the 203 individuals screened, 78.3% had a BMI within the normal range (18.5 – 24.9 kg/m^2). A total of 84.7% of the participants had suboptimal vitamin D status, with 20.2% classified as insufficient and 64.5% classified as deficient. The mean serum 25(OH)D level was 12.99 ± 8.82 ng/mL . About 30% were staff (including doctors, nurses, researchers and technicians), whereas 72% were students, primarily from nursing and doctoral programs. The results of 108 participants with deficient/insufficient vitamin D status enrolled in the dose-response trial is also provided in Table 1 and the mean serum 25(OH)D level was 9.83 ± 3.44 ng/mL .

Biochemical markers, BMD, nutrient intake and sun exposure

Among 108 participants with deficient/insufficient vitamin D status, the association between bone biomarkers and nutrient intake were examined. Table 2 summarizes the descriptive statistics of biomarkers of bone health, DEXA-derived BMD and BMC, usual nutrient intake, food group consumption and sun exposure. The mean usual energy intake was 1565.7 ± 267.7 kcal, and protein intake was 51.9 ± 8.5 g. The mean usual intakes of calcium and phosphorus were 480.6 ± 117.7 mg and 1067.4 ± 177.3 mg, respectively, with a calcium-to-phosphorus (Ca:P) ratio of 0.5:1. The mean BMD (1.09 ± 0.08 g/cm^2), T score (0.10 ± 0.79) and Z score (0.51 ± 0.82) were within normal reference ranges. The total body BMC averaged 1968.69 ± 219.00 g. 75% of the participants reported less than 15 min of daily sun exposure.

Correlations between 25(OH)D, bone biomarkers, nutrient intakes and BMD

Serum 25(OH)D levels were negatively correlated with PTH ($\rho = -0.364$, $p < 0.001$) and osteocalcin ($\rho = -0.342$, $p < 0.001$) but were not significantly correlated with BSAP ($\rho = 0.024$, $p = 0.803$), BMD ($\rho = 0.132$, $p = 0.175$)

Variable	Screened participants (n=203)	Participants included in baseline assessment of dose–response trial (n=108)
Demographic and anthropometry ¹		
Age (years)	22.80 ± 4.60	20.50 ± 1.10
Height (cm)	157.40 ± 5.60	158.38 ± 5.34
Weight (kg)	55.52 ± 8.63	53.27 ± 5.73
BMI (kg/m ²)	22.42 ± 3.40	21.22 ± 1.91
BMI (WHO classification) ²		
Underweight, n (%)	7 (3.45)	0 (0.00)
Normal, n (%)	159 (78.30)	108 (100.00)
Overweight, n (%)	37 (18.23)	0 (0.00)
Obese, n (%)	0 (0.00)	0 (0.00)
Occupation		
Staff (doctors, nurses, research)	58 (28.57)	3 (2.78)
Students (nursing, doctoral, other)	145 (71.43)	105 (97.22)
Vitamin D (25(OH)D) Status (ng/mL)		
Deficient (< 12 ng/mL), n (%)	131 (64.53)	83 (76.85)
Insufficient (12–20 ng/mL), n (%)	41 (20.20)	25 (23.15)
Vitamin D Total (25(OH)D, ng/mL)	12.99 ± 8.82	9.83 ± 3.44

Table 1. Baseline characteristics of the participants. ¹Values are expressed as the mean ± standard deviation (SD) for continuous variables. ²Values are expressed as numbers (percentages, %) for categorical variables. *BMI* body mass index, *WHO* World Health Organization, *25(OH)D* 25-hydroxyvitamin D.

or BMC ($\rho = -0.009$, $p = 0.930$). PTH, BSAP and osteocalcin were also not significantly correlated with each other. However, a significant negative correlation was observed between osteocalcin and total BMD ($\rho = -0.255$, $p = 0.008$), indicating an inverse association. Similarly significant negative association was noted between osteocalcin and BMD of the total arm ($\rho = -0.238$, $p = 0.01$). No significant correlation was observed between BSAP and BMD ($\rho = -0.189$, $p = 0.050$) or between osteocalcin ($\rho = 0.052$, $p = 0.590$) or BSAP ($\rho = -0.107$, $p = 0.271$) and BMC.

Correlation between the dietary intake of calcium, phosphorus and vitamin D with bone biomarkers were examined. Significant positive correlations was observed between osteocalcin with calcium intake ($\rho = 0.228$, $p < 0.01$; Fig. 1A) and calcium-to-phosphorus (Ca: P) intake ratio ($\rho = 0.240$, $p < 0.01$; Fig. 1B). The correlation between osteocalcin and phosphorus intake was positive but not statistically significant ($\rho = 0.185$, $p = 0.057$). No significant correlations were found between dietary calcium, phosphorus or the Ca: P ratio with serum 25(OH)D, PTH or BSAP levels. Calcium and phosphorus intake was significantly correlated with milk and milk product consumption ($\rho = 0.824$, $p < 0.001$ and $\rho = 0.499$, $p < 0.001$, respectively), whereas serum osteocalcin was positively correlated with milk consumption ($\rho = 0.226$, $p < 0.01$; Fig. 1C).

Association between dietary intakes with serum 25OHD and osteocalcin

Multiple linear regression analyses was performed to assess the association between dietary intakes of calcium, Phosphorus, milk and milk products including Ca: P Ratio with serum osteocalcin levels after adjusting for age and total energy intake (Table 3). There was no significant association observed between intakes of calcium, phosphorus, milk and milk products (g) and the Ca: P ratio with serum 25(OH)D concentrations. Although statistically not significant, positive association was noted between osteocalcin levels with calcium intake [$\beta = 0.018$, 95% CI: -0.003 to 0.039, $p = 0.091$], milk intake [$\beta = 0.032$, 95% CI: -0.001 to 0.065, $p = 0.058$] and Ca: P ratio [$\beta = 23.579$, 95% CI: -0.614 to 47.771, $p = 0.056$]. Phosphorus intake was not associated with osteocalcin.

Discussion

This study highlights the high prevalence of vitamin D insufficiency and deficiency among young urban women in Bangalore, South India. Nearly 85% of the participants screened had serum 25(OH)D levels less than 20 ng/mL, despite the region's tropical climate and year-round sunlight. These findings align with recent studies in India, which reported a high vitamin D deficiency prevalence of 58% – 85% among children, adolescents and adults^{30,31}. The high prevalence observed in our study can be attributed to multiple factors. Only 50% of the participants in the study reported 5–15 min of daily sun exposure, whereas 25% reported less than 5 min per day of direct sun exposure. These findings are similar to earlier reports of inadequate sun exposure because of lifestyle choices, indoor occupations and cultural clothing practices⁴. In southern India, optimal cutaneous vitamin D synthesis occurs between 11 AM and 2 PM³². Sun exposure for 30–45 min on 12–18% of the body surface can generate 600–1000 IU of vitamin D, which is equivalent to the amount needed to meet daily requirements³³. However, nearly 75% of the participants had sun exposure less than 15 min per day, and only 54% of the participants reported sun exposure between 11 AM and 2 PM. Another factor that could have contributed to lower exposure is that the data collection took place largely between the monsoon season and early winter (August–December 2021), with higher cloud cover and lower UV-B availability, contributing to inadequate vitamin D synthesis³⁴.

Variables	Participants included in baseline assessment (n = 108)
Biochemical bone markers ¹	
PTH serum (pg/mL)	47.83 ± 17.82
BSAP serum (U/L)	20.21 ± 9.63
Osteocalcin serum (ng/mL)	25.02 ± 9.10
DEXA-total body (ancillary results) ²	
BMD total body (g/cm ²)	1.09 ± 0.08
Young adult T-score (SD) total body ³	0.10 ± 0.79
Age-matched Z-score (SD) total body ³	0.51 ± 0.82
BMC (g)	1968.69 ± 219.00
Daily usual nutrient and food group intake ¹	(n = 106)*
Nutrients	
Energy (kcal/day)	1565.70 ± 267.70
Protein (g/day) ⁴	51.90 ± 8.50
Carbohydrate (g/day)	228.33 ± 46.50
Fat (g/day)	49.90 ± 12.30
Calcium (mg/day) ⁴	480.60 ± 117.70
Phosphorus (mg/day) ⁴	1067.40 ± 177.30
Vitamin D (µg/day) ⁴	1.14 ± 0.99
Food groups	
Cereals (g/day)	180.70 ± 29.60
Legumes (g/day)	33.60 ± 11.80
Vegetables (g/day)	157.90 ± 40.50
Fats and Oils (g/day)	26.00 ± 8.50
Milk (g/day)	152.90 ± 92.83
Nonvegetarian (Meat/Poultry/Fish/Egg) (g/day) ⁵	0.68 ± 0.47
Sun exposure habits n(%)	(n = 106)*
Average daily sun exposure in the past 4-weeks ²	
< 5 min per day	27 (25.00)
5–15 min per day	54 (50.00)
15–30 min per day	21 (19.40)
> 30 min per day	4 (3.70)

Table 2. Participant baseline biochemical bone markers, bone mineral density, dietary intake, and sun exposure habits (n = 108). ¹Values are expressed as the means ± SDs for continuous variables. ²Values are expressed as numbers (percentages, %) for categorical variables. ³Values for YA-T score: number of SDs a participant's total body BMD deviation from the peak bone mass of a healthy young adult (age ≥ 20 years; n = 92); values for AM Z score (total body): number of SDs a participant's total body BMD differs from that of the age-matched reference population (age < 20 years; n = 16). ⁴Reference values for estimated average requirements (EAR)/day for a sedentary adult Indian woman: protein = 36 g; calcium = 800 mg; phosphorus = 600 mg; vitamin D = 10 µg. ⁵Nonvegetarian (meal/poultry/fish/egg) intake (g/day) was computed by aggregating the frequencies of all nonvegetarian food items to derive a consolidated value. *Two out of 108 participants (study dropouts) did not complete the diet recall and sun exposure questionnaires; hence, the sample size for these variables is n = 106. PTH parathyroid hormone, BSAP bone-specific alkaline phosphatase, DEXA dual-energy X-ray absorptiometry, BMD bone mineral density, YA young adult, AM age-matched, BMC bone mineral content.

The significant inverse correlation observed between 25(OH)D and PTH is consistent with vitamin D deficiency³⁵. When vitamin D levels are low, PTH secretion increases to maintain calcium homeostasis by enhancing bone resorption and renal activation of 25(OH)D to 1,25(OH)₂D. Chronic PTH elevation is associated with increased bone turnover, reduced BMD and increased fracture risk in vulnerable populations^{36,37}. Maintaining adequate vitamin D levels is therefore crucial for suppressing excessive PTH level and preserving bone health, especially in younger women at risk of early osteopenia or osteoporosis. An inverse correlation between osteocalcin and 25(OH)D is also suggestive of increased bone turnover in vitamin D-deficient individuals. Higher osteocalcin levels were also associated with lower BMD supporting previous findings that elevated bone turnover is related to lower bone mass in vitamin D-deficient populations³⁸.

In addition to physiological factors, the dietary intake of calcium, phosphorus and vitamin D is a key determinant of bone health. Inadequate dietary intake is a major contributor to poor vitamin D status. In this study, the mean usual calcium intake was 480.6 ± 117.7 mg/day, which is well below the EAR of 800 mg/day for Indian adult women²⁶. Based on the intake distribution (mean ± 2SD), over 95% of the participants had calcium

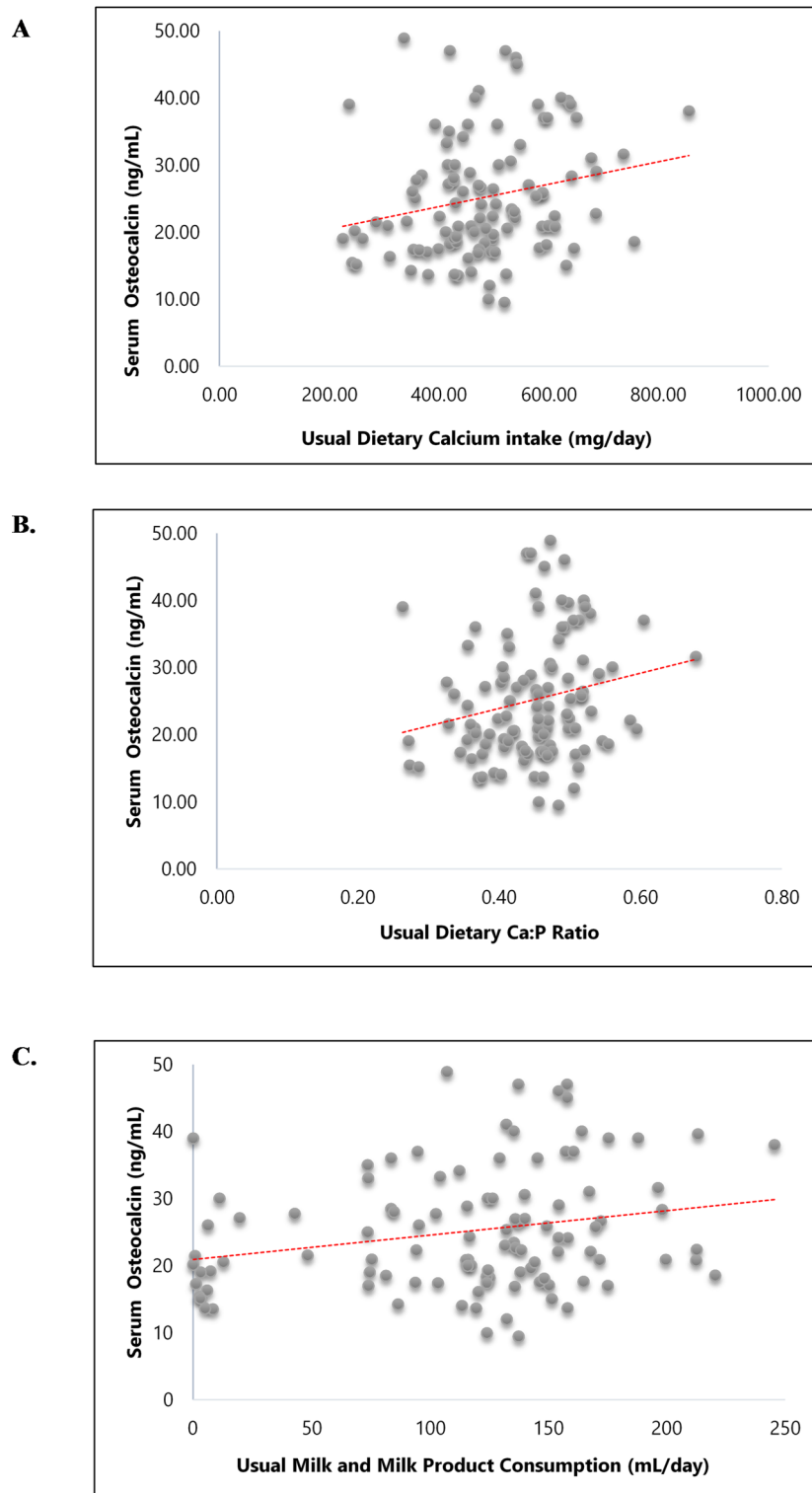


Fig. 1. Correlation between serum osteocalcin levels and dietary variables among vitamin D-deficient and vitamin D-insufficient women. **(A)** Correlation between usual dietary calcium intake (mg/day) and serum osteocalcin levels (ng/mL). **(B)** Correlation between usual dietary calcium-to-phosphorus (Ca: P) intake ratio and serum osteocalcin levels (ng/mL). **(C)** Correlation between usual dairy (milk and milk product) intake (mL/day) and serum osteocalcin levels (ng/mL). Each dot represents one participant ($n = 106$). Red dashed lines indicate linear regression fits. *Ca: P* calcium-to-phosphorus ratio.

Independent variables ²	Serum 25(OH)D ¹				Serum osteocalcin ¹			
	β^*	SE \dagger	95% CI for β	<i>p</i> value	β^*	SE \dagger	95% CI for β	<i>p</i> value
Calcium intake (mg)	-0.004	0.004	[-0.012, 0.004]	0.268	0.018	0.011	[-0.003, 0.039]	0.091
Phosphorus intake (mg)	-0.001	0.005	[-0.010, 0.009]	0.874	-0.004	0.014	[-0.030, 0.023]	0.786
Ca: P Ratio	-6.550	4.742	[-15.955, 2.856]	0.170	23.579	12.197	[-0.614, 47.771]	0.056
Milk and Milk Products (g)	-0.008	0.006	[-0.020, 0.005]	0.243	0.032	0.017	[-0.001, 0.065]	0.058

Table 3. Association between dietary intakes with serum 25(OH)D and osteocalcin concentrations.

¹Dependent variable included in the analysis. ²Independent variables were adjusted for age (years, continuous) and usual energy intake (kcal/day, continuous). *Regression coefficient (B); standard error (SE \dagger). 25(OH)D 25-hydroxyvitamin D, CI confidence interval, B unstandardized regression coefficient, SE standard error, Ca: P calcium-phosphorus ratio.

intakes between 245 and 716 mg/day, indicating widespread inadequacy. This aligns with global and regional estimates indicating that many countries in South and East Asia, including India, have average calcium intakes well below recommended levels, often < 500 mg/day³⁹. Low calcium intake in India is partly due to limited dairy consumption, which is shaped by traditional dietary habits, economic constraints and intrahousehold food distribution patterns. Unlike Western populations that rely on dairy, Indian diets are predominantly plant-based and often high in phytates and oxalates, which inhibit calcium absorption. Given the role of calcium in bone health, particularly in vitamin D-deficient individuals, these findings highlight the need for dietary diversification and fortification³⁹. The calculated Ca: P ratio in this study was 0.5:1, reflecting a diet skewed towards phosphorus-rich foods, likely due to the high intake of cereals and legumes. Typical Indian staples such as wheat, rice bran, maize, millet and lentils contribute to high phosphorus intake, which, when coupled with low calcium intake, has been linked to adverse bone health outcomes⁴⁰. Studies have demonstrated that diets with a low Ca: P ratio lead to increased PTH secretion, which can result in bone demineralization and altered calcium homeostasis^{40,41}. To mitigate these effects, adequate calcium intake and a balanced Ca: P ratio, such as that naturally found in dairy products, are recommended⁴². Although statistically not significant, a positive association was noted for calcium intake and osteocalcin suggesting a possible role of calcium in regulating bone turnover in these vitamin D deficient/in sufficient women. Furthermore, in our study population, dietary vitamin D intake was critically low (45.6 ± 39.6 IU/day) compared with the EAR of 600 IU/day, reaffirming previous findings that dietary food sources contribute minimally to vitamin D status in Indian populations¹⁰.

In our study, the average intake of milk and milk products was only 152 ml/day, which is usually diluted prior to consumption, further highlighting the inadequate contribution of dairy to total calcium and vitamin D intake in this population. While non-dairy sources such as finger millet (*Eleusine coracana* L.), which contains 344 mg of calcium per 100 g, is one alternative, their bioavailability is reduced by antinutritional factors such as phytates (0.48%), polyphenols, tannins (0.61%) and trypsin inhibitors⁴³. As a result, relying solely on plant-based sources may be insufficient to meet daily calcium requirements. These findings underscore the need for targeted public health strategies that include dietary diversification, food fortification and awareness campaigns to improve calcium and vitamin D status. The strengths of the study includes the use of both standardized biomarker assays and dietary intake methods, and its focus on young Indian women at a critical stage of peak bone mass. This study provides important insights into the interplay between vitamin D deficiency, dietary intake and bone health, highlighting areas for future research and intervention. The findings from this study would help in designing more detailed research by incorporating diverse population groups age categories, occupation, or lifestyle, and include more outcome measures to explore new patterns or relationships.

Study limitations

While the study offers important insights, it is not without limitations. A key limitation is that participants were recruited on campus primarily students due to COVID-19 restrictions during Bangalore's second wave, which limited screening to onsite locations and prevented broader recruitment. This may have introduced homogeneity in dietary calcium intake, as hostel residents typically consumed meals from the same facilities. Furthermore, as the study population comprised predominantly young female students from a single urban institution, their dietary patterns, lifestyles, and sun exposure practices may not represent those of women in other settings, such as rural areas, older age groups, or different socio-economic strata. This may limit the generalizability of findings to the broader population and therefore should be interpreted with caution. Another limitation is the reported dietary recalls and sun exposure questionnaires, which may introduce recall bias and measurement error. To improve accuracy, three non-consecutive 24-hour recalls with standardized portion-size tools were collected, and nutrient intakes were computed using a validated Indian dietary assessment software²⁵ together with the ICMR-NIN 2020 food composition Table²⁶. Sun exposure was assessed using a structured questionnaire adapted from Patwardhan et al.²⁷. Nevertheless, these tools were not formally validated in this cohort. Another limitation relates to the bone mineral density measurements. T- and Z-scores were generated using the GE Lunar Prodigy Advance system reference database (USA Combined NHANES/Lunar, Female, v113), which is derived predominantly from Caucasian populations. As there is no normative database currently available for Indian women, the use of this reference may have led to under- or overestimation of BMD status in our study population. Lastly, the study lacked direct UV-B exposure quantification, which would have provided

an assessment of sunlight exposure and its contribution to vitamin D synthesis. Similarly physical activity and socio-economic status was not assessed.

Conclusions

Our findings highlight widespread vitamin D insufficiency and deficiency coupled with low calcium intake among young urban Indian women. The observed associations between osteocalcin, dietary calcium and milk intake suggest that dietary patterns may influence bone health in vitamin D-deficient populations. While sunlight remains the most potent source of vitamin D, factors such as lifestyle, clothing practices and urbanization limit sunlight exposure, making dietary intake and fortification critical for bridging this gap. Strategies such as increasing dairy consumption, improving the Ca: P balance in diets and promoting safe sun exposure habits could be explored in future research to help mitigate the high prevalence of deficiency and its associated health risks.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Chandni Peris (CP) collected and curated the data, conducted the investigation, and prepared the original draft of the manuscript. Sumithra Selvam (SSelvam) was responsible for the formal analysis and contributed to the data visualization. Prashanth Thankachan (PT) contributed to the study methodology, supervised the research, and managed project administration. Sumathi Swaminathan (SSwaminathan), PT, Ravi Rangarajan (RR), Tony Raj (TR), and SSelvam contributed to the review and editing of the manuscript. TR provided oversight, secured resources, and acquired funding for the study. PT, RR, and TR jointly conceptualized and designed the study. SSelvam, SSwaminathan, and CP contributed to data visualization. All the authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

This study adhered to the principles outlined in the Declaration of Helsinki, with approval from the Institutional Review Board (IRB) of St. John's Medical College and Hospital, Bangalore, India (protocol code

IEC Study Ref No: 370/2018; date of approval: 7 January 2019), for all procedures involving participants. Written informed consent was obtained from all individuals involved in the study.

Additional information

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