RESEARCH PAPER

Impact of Two Regimens of Vitamin D Supplementation on Calcium - Vitamin D - PTH Axis of Schoolgirls of Delhi

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Objective: To determine the efficacy of supplementation with oral vitamin D_3 (cholecalciferol) on bone mineral biochemical parameters of school-going girls.

Setting: Government school (government-aided) and Private school (fee paying) in Delhi.

Design: Randomized controlled trial.

Intervention: Cholecalciferol granules (60,000 IU) orally with water, either once in two months (two-monthly D_3 group) or once a month (one-monthly D_3 group) for one year.

Participants: 290 healthy schoolgirls (6-17 y), 124 from lower socioeconomic strata (LSES) (attending government schools) and 166 from upper socioeconomic strata (USES) (attending private schools).

Outcome measures: Serum 25(OH)D, calcium, phosphorus, parathyroid hormone, and alkaline phosphatase levels at 6 and 12 months after start of supplementation.

Results: At baseline, 93.7% schoolgirls were vitamin D deficient [25(OH)D<50 nmol/L]. While significant increase in serum calcium and decrease in alkaline phosphatase levels was noted in both groups with both interventions, PTH response was inconsistent. In LSES subjects, two-monthly D_3 and one-monthly D_3 supplementation resulted in a significant increase in serum 25(OH)D levels by 8.3 nmol/L and 11.0 nmol/L, respectively at 6 months ($P<0.05$). Similarly, the increase in the two intervention arms in USES subjects was 10.5 nmol/L and 16.0 nmol/L, respectively ($P<0.05$). In both groups, this increase in serum 25(OH)D levels persisted at 12 months ($P<0.05$). Despite supplementation with 60,000 IU of Vitamin D_3 (monthly or two-monthly), only 47% were vitamin D sufficient at the end of one year.

Conclusions: 60,000 IU of cholecalciferol, monthly or two-monthly, resulted in a significant increase in serum 25(OH)D levels in vitamin D deficient schoolgirls.

Key words: *Cholecalciferol, India, Supplementation, Schoolgirls, Vitamin D.*

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Adequate vitamin D status for optimum bone health has received increased recognition in recent years. Vitamin D insufficiency has been reported in healthy children, adolescents and adults worldwide(1), which has been attributed to low vitamin D intake and inadequate sunlight exposure(2,3). Rickets is seen in children with severe vitamin D deficiency, but previous studies have shown that even mild vitamin D insufficiency can have detrimental effects on bone mineral acquisition(4,5) and bone remodeling(6,7) in adolescence. Nutrition guidelines for optimizing bone health in children and adolescents have focused on calcium and exercise, but have neglected vitamin D(8,9). Furthermore, the optimal serum 25(OH)D concentrations for children and adolescents is still a subject of debate. While most professional bodies have recommended a daily vitamin D intake of 10mg(10), the Indian Council of Medical Research has made no recommendation for India, with the view that adequate sunlight exposure would provide the necessary daily vitamin D requirement(11). However, increasing awareness of wide prevalence of vitamin D deficiency in Asian Indian adults(12-17) and children(18-20) has prompted a renewed discussion on the desired vitamin D intake to ensure optimal bone health.

In the absence of results of vitamin D fortification studies, it is not possible to evaluate the merits of food fortification relative to the systemic use of vitamin D containing dietary supplements(21). While several intervention trials have evaluated the role of calcium on the bone health of growing children and adolescents(22,23), infor-mation on the effect of vitamin D supplementation remains limited. We planned this study with the primary objective to assess the impact of oral vitamin D_3 (cholecalciferol) supplementation for one year, on bone mineral biochemical parameters, in healthy school-going girls (6-17 years) from two different socioeconomic strata, residing in Delhi, India.

METHODS

Subjects

Three hundred and fifty five healthy school girls, aged 6-17 years, who responded to a request to participate, were recruited from two schools of Delhi, which were located in geographic proximity to the principal investigator's institution. Socioeconomic stratification of the subjects was based on the type of school attended. Girls studying in the government-aided school were considered to represent the lower socioeconomic strata (LSES, *n*=165), while those enrolled in the fee-paying private school represented the upper socioeconomic strata (USES, *n*=190). A convenient sample was selected because available information in literature was limited and was based on populations receiving food items fortified with vitamin D and with different baseline 25(OH)D levels. Children with systemic illness, endocrine disorders and drugs affecting bone mineral health were excluded. Each class in a school had 5 sections, and one section from each class was randomly selected. Of the 355 girls screened, 290 girls were enrolled for the intervention after excluding 65 girls. These 290 subjects underwent baseline assessment in summer months (July-August, 2006).

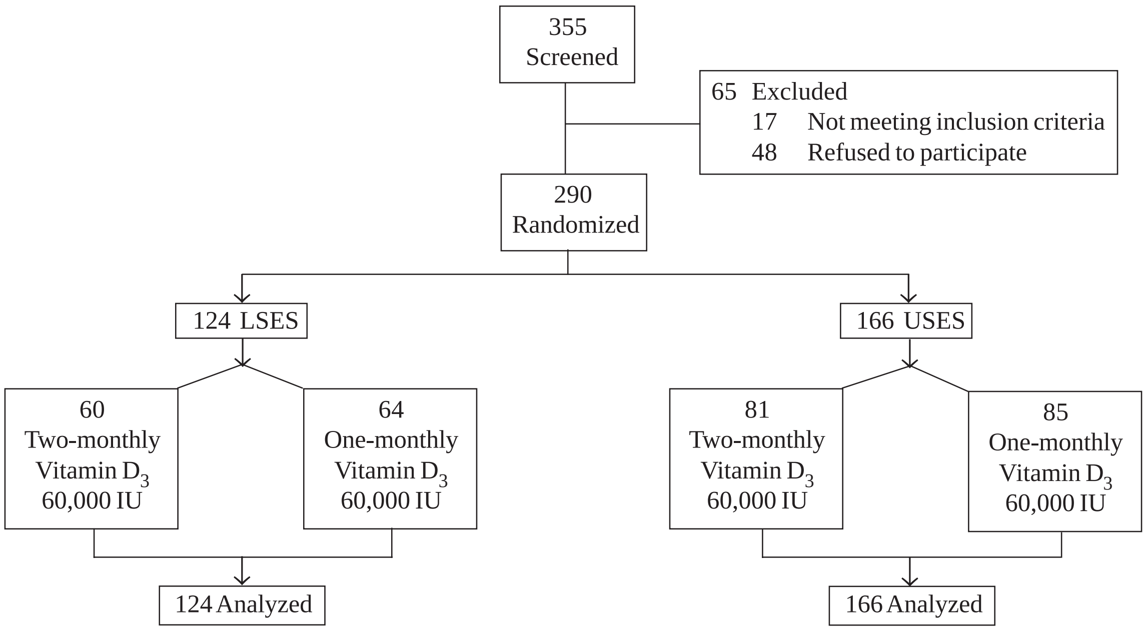
The study protocol was approved by the institutional ethics committee of the Institute of Nuclear Medicine and Allied Sciences (INMAS). A prior written consent for the study was taken from the school administration and from the parents. The details of the study design are shown in ***Fig* 1**.

Data collection

Anthropometric measurements and dietary infor-mation were collected from the subjects at baseline. Standing height was recorded to the nearest 1mm without wearing shoes and by using a wall stadiometer. Weight was recorded to the nearest 0.1kg by using a clinical balance, with the subject wearing light clothing. Body mass index was calculated using the formula weight (in kg) / height (in m^2). Every morning, the scale and stadiometer were calibrated with standard weight and height, respectively.

Dietary information was collected using 24 hour dietary recall and food frequency questionnaire (FFQ). The daily intake of various food groups (cereals, pulses, fruits, vegetables, milk and milk products, animal foods and fat) was determined using standardized recipes of the food preparations(25). The dietary intake of nutrients was calculated as per ICMR recommendations(11,26). Calculations for vitamin D intake were based on US Department of Agriculture tables(27).

Blood samples were collected from subjects in the fasting state at 0800 hours without venostasis under basal conditions for estimation of serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), 25-hydroxyvitamin D [25(OH)D], and immunoreactive parathyroid hormone (PTH) prior to initiating intervention. Serum calcium, phosphorus, and ALP were estimated on the same day, two aliquots were stored at –20ºC until PTH and 25(OH)D were estimated. Serum calcium (Randox Laboratory Ltd, Crumlin, UK) and phosphorus (Clonital; Ampli Medical SPA, Milan, Italy) were measured by colorimetric method and ALP by liquid kinetic method (Clonital; Ampli Medical SPA, Milan, Italy). Normal laboratory range for serum



LSES: Lower socioeconomic strata; USES: Upper socioeconomic strata

FIG.1 *Study design.*

calcium was 2.02-2.60 mmol/L (8.10-10.40 mg/dL) and for serum phosphorus in adults was 0.81-1.55 mmol/L (2.5-4.8 mg/dL), according to the kit manufacturers. The reported upper limit of serum P in mid-childhood is 1.87 mmol/L (5.8 mg/dL)(28). Normal laboratory range for serum ALP at 37ºC was 100-275 IU/L in adults and 180-1200 IU/L in children before epiphyseal closure.

Serum iPTH was measured by an immunoradiometric assay (Diasorin, Stillwater, Min. U.S.A.; normal range13-66 pg/mL, intra and inter-assay CV 4% and 8%, respectively). Serum concentration of 25(OH)D were estimated by radioimmunoassay (Diasorin; reference range 22.4-93.6 nmol/L (9.0-37.6 ng/mL). The lowest concentration of 25(OH)D measurable by this kit, defined as the lowest quantity differentiated from zero at 2 SDs below the mean counts per min of the zero standard, is 3.7 nmol/L (1.5 ng/mL). Hypovitaminosis D was defined as serum concentration of 25(OH)D below 50 nmol/L (20 ng/mL).

Intervention

Supplementation was initiated in the winter (December, 2006) of the same year for all subjects and was carried out for a period of one year. The subjects were randomized to two intervention groups receiving either 60,000 IU of cholecalciferol granules (Calcirol, Cadila Pharmaceuticals, Ahmedabad) every two months (two-monthly D_3 group; equivalent to 1000 IU/day) or every month (one-monthly D_3 group; equivalent to 2000 IU/day), for a period of one year. These doses represent 25% and 50%, respectively of the lowest adult dose considered likely to cause adverse effects(29).

The cholecalciferol granules were administered at the study site, under the direct supervision of an investigator to ensure compliance. All cholecalciferol sachets were from one batch of manufacture. The cholecalciferol granules were administered orally by the investigator followed by a drink of 100 mL of water to facilitate swallowing. In view of our earlier studies consistently showing high prevalence of hypovitaminosis D(20,21), and limitation of funds, it was decided not to include a separate group receiving placebo. Estimation of serum calcium, phosphorus, ALP, 25(OH)D and PTH were repeated at 6 and 12 months after initiating intervention. Baseline and 6 month post-supplementation values were taken in summer months, while 12 month post-supplementation samples were collected in winter months.

Statistical Analysis

Statistical analysis was carried out using STATA 9.0 (College Station, Texas, U.S.A.). Baseline data are presented as mean (SD) or number (percentage) as appropriate. Effect of vitamin D_3 supplementation (once in two months and once a month) on serum Ca, P, ALP, 25(OH)D and PTH was analyzed using generalized estimating equation (GEE). *P* values <0.05 were considered significant.

RESULTS

Baseline characteristics from each socioeconomic stratum randomized to the two intervention groups are presented in ***Table* I**. The two groups were comparable for age. The height, weight and BMI of USES subjects were significantly higher than LSES subjects at all ages. USES subjects had significantly higher dietary intake of energy, protein, fat, calcium, phosphorus and vitamin D than LSES subjects, while they had a significantly lower intake of carbohydrate, phytate and fiber than LSES

TABLE I BASELINE ANTHROPOMETRIC AND DIETARY PARAMETERS OF THE STUDY SUBJECTS [MEAN(SD)]

	LSES		USES	
Variable	Two-monthly D_3 group (*n*=60)	One-monthly D_3 group (*n*=64)	Two-monthly D_3 group (*n*=81)	One-monthly D_3 group (*n*=85)
Anthropometric				
Age	12.0 (2.8)	11.4 (3.0)	11.6 (2.7)	11.7 (2.8)
Height (cm)	138.2 (12.4)	136.8 (14.3)	146.2 (14.9)	144.7 (13.3)
Weight (kg)	32.7 (10.1)	31.5 (11.0)	39.3 (12.8)	39.9 (11.6)
Height (*Z* score)*	–0.9 (1.0)	–0.6 (0.9)	0.7 (0.9)	0.3 (0.9)
Weight (*Z* score)*	–0.7 (0.7)	–0.6 (0.7)	0.4 (0.9)	0.4 (0.9)
BMI (kg/m^2)	16.6 (2.8)	16.3 (3.0)	17.8 (3.2)	18.6 (3.3)
Dietary Intake				
Energy (Kj)	5324.1 (758.3)	5426.1 (738.6)	5758.4 (594.8)	5817.3 (726.9)
Protein (g)	36.1 (6.6)	36.5 (6.6)	43.2 (6.7)	43.9 (7.5)
Carbohydrate (g)	195.7 (32.8)	195.7 (30.9)	189.5 (28.2)	193.1 (32.1)
Fat (g)	37.8 (7.9)	39.5 (7.8)	49.3 (5.8)	48.8 (7.1)
Dietary fiber (g)	12.6 (7.5)	12.8 (6.7)	9.6 (6.7)	9.3 (5.8)
Phytate (mg)	97.2 (57.9)	99.6 (57.3)	74.8 (49.4)	86.4 (53.5)
Calcium (mg)	480.8 (191.4)	456.3 (170.4)	707.3 (162.9)	670.5 (180.1)
Phosphorous (mg)	863.5 (174.5)	850.4 (165.2)	976.3 (141.5)	955.4 (174.4)
Vitamin D (μg)	1.7 (1.3)	1.5 (1.2)	3.0 (1.3)	2.8 (11.4)
Cereals (g/d)	190.2 (38.0)	194.5 (41.5)	147.1 (38.4)	162.7 (44.9)
Pulses (g/d)	29.6 (23.1)	34.2 (22.0)	41.4 (25.3)	35.3 (23.4)
Vegetables (g/d)	95.8 (54.8)	71.1 (39.0)	108.8 (48.9)	110.5 (54.3)
Fruits (g/d)	20.9 (6.2)	23.0 (6.6)	98.4 (79.0)	75.5 (58.3)
Milk (g/d)	234.1 (135.4)	212.2 (108.1)	379.4 (122.4)	361.5 (138.6)
Animal foods (g/d)	1.7 (7.8)	3.2 (11.4)	9.7 (23.2)	17.5 (29.6)
Fats (g/d)	20.9 (6.2)	22.9 (6.6)	23.4 (6.1)	23.9 (6.2)

*LSES: Lower socioeconomic strata; USES: Upper socioeconomic strata; BMI: Body Mass Index; *Height and weight Z scores were calculated based on the reference values provided by Agarwal, et al.(24).*

subjects. USES had significantly higher consumption of pulses, milk, animal foods, fruits and fat in their daily diets as compared to LSES girls where cereals formed the major constituent; nevertheless both the groups had daily intakes less than the ICMR recommendations.

The effect of vitamin D supplementation on biochemical and hormonal parameters is depicted in ***Table* II**. At baseline, 15.3% LSES and 0.6% USES subjects, respectively(P=0.001), had serum calcium values below the kit normal range. While serum calcium improved with vitamin D supplementation, there was no significant difference in the effect of the two intervention regimens on serum calcium within LSES and USES groups.

Elevated ALP (>1200 IU/L) was noted in 1.6% LSES subjects and none of the USES subjects. ALP levels decreased significantly after supplementation at 6 and 12 months with reference to baseline in both intervention groups and in both SES subjects (***Table* II**). Further, no significant difference was found between the two intervention arms in both LSES and USES.

In the study population, 93.7% girls (97.5% vs. 90.9% in LSES and USES, respectively) were found to be vitamin D deficient at baseline. In LSES group, this prevalence declined from 98% to 74% to 38% in two-monthly D_3 group and from 97% to 69% to 28% in one-monthly D_3 group at 6 and 12 months post-supplementation, respectively. However, in USES strata, the percentage of vitamin D deficient girls decreased from 94% to 84% to 80% in two-monthly D_3 group and 88% to 68% to 57% in one-monthly D_3 group at 6 and 12 months of supplementation, respectively.

Mean serum PTH noted at initiation of study in LSES and USES subjects was 37.14 (SD 18.77) pg/mL and 34.70 (SD 20.84) pg/mL, respectively (P=0.30). A similar proportion of LSES (8.1%) and USES (7.2%) subjects had elevated PTH values.

DISCUSSION

Several studies from across the world, including India, have shown a high prevalence of vitamin D deficiency in children and adolescents(1,20,21). In India, where there is no fortification of food with vitamin D, supplementation remains an important alternative for improving the vitamin D status of individuals. As reviewed by Vieth(30), there are several studies evaluating the efficacy of vitamin D supplementation in adults. However, limited information is available assessing the impact of vitamin D supplementation on bone mineral parameters in children and adolescents(31-39).

As reviewed recently, most experts agree that in the absence of adequate sun exposure, children and adults need 800-1000 IU of vitamin D/day(1). Since data from several workers in India suggest a high prevalence of vitamin D deficiency(12-21), we considered evaluating two dose strengths; namely, the equivalent of 1000 units/day and 2000 units/day. Oral vitamin D_3 supplementation with a dose equivalent to 1000-2000 units/day for 1 year was safe, and increased serum 25(OH)D concentrations significantly in both upper and lower socio-economic strata. However, despite the supplementation, only 47% of schoolgirls studied became vitamin D sufficient. The mode and frequency of administration of vitamin D_3, and the extent and possible chronicity of hypovitaminosis D in the study population may in part explain this observation.

Two important observations need to be highlighted. It is an established fact that serum 25(OH)D levels in volunteers in Delhi are lower when measured in winter as compared with summer(17). This anticipated decline in serum 25(OH) D levels during winter was overcome by the vitamin D supplementation, especially in the LSES group. Secondly, monthly supplementation (equivalent to 2000 IU D_3/day) was superior to two-monthly supplementation (equivalent to 1000 IU D_3/day), though this effect was significant only in the USES.

The superiority of a higher dose of vitamin D was also reported by Fuleihan, *et al*.(34) and Maalouf, *et al*.(36) comparing 200 IU *vs*. 2000 IU of vitamin D/day, and by Viljakainen, *et al*.(35), comparing 200 IU *vs*. 400 IU of vitamin D per day. Maalouf, *et al*.(36) reported that in children and

TABLE II EFFECT OF VITAMIN D SUPPLEMENTATION ON SERUM CALCIUM, PHOSPHOROUS, ALKALINE PHOSPHATASE, 25(OH)D AND PARATHYROID HORMONE

	Lower socioeconomic group					Upper socioeconomic group				
Parameter [Mean (SE)]	Two-monthly D_3 group (*n*=60)	One-monthly D_3 group (*n*=64)	Mean difference	(95% CI)	*P* value	Two-monthly D_3 group (*n*=81)	One-monthly D_3 group (*n*=85)	Mean difference	95% CI	*P* value
Serum calcium (mmol/L)										
Baseline	2.23(0.03)	2.25(0.02)	-0.01	(-0.09, 0.06)	0.68	2.30(0.01)	2.32(0.01)	-0.01	(-0.04,0.01)	0.19
6 mo	2.28(0.02)	2.32(0.02)*	-0.04	(-0.11, 0.01)	0.17	2.50(0.01)*	2.50(0.01)*	-0.01	(-0.04, 0.03)	0.76
12 mo	2.52(0.01)$^{\#,\$}$	2.53(0.01)$^{\#,\$}$	-0.01	(-0.04, 0.01)	0.37	2.61(0.01)$^{\#,\$}$	2.63(0.01)$^{\#,\$}$	-0.01	(-0.03, 0.01)	0.30
Serum phosphorous (mmol/L)										
Baseline	1.55(0.04)	1.50(0.03)	0.05	(-0.05, 0.1)	0.35	1.37(0.02)	1.35(0.020	0.02	(-0.04, 0.08)	0.51
6 mo	1.47(0.04)	1.49(0.03)*	-0.02	(-0.124, 0.086)	0.72	1.43(0.02)*	1.44(0.02)*	-0.01	(-0.07, 0.05)	0.80
12 mo	1.56(0.03)$^{\$}$	1.59(0.03)$^{\#,\$}$	0.03	(-0.121, 0.064)	0.55	1.47(0.02)$^{\#,\$}$	1.45(0.02)$^{\#}$	0.03	(-0.04, 0.09)	0.43
Serum alkaline phosphatase (IU/L)										
Baseline	576.38(34.62)	506.29(27.16)	70.08	(-16.16, 156.3)	0.11	353.82(18.10)	371.13(17.70)	-17.30	(-66.9, 32.3)	0.49
6 mo	375.46(21.65)*	361.78(20.46)*	13.67	(-44.72, 72.07)	0.65	213.40(13.12)	220.81(13.40)	-7.45	(-44.27, 29.36)	0.691
12 mo	283.22(17.55)$^{\#,\$}$	269.60(15.25)$^{\#,\$}$	13.61	(-31.97, 59.19)	0.56	222.70(13.60)	204.83(11.40)	-17.85	(-16.8, 52.56)	0.313
Serum 25(OH)D(nmol/L)										
Baseline	31.20(1.68)	32.93(1.37)	-1.72	(-5.98, 2.53)	0.43	29.13(1.54)	30.80(1.39)	-1.66	(-5.74, 2.41)	0.426
6 mo	39.53(2.01)*	43.90(1.50)*	-4.36	(-9.30, 0.56)	0.08	39.55(1.24)*	46.81(1.45)*	-7.25	(-11.00, -3.51)	0.001
12 mo	53.0(3.05)$^{\#,\$}$	59.33(2.64)$^{\#,\$}$	-6.34	(-14.27, 1.58)	0.12	38.25(2.13)$^{\#}$	49.94(2.01)$^{\#}$	-11.69	(-17.44, -5.95)	0.001
Serum parathyroid hormone (pg/mL)										
Baseline	36.41(2.63)	37.64(2.19)	-1.23	(-7.95, 5.48)	0.72	34.40(2.00)	34.98(2.51)	-0.58	(-6.88, 5.72)	0.86
6 mo	29.10(2.35)*	30.87(1.82)*	-1.77	(-7.61, 4.05)	0.55	26.90(1.77)*	28.35(1.74)*	-1.44	(-6.31, 3.40)	0.56
12 mo	60.81(4.07)$^{\#,\$}$	55.96(3.08)$^{\#,\$}$	4.84	(-5.16, 1.48)	0.34	34.66(2.54)$^{\$}$	35.01(2.58)$^{\$}$	-0.35	(-7.45, 6.75)	0.92

*LSES: Lower socioeconomic strata; USES: Upper socioeconomic strata; Two-monthly D_3 group: Vitamin D (60,000 IU) once in two months; One-monthly D_3 group: Vitamin D (60,000IU) once a month; * = $P<0.05$ for baseline vs. 6 month; $^{\#}$ = $P<0.05$ for baseline vs. 12 month; $^{\$}$ = $P<0.05$ for 6 months vs. 12 month.*

WHAT THIS STUDY ADDS?

- 60,000 IU of cholecalciferol, monthly or two-monthly, results in a significant increase in serum 25(OH)D levels in vitamin D deficient school girls.

adolescents with serum 25(OH)D concentration below 20 ng/mL (*i.e.* <50 nmol/L), a vitamin D_3 dose equivalent to 2000 IU/day resulted in desirable vitamin D levels. In a study assessing only winter time vitamin D_2 supplementation in Finnish girls, prevalence of hypovitaminosis D was significantly reduced in those receiving vitamin D(4). The supplementation had more effect on those with severe hypovitaminosis D than those with normal vitamin D levels at baseline. In a similar study assessing the impact of winter time supplementation of vitamin D_3 in French male adolescents, Guillemant, *et al*.(31), reported that a monthly dose of 50,000 IU was adequate to prevent the decline in serum 25(OH)D levels usually observed in winter.

The increment in serum 25(OH)D levels in response to monthly D_3 supplementation in the present study was significantly less than that reported by Maalouf, *et al*.(36), using a comparable dose of D_3. The possible reasons for the differences in outcome between the two studies could be as follows: use of oil containing vitamin D_3 preparation by Maalouf, *et al*.(36), in contrast to the direct ingestion of granulated vitamin D_3 followed by water in our study; weekly delivery versus monthly delivery of vitamin D_3; and, differences in the mean baseline calcium intake and severity of hypovitaminosis D in the study population.

Vitamin D supplementation resulted in a significant increase in serum calcium in both intervention arms (two-monthly and one-monthly D_3 groups) of both socioeconomic strata. This has also been reported by other workers(33,35). Similarly, both interventions resulted in a decline in ALP in both SES, with the maximum decline observed at 6 months in both groups. However, Maalouf, *et al*.(36) also showed a significant reduction in ALP at 12 months with both low (200 IU/day) and high (2000 IU/day) doses of vitamin D_3 but the reduction at 6 months was not significant. In contrast, Rajakumar, *et al*.(33) showed decline in ALP with a lower vitamin D dose of 400 IU/day, after one month of intervention.

The inconsistent PTH response showing a decline at 6 months, but tendency to rise at 12 months, underscore the fact that PTH levels are under multi-factorial regulation(40). One possible explanation could be that PTH-mediated raised bone turnover is an essential component of the maturation process during this life stage. In a study involving Finnish adolescents, vitamin D supplementation reduced PTH levels only if the subjects were vitamin D insufficient at baseline(35). In contrast, using a similar dose of vitamin D, Rajakumar, *et al*.(33) showed no effect of vitamin D supplementation on PTH levels, even in those who were vitamin D insufficient. In view of these varied observations, there is a need for more studies to evaluate the response of PTH to vitamin D supplementation in different regimens, age groups and severity of underlying vitamin D deficiency.

Certain limitations of the present study need to be highlighted. Firstly, due to constraints in the study conditions, the cholecalciferol granules had to be given to the subjects with water instead of milk or an oily preparation. Secondly, due to lack of permission from the school authorities, pubertal staging could not be carried out. Thirdly, due to procedural delay, the intervention was initiated in the winter, while the baseline assessment was performed in summer months. Finally, since serum albumin was not measured, we have not provided corrected values of serum calcium. Thus, in the absence of food fortified with vitamin D, monthly supplementation providing the equivalent of 2000 IU of Vitamin D_3/day may be the preferred approach to combat the prevalence of hypovitaminosis D in Indian school girls.

Contributors: RKM and NT: design of the study, collection and analysis of data, and writing of the manuscript; NA: collection and analysis of data and writing of the manuscript; SP: design of the study, analysis

and writing of the manuscript; RA: collection of data and quality assurance; KM: design of the study and analysis of data; and SS: laboratory assays. RKM and NT are to be considered as joint first authors.

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