British Journal of Nutrition (2019), 121, 538–548
doi:10.1017/S0007114518003690

Impact of three different daily doses of vitamin D_3 supplementation in healthy schoolchildren and adolescents from North India: a single-blind prospective randomised clinical trial

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(Submitted 7 April 2018 – Final revision received 27 November 2018 – Accepted 3 December 2018 – First published online 29 January 2019)

Abstract

In India, there is a lack of information about the adequate daily dose of vitamin D_3 supplementation in school children. Hence, we undertook this study to evaluate the adequacy and efficacy of different doses of vitamin D_3 in schoolchildren. A total of 1008 vitamin D-deficient (VDD) children, aged 6–16 years with serum 25-hydroxyvitamin D (25(OH)D) levels <50 nmol/l, were cluster randomised into three groups (A-344, B-341 and C-232) for supplementation (600, 1000 and 2000 IU daily) of vitamin D_3 under supervision for 6 months. Of the 1008 subjects who completed the study, 938 (93 %) were compliant. Baseline and post-supplementation fasting blood and urine samples were evaluated for Ca, phosphates, alkaline phosphatase, 25(OH)D and parathormone and urine Ca:creatinine ratio. The mean age of the subjects was 11·7 (SD 2·4) years, and the overall mean baseline serum 25(OH)D level was 24·3 (SD 9·5) nmol/l. Post-supplementation rise in serum 25(OH)D in compliant group was maximum with 2000 IU (70·0 (SD 30·0) nmol/l), followed by 1000 IU (46·8 (SD 22·5) nmol/l) and 600 IU (36·5 (SD 18·5) nmol/l), and serum 25(OH)D levels of ≥50 nmol/l were achieved in 71·5, 81·8 and 92·9 % by groups A, B and C, respectively. Secondary hyperparathyroidism decreased from 31·7 to 8·4 % post-supplementation. Two participants developed hypercalciuria, but none developed hypercalcaemia. Children with VDD benefit maximum with the daily supplementation of 2000 IU of vitamin D_3. Whether recommendations of 400 IU/d by Indian Council of Medical Research or 600 IU by Indian Academy of Pediatrics or Institute of Medicine would suffice to achieve vitamin D sufficiency in children with VDD remains debatable.

Key words: Vitamin D_3 supplementation: Vitamin D deficiency: Secondary hyperparathyroidism: Children and adolescents

Vitamin D is an important micronutrient required for not only maintaining Ca balance and safeguarding skeletal integrity but also overall health and well-being of all age groups[1]. Presently, vitamin D deficiency (VDD) is recognised as a global epidemic[2,3]. Despite, adequate sunshine throughout the year, VDD has been reported among all age groups and both sexes from different parts of India[4–9]. This has been primarily attributed to poor sun exposure due to cultural avoidance of skin exposure, crowded houses with limited sun exposure, work culture of staying indoors, dark skin complexion, atmospheric pollution, vegetarian foods habits, absence of food fortification with vitamin D and poor intake of vitamin D supplements[4,10]. Though vitamin D is synthesised in the skin on exposure to UV radiation, it is difficult for children to achieve vitamin D sufficiency in all seasons solely through sun exposure, as observed in two of our studies[11,12]. Fortification of

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ALP, alkaline phosphatase; IAP, Indian Academy of Pediatrics; ICMR, Indian Council of Medical Research; IOM, Institute of Medicine; PTH, parathyroid hormone; UCaCrR, urinary calcium:creatinine ratio; VDD, vitamin D deficiency.

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widely consumed staple foods offers a simple, practical, effective and safe alternative for combating VDD and is being practised all over world[13]. The food fortification program in India is still in the stage of infancy[4,14]. Food Safety and Standard Authority of India under section 16(5) of Food Safety and Standards Act (2006) relating to standards for food fortification has permitted voluntary fortification of milk and oil with vitamins A and D vide their letter dated 19 May 2017. Our own study in Indian schoolchildren clearly showed that providing milk fortified with vitamin D is an effective and safe strategy to deal with public health issue[15]. Although there are several studies in literature evaluating the impact of vitamin D_3 supplementation in adults[16], studies in children and adolescents are limited[17–25], particularly from India[26]. Duration of the available studies in children varied from 8 weeks[19,24] to 1 year[21,26], with supplemental doses ranging from 200[21,23] to 60 000 IU[26] administered daily[17,19–24], weekly[19,25], bimonthly[18], monthly[18,26] or once in 2 months[26]. Supplementation with lower doses of 200–600 IU/d did not achieve vitamin D sufficiency in majority of VDD subjects[15,16,27]. Indian Council of Medical Research (ICMR) recommends daily allowance of 400 IU for Indian children and adolescents[28] in contrast to 600 IU/d recommended by the Indian Academy of Pediatrics (IAP)[29] and Institute of Medicine (IOM)[30]. In the absence of information with regard to adequate daily dose of vitamin D_3 required for Indian children with VDD[4], we undertook this study with the primary aim to evaluate the adequacy and efficacy of daily supplementation of 600, 1000 and 2000 IU of vitamin D_3 on serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) levels in schoolchildren and adolescents with VDD.

Methods

Subjects

This randomised study was performed between July 2015 and December 2017. Two schools underwent supplementation in the year 2016 and the other two in the year 2017. A total of 1112 schoolchildren, aged 6–16 years, who responded to our request to participate, were recruited from four fee paying schools in Delhi (Latitude North 28.38°, East 77.12°), India, representing mid socio-economic strata. The consent was obtained from school management, parents/guardians and verbal assent from children before undertaking this study. Parents were asked to sign the consent form, after they were provided with the details of the study in the patient information sheet and interaction of the first author with the parents to clear their doubts. These children and adolescents had minimal interrupted sun exposure (10–30 % body surface area for approximately 30 min/d during 09.00–16.00 hours. The dietary intake of vitamin D_3 was minimal as most commonly consumed Indian foods contain negligible amount of vitamin D[14]. However, mean dietary intake of Ca (boys: 958 (SD 566) mg/daily; girls 796 (SD 436) mg/daily) was adequate and met the RDA as advised by ICMR[28]. These subjects were not advised any change in lifestyle during the study period. The details of screening and selection of subjects for the study is given in Fig. 1. Children and adolescents who were either on drugs affecting bone mineral metabolism such as Ca, vitamin D, glucocorticoids, anti-tubercular or anti-epileptics or suffering from any systemic illness were excluded from the study. A total of forty-nine children were excluded as they did not meet the inclusion criteria, and the rest (1063) underwent baseline investigations. A total of fifty-five children had serum 25(OH)D >50 nmol/l and therefore excluded from the study. The remaining 1008 were finally recruited to participate in the study. Clinical trial registration number CTRI:2017/01/007681.

The students were recruited from class one to nine, with three sections per class. Cluster randomisation was done within each class, taking each section as a cluster, using draw of lots to maintain age parity within each group. Within a class, three sections (clusters) were allocated for interventions (daily 600 IU (A), 1000 IU (B) and 2000 IU (C) of vitamin D_3) for 6 months separately. The randomly allocated interventions were neither shared with class teachers nor shared with the students within each class till the end of the study. Three interventions were procured as tablets of same shape and colours packed in yellow, green and red bottles, content of which were not known to class teacher or students. The class teachers were handed over the respective allocated intervention to be given under supervision. Investigators were aware about the intervention allocation to sections, though the people involved in the laboratory analysis were blinded to the intervention status. The vitamin D_3 capsules were manufactured and supplied every month by USV Private Ltd. The study protocol was approved by Institute Ethical committee of All India Institute of Medical Sciences, New Delhi. This trial was registered as Clinical trial registration number: CTRI-2017/01/007681. We did not include a placebo arm since only vitamin D-deficient children were included in the current study, and it would be unethical to supplement these children with placebo.

Data collection

Anthropometry measurements such as height, weight and BMI were noted at baseline. Height was measured to the nearest 0·1 cm using portable wall-mounted stadiometer (Holten's stadiometer, 200 cm/78 inches, Model WS045; Narang Medical Limited), with subjects standing straight with head held in the Frankfurt plane. Weight without shoes and light clothes on was measured to the nearest 0·1 kg, using an electronic scale (EQUINOX Digital weighing machine, Model EB6171; Equinox Overseas Private Limited). BMI, defined as the ratio of body weight to height square, was expressed in kg/m^2. Weight categories were defined based on the revised criteria as given by the IAP. Participants above adult equivalent of BMI of 23 kg/m^2 were defined as overweight and those above adult equivalent of BMI of 27 kg/m^2 were defined as obese[31].

Blood samples were collected in the fasting state between 08.00 and 09.00 hours, centrifuged and serum separated into three aliquots at the study site and transported in dry ice to the laboratory. Serum Ca, P and alkaline phosphatase (ALP) were estimated within 2 d of collection and the other two aliquots were frozen at –20°C for estimation of serum 25(OH)D and PTH

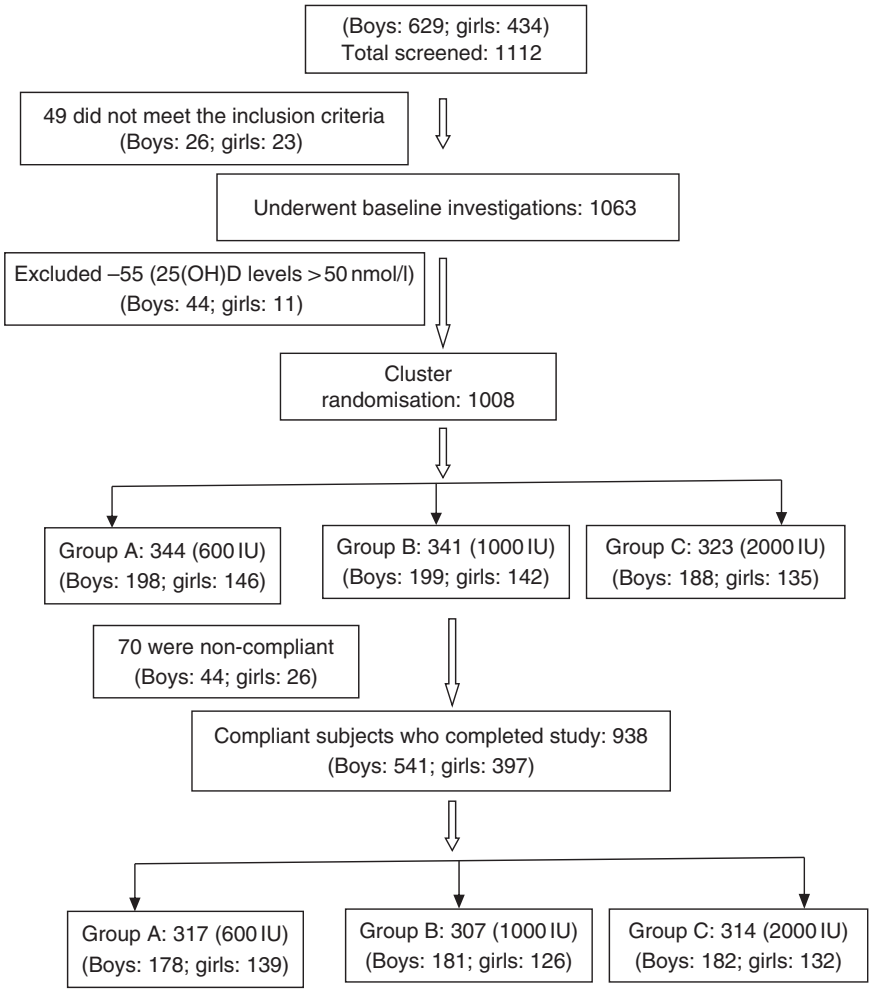

Fig. 1. CONSORT flow diagram. 25(OH)D, 25-hydroxyvitamin D.

at a later date. Serum Ca, serum phosphate and ALP were measured by commercially available kit using automated biochemistry analyser Cobasc-501 (Roche Diagnostics). The normal range for serum total Ca for 2- to 12-year-olds was 2·2–2·7 mmol/l and 2·1–2·6 mmol/l for 12- to 18-year-old children with analytical sensitivity of 0·05 mmol/l, inorganic phosphorus was 1·0–1·7 mmol/l in 7- to 12-year-olds and 0·9–1·5 mmol/l in 13- to 16-year-old children with analytical sensitivity of 0·1 mmol/l; and among girls, ALP was 129–417 U/l in 10– <13 years, 57–254 U/l in 13– <15 years and 50–117 U/l in 15– <18 years; and among boys it was 129–417 U/l in 10– <13 years, 116–468 U/l in 13– <15 years and 82–331 U/l in 15– <18 years with an analytical sensitivity of 5 U/l. The serum 25(OH)D was assayed using chemiluminescence method (Diasorin) and PTH (reference range: 10–65 pg/ml, analytical sensitivity 0·7 pg/ml) using electrochemiluminiscence assay (Roche Diagnostics), respectively. Intra- and inter-assay CV was 3·5 and 5 % for serum 25(OH)D and 2·4 and 3·6 % for serum PTH. Serum 25(OH)D level of <50 nmol/l was defined as VDD[30]. VDD was further classified as severe (25(OH)D <12·5 nmol/l), moderate (25(OH)D <25 nmol/l) and mild (25(OH)D <50 nmol/l)[32]. Urinary samples were also collected for the random urinary Ca: creatinine ratio (UCaCrR – both Ca and creatinine measured in mg)) and was performed using Cobas-C III (Roche Diagnostics). Both blood and urine samples were repeated 6 months after intervention. However, in the absence of established Indian standards, diagnosis of hypercalciuria was made when random UCaCrR exceeded 0·21[33].

Intervention

Supplementation was initiated in the month of July 2016 and 2017, every day for a period of approximately 6 months, under supervision of teachers and investigating staff at the study site for 6 working days/week and the records were maintained. Required numbers of vitamin D capsules were provided to the parents/guardians along with a record sheet to be maintained by the parents for sundays and planned holidays as per school calendar. For unplanned holidays, parents were advised to collect their requirement from school. Subjects were labelled as non-compliant when they either missed taking vitamin D for more than 7 d or were regularly absenting themselves from school during the period of supplementation. Although seventy

participants (7·0 %) were labelled as non-compliant, they completed the study.

Sample size calculation

We expect that 70, 80 and 90 % children would achieve a serum level of 25(OH)D ≥50 nmol/l after 6 months of supplementation with 600, 1000 and 2000 IU/d cholecalciferol, respectively. This was based on our earlier study where 70 and 81 % children achieved serum 25(OH)D of ≥50 nmol/l when supplemented with a daily dose of 600 and 1000 IU of vitamin D_3 for 3 months[15]. To detect a significant difference among the three groups in a two-sided test with a 5 % α error and 80 % power, seventy-four patients per group were required. Considering 10 % loss during the follow-up period, a sample size of eighty-two per group was considered. The increase in sample size in this study, however, was due to the fact that we had approached all children in schools to participate and we could not refuse any child's participation.

Statistical analysis

Analysis was performed using Stata 12.0. Descriptive statistics were calculated as means and standard deviations and median (min–max). Difference in the means of various parameters (continuous variables) and difference in the proportions were compared among the three study groups using ANOVA and χ^2 test for trend. The primary outcome (serum 25(OH)D ≥50 nmol/l) and secondary outcomes such as changes in serum 25(OH)D (nmol/l) and serum PTH (pg/ml) were analysed by both intention-to-treat (ITT) and per-protocol method. The missing values were imputed using the baseline observation carried forward technique for ITT analysis. The differences in percentage of serum 25(OH)D ≥50 nmol/l across the groups were compared using regress (adjusted for age) and 'svy regress' command to account for cluster randomisation. The results were presented as difference (95 % CI). Paired '*t*' test was applied to calculate the significance level of various parameters pre- and post-supplementation. Serum PTH and UCaCrR were not normally distributed. These parameters were analysed with Kruskal–Wallis test followed by Wilcoxon rank sum test and Wilcoxon signed rank test was used to assess the change in PTH and UCaCrR pre- and post-supplementation. Pearson's correlation was used to evaluate the relation between various parameters and change in serum 25(OH)D and PTH levels. Multiple linear regression analysis was carried out on delta change in hormonal parameters after adjusting for age, BMI and basal 25(OH)D levels. A *P*-value <0·05 was considered statistically significant.

Results

The baseline anthropometric and biochemical characteristics of the participants is shown in Table 1. The mean age and BMI of the children were 11·7 (SD 2·4) years (boys: 11·8 (SD 2·5) years; girls: 11·6 (SD 2·3) years) and 18·1 (SD 3·7) kg/m^2 (boys: 18·2 (SD 3·8) kg/m^2; girls: 17·8 (SD 3·6) kg/m^2), respectively. Among the three study groups, there was no significant difference in various parameters except for age and serum Ca levels (Table 1). The mean age of group C was significantly higher than those in groups A and B. Bony deformities (genu valgum/varum) were present in 15·1 % (152) participants. A total of eighty-seven participants (8·6 %) were obese (boys: 67 (11·5 %); girls: 20 (4·7 %)) and 187 (18·7 %) were overweight (boys: 113 (19·3 %); girls 74 (17·5 %)). Number of participants with obesity and overweight did not differ significantly between trial groups (obesity: 9·6, 8·8 and 7·4 %; overweight: 16·3, 19·6 and 19·8 % in groups A, B and C, respectively). In all, 14·6 % (147) participants had severe, 46·8 % (472) had moderate and 38·6 % (389) had mild VDD.

Vitamin D status

The overall mean baseline serum 25(OH)D level of 24·3 (SD 9·5 nmol/l (boys: 26·3 (SD 9·8) nmol/l; girls: 21·8 (SD 8·8) nmol/l; $P<0·0001$) increased significantly to 77·8 (SD 27·5) nmol/l ($P<0·001$) with no appreciable difference in the post-supplementation serum 25(OH)D levels between boys and girls (boys: 75·5 (SD 25·8); girls 75·3 (SD 29·5) nmol/l; $P=0·842$). Overall, 84·1 % (789) participants achieved serum 25(OH)D levels of ≥50 nmol/l (boys: 86·7 %; girls: 80·6 %). As shown in Table 2, in the ITT analysis, the percentage of subjects achieving serum 25(OH)D levels ≥50 nmol/l increased significantly from group 'A' to group 'C' (71·5, 81·8 and 92·9 %, $P<0·0001$), respectively. The results did not change even after adjustment for age (71·2, 81·4 and 93·6 %). The differences in the percentage of subjects achieving serum levels of 25(OH)D ≥50 nmol/l between the supplementation groups, A *v.* B, A *v.* C and B *v.* C were 10·3 (95 % CI 4·7, 15·9), 21·4 (95 % CI 15·6, 27·1) and 11·1 (95 % CI 5·3, 16·8), respectively. After accounting for cluster randomisation, the difference between A *v.* B, A *v.* C and B *v.* C was 10·3 (95 % CI 0·87, 19·7), 21·4 (95 % CI 11·7, 31·0) and 11·1 (95 % CI 2·2, 19·9), respectively. Similarly, significant differences were observed between A and C and A and B except for B and C in the per-protocol analysis. Those who did not achieve serum 25(OH)D levels ≥50 nmol/l (149 children (15·9 %)) had higher BMI (18·6 (SD 3·6) *v.* 17·8 (SD 3·7) kg/m^2, $P=0·016$), lower baseline serum 25(OH)D (21·3 (SD 8·0)) *v.* 24·8 (SD 9·8) nmol/l, $P<0·0001$) and higher PTH (79·3 (SD 81·3) *v.* 63·9 (SD 60·5) pg/ml, $P=0·007$) when compared with those who achieved serum 25(OH)D >50 nmol/l.

A significant rise in serum 25(OH)D following supplementation was observed in all the three groups both in ITT and in per-protocol analysis. Significant incremental responses in the mean serum 25(OH)D and percentage increase in serum 25(OH)D levels were observed among the three groups (A–C) (Table 3).

Increase in serum 25(OH)D levels was negatively correlated with age (r –0·045, $P=0·169$), BMI (r –0·091, $P=0·005$) and baseline 25(OH)D (r –0·235, $P<0·0001$). Serum 25(OH)D increase among three groups (means with their standard errors) remained significant even after adjusting for age, BMI and baseline 25(OH)D levels ('A': 36·3 (SD 1·3), 'B': 46·3 (SD 1·3), 'C': 70·8 (SD 1·3) nmol/l; $P<0·0001$). Serum 25(OH)D increase was significantly higher in pre-pubertal than in post-pubertal children, in girls than in boys and in severe than in mild VDD subjects (Table 4).

Table 1. Baseline demographic details and biochemical parameters
(Mean values, standard deviations and 95 % confidence intervals)

	Vitamin D supplementation groups									
	600 IU (*n* 344)			1000 IU (*n* 341)			2000 IU (*n* 323)			
Baseline characteristics	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI	$P_{for\ trend}$
Age (year)	11·5	2·4	11·3, 11·8	11·5	2·4	11·2, 11·7	12·1	2·4	11·8, 12·4	0·001
BMI (kg/m^2)	18·0	3·5	17·7, 16·4	17·9	4·0	17·5, 18·3	18·2	3·6	17·8, 18·6	0·531
Serum 25(OH)D (nmol/l)	24·0	9·5	23·0, 25·0	24·3	9·8	23·3, 25·3	24·5	9·5	23·5, 25·5	0·823
Serum PTH (pg/ml)										0·911
Median		53·2			51·5			52·7		
Range		12·6–764·3			15·0–613·4			16·8–845·5		
Serum Ca (mmol/l)	2·48	0·13	2·45, 2·48	2·48	0·10	2·45, 2·48	2·45	0·13	2·43, 2·45	0·010
Serum phosphates (mmol/l)	1·55	0·23	1·52, 1·58	1·55	0·19	1·52, 1·58	1·52	0·19	1·49, 1·52	0·041
Serum ALP (U/l)	275·3	100·1	263·3, 285·8	274·0	109·7	264·9, 289·5	273·4	121·6	259·0, 285·9	0·975
UCaCrR (mg/mg)										0·126
Median		0·027			0·022			0·020		
Range		0·0006–0·129			0·0004–0·125			0·0008–0·151		

25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; ALP, alkaline phosphatase; UCaCrR, urinary Ca:creatinine ratio.

Table 2. Comparison of percentage of serum 25-hydroxyvitamin D (25(OH)D) levels ≥50 nmol/l (primary outcome) after vitamin D supplementation in the three groups by intention-to-treat and per-protocol analysis
(Numbers, percentages and 95 % confidence intervals)

	600 IU (A)		1000 IU (B)		2000 IU (C)		
Serum 25(OH)D (nmol/l)	*n*	95 % CI	*n*	95 % CI	*n*	95 % CI	*P*
Intention to treat (*n* 1008)	344		341		323		
≥50 nmol/l	246		279		300		<0·0001
%	71·5		81·8		92·9		
Difference	Between A and B		Between A and C		Between B and C		
Unadjusted	10·3	4·7, 15·9	21·4	15·6, 27·1	11·1	5·3, 16·8	
P	<0·0001		<0·0001		<0·0001		
Adjusted for age	10·2	4·6, 15·9	22·4	16·7, 28·2	12·2	6·5, 17·9	
P	<0·0001		<0·0001		<0·0001		
Adjusted for cluster	10·3	0·87, 19·7	21·4	11·7, 31·0	11·1	2·2, 19·9	
P	<0·0001		<0·0001		<0·0001		
Per protocol (*n* 938)	317		307		314		
≥50 nmol/l	246		279		300		<0·0001
%	77·6		90·9		95·5		
Difference	Between A and B		Between A and C		Between B and C		
Unadjusted	13·3	8·3, 18·3	17·9	13·0, 22·9	4·6	–0·3, 9·7	
P	<0·0001		<0·0001		0·067		
Adjusted for age	13·2	8·2, 18·2	18·4	13·4, 23·4	5·2	0·1, 10·2	
P	<0·0001		<0·0001		0·045		
Adjusted for cluster	13·3	7·3, 19·2	17·9	10·6, 25·2	4·6	–0·09, 9·4	
P	<0·0001		<0·0001		0·054		

Serum parathyroid hormone status

The median serum baseline PTH decreased from 52·3 (12·6–845·5) pg/ml (boys: 49·5 (12·6–845·5) pg/ml, girls: 57·3 (16·8–764·3) pg/ml) to 39·8 (9·8–159·7) pg/ml (boys: 33·5 (9·8–159·7) pg/ml, girls: 38·6 (12·3–129·4) pg/ml) following 6 months of vitamin D_3 supplementation ($P < 0·0001$). This decrease was observed in all three groups in both ITT and per-protocol analysis categories (Table 3). Secondary hyperparathyroidism (PTH >65 pg/ml) was seen in 31·7 % (320) participants (boys: 25·6 % (150), girls: 40·2 % (170); $P < 0·0001$) at baseline, decreased to 79 (8·4 %) post-supplementation (boys: 7·2 % (39), girls: 10·1 % (40); $P = 0·075$). The median decrease in serum PTH was not significant but the percentage decrease was significant among three groups in both categories (Table 3). Decrease in serum PTH was higher in post-pubertal adolescents than in per-pubertal children, in girls than in boys, in severe than in mild VDD and in those with secondary hyperparathyroidism (Table 4).

Other biochemical parameters

Though the mean serum Ca and ALP decreased while serum phosphate increased significantly post-supplementation, their values were still within normal ranges. The median UCaCrR increased from 0·022 (0·0003–0·152) to 0·032 (0·001–0·245) mg/mg following 6 months of supplementation

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Table 3. Comparison of mean serum levels of serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) in the three groups by intention-to-treat and per-protocol analysis
(Mean values and standard deviations; means and 95 % confidence intervals; medians and ranges)

	600 IU (A)		1000 IU (B)		2000 IU (C)		
Outcome measures	*n* 344		*n* 341		*n* 323		
Intention to treat (*n* 1008)	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*
Serum 25(OH)D (nmol/l)							
Baseline							0·838
Mean	24·0		24·3		24·5		
SD	9·5		9·8		9·5		
Post-supplementation							<0·0001
Mean	58·8		66·8		92·8		
SD	18·5		24·3		31·0		
P (paired)	<0·0001		<0·0001		<0·0001		
Mean increase	34·5	32·5, 36·8	42·5	39·8, 45·3	68·0	64·8, 71·5	<0·0001
Percentage increase	183	166·8, 198·9	229	208·0, 250·5	342	315·0, 369·3	<0·0001
Serum PTH (pg/ml)							
Baseline							0·911
Median	53·2		51·5		52·7		
Range	12·6–764·3		15·0–613·4		16·8–845·5		
Post-supplementation							0·112
Median	37·5		34·9		34·9		
Range	12·3–126·3		12·2–159·7		9·8–109·0		
P (paired)	<0·0001		<0·0001		<0·0001		
Median decrease	15·7	–6·1, 170·4	16·6	–3·6, 362·9	17·8	–4·3, 753·6	0·223
Percentage decrease	27·6	25·3, 29·9	30·9	28·5, 33·3	31·3	28·9, 33·8	0·032
	n 317		*n* 307		*n* 314		
Per protocol (*n* 938)	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*
Serum 25(OH)D (nmol/l)							
Baseline							0·796
Mean	24·3		24·0		24·5		
SD	9·5		9·8		9·8		
Post-supplementation							<0·0001
Mean	60·8		70·8		94·5		
SD	17·8		21·8		29·5		
P (paired)	<0·0001		<0·0001		<0·0001		
Mean increase	36·5	34·5, 38·5	46·8	44·3, 49·5	70·0	66·5, 73·3	<0·0001
Percentage increase	192	175·3, 209·2	251	228·7, 272·9	351	323·8, 372·3	<0·0001
Serum PTH (pg/ml)							
Baseline							0·934
Median	52·2		51·5		52·1		
Range	12·6–764·3		15·0–613·4		16·8–845·5		
Post-supplementation							0·049
Median	37·8		34·1		34·5		
Range	12·3–126·3		12·3–159·7		9·8–109·0		
P (paired)	<0·0001		<0·0001		<0·0001		
Median decrease	15·7	–6·1, 170·4	16·6	–3·6, 362·9	17·8	–4·3, 753·6	0·223
Percentage decrease	27·1	24·7, 29·5	31·3	28·8, 33·8	31·3	28·8, 33·8	0·012

($P < 0·001$). The decrease in the serum Ca, ALP and increase in serum phosphates and UCaCrR post-supplementation was no different among the three groups (Table 5). Even though none of the subjects in this study developed hypercalcaemia, two participants from group 'B' developed hypercalciuria following supplementation.

Discussion

In the absence of universal food fortification with vitamin D, supplementation is an effective alternate strategy to improve serum 25(OH)D status in India, as it has greater specificity of intervention and permits dose adjustment. There are several studies assessing the efficacy of vitamin D_3 supplementation in adults[16]. However, only limited studies are available in children[17–25]. Furthermore, there are even fewer studies assessing the adequacy and efficacy of different daily doses of vitamin D_3 supplementation in increasing the serum 25(OH)D levels in children and adolescents with VDD[19,20,24] compared with those without VDD[17,22–24].

A report of an expert group from ICMR recommended 400 IU/d of vitamin D daily for Indians of all age groups[28] as against 600 IU/d recommended by IAP[29] and IOM, USA[30] and several other countries[34]. There is no definite data on how much daily vitamin D is required to prevent

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Table 4. Parameters affecting changes in 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) (Mean values, standard deviations and 95 % confidence intervals)

		25(OH)D increase (nmol/l)			PTH decrease (pg/ml)		
Parameters	*n*	Mean	SD	95 % CI	Mean	SD	95 % CI
Age (years)							
Pre-pubertal (<10 years)	232	55·8	34·0	51·3, 60·0	15·0	23·7	12·0, 18·1
Post-pubertal (>10 years)	706	49·5	25·8	47·8, 51·5	31·0	64·3	26·3, 35·8
P			0·004			<0·0001	
Sex							
Boys	541	49·5	27·3	47·0, 51·8	21·0	45·0	17·2, 24·8
Girls	397	53·3	29·0	50·5, 56·3	35·4	70·1	28·4, 42·3
P			0·033			<0·0001	
Weight (kg)							
Normal	689	52·0	29·0	49·8, 54·0	28·8	64·1	24·1, 33·6
Overweight	170	49·8	26·3	45·8, 53·8	20·9	24·5	17·2, 24·6
Obese	79	46·3	23·8	41·0, 51·5	24·7	43·7	14·9, 34·5
P			0·181			0·748	
Vitamin D deficiency							
Mild	356	44·5	28·5	41·5, 47·5	12·0	15·5	10·4, 13·7
Moderate	444	52·8	27·0	50·3, 55·3	26·6	52·2	21·8, 31·5
Severe	138	62·5	25·8	58·0, 66·8	67·2	104·4	49·6, 84·7
P			<0·0001			<0·0001	
Secondary hyperparathyroidism							
Present	79	36·3	16·3	32·5, 40·0	65·3	129·4	36·3, 94·3
Absent	859	52·3	28·5	50·5, 54·3	23·5	43·5	20·6, 26·5
P			<0·0001			<0·0001	

VDD and whether recommended daily allowance of 400 or 600 IU/d will suffice to combat widely prevalent VDD in India[4]. We, therefore, undertook to supplement a large cohort of schoolchildren with different daily doses of vitamin D and evaluated their adequacy and efficacy. We chose the daily supplementation dose of 600 IU as it is a widely recommended RDA in literature, a higher dose of 1000 IU as per our earlier reported prediction equation[7] and 2000 IU, as the estimated daily intake of vitamin D shown to achieve serum 25(OH)D levels of ≥50 nmol/l in 97·5 % of subjects[35].

The dose-dependent increase in serum 25(OH)D following daily supplementation in consistent with the reports in literature with[19,20,24] or without VDD[17,22–24] and with different time durations[17–26]. We, in one of our earlier studies evaluating the impact of supplementing milk fortified with 600 and 1000 IU of vitamin D_3 in schoolchildren with VDD every day for 12 weeks, showed almost similar increase of 28·6 and 39·2 nmol/l, respectively. Likewise, percentages of children (70 and 81 %) who had achieved serum 25(OH)D of ≥50 nmol/l with 600 and 1000 IU of vitamin D in our previous study were very similar to that observed in the present study (71·5 and 81·8 %)[15]. Though there is a 3-month difference in the duration of the two studies, a recent study reported little change in the mean serum 25(OH)D levels following 3 or 6 months of daily supplementation[17]. Talib *et al.*[19] from New York (USA), who carried out a study in 183 vitamin D-deficient children (mean age 16·6 (SD 2·2) years) with three doses of 50 000 IU/weekly, 5000 IU/daily and 1000 IU/daily, also observed a dose-dependent mean increase in 60·3, 52·5 and 15·5 nmol/l in serum 25(OH)D, respectively.

Dong *et al.*[24] compared 400 and 2000 IU given for 16 weeks to forty-nine black boys and girls aged 16·3 (SD 1·4) years with VDD (mean baseline serum 25(OH)D of 34 nmol/l) also did show a higher increase with 2000 IU than 400 IU/d. The mean increase in serum 25(OH)D of 60 nmol/l with 2000 IU dose was almost similar to the rise in serum 25(OH)D in the present study. Similar observation was made by Al-Shaar *et al.*[21] in 336 Lebanese adolescents aged 13 (SD 2) years while studying the impact of low- (200 IU) and high-dose (2000 IU) vitamin D supplementation. The mean baseline serum 25(OH)D increased from 37·5 (SD 17·5) to 90·8 (SD 55·8) nmol/l with 2000 IU and to 46·5 (SD 16·5) nmol/l with 200 IU and the percentage of vitamin D-deficient Lebanese children achieving sufficiency (96 %) after 1 year of supplementation with 2000 IU/d was the same as that achieved in the present study.

Similarly, increase in serum 25(OH)D with increasing doses of vitamin D supplementation in vitamin D-sufficient children was also noted in a recent study by Sacheck *et al.*[17] who evaluated the impact of three doses of vitamin D_3 on serum 25(OH)D in at-risk schoolchildren where the mean baseline serum 25(OH)D was 55·0 (SD 17·0) nmol/l. In addition, the GAPI (Georgia, Purdue, and Indiana University) trial (multicentre randomised dose–response trial) conducted in children aged 9–13 years, with the mean baseline serum 25(OH)D of 70·0 nmol/l and supplementation doses ranging from 400- to 4000-IU/d, also showed a dose-dependent increase[22].

The response to supplementation in VDD subjects in the present study as well as other studies was significantly greater in terms of rise in serum 25(OH)D levels[21,24] as compared with subjects with baseline vitamin D sufficiency[17,22]. The response to supplementation with 2000 IU of vitamin D_3 per d in the current study (68·0 (SD 31·3) nmol/l) was similar to that reported by Al-Shaar *et al.*[21] (60·0 nmol/l) and Dong *et al.*[24] (52·5 nmol/l) in VDD subjects in contrast to not very large rise (26·8 nmol/l) in a recent study by Sacheck *et al.*[17] and (38 nmol/l) in an earlier

Table 5. Showing effect of vitamin D supplementation on serum levels of serum calcium, phosphates, alkaline phosphatase (ALP) and urinary calcium: creatinine ratio (UCaCrR) in the three groups
(Mean values, standard deviations and 95 % confidence intervals; medians and ranges)

	600 IU (A)			1000 IU (B)			2000 IU (C)			
	n 317			*n* 307			*n* 314			
Other outcomes	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI	*P*
Serum Ca (mmol/l)										
Baseline	2·48	0·13	2·45, 2·48	2·48	0·10	2·45, 2·48	2·45	0·13	2·43, 2·45	0·015
Post-supplementation	2·43	0·08	2·40, 2·43	2·43	0·08	2·40, 2·43	2·54	0·08	2·43, 2·45	0·107
P		<0·0001			<0·0001			0·090		
Serum phosphates (mmol/l)										
Baseline	1·55	0·23	1·52, 1·58	1·55	0·19	1·52, 1·58	1·52	0·19	1·49, 1·52	0·013
Post-supplementation	1·58	0·19	1·58, 1·62	1·58	0·19	1·55, 1·58	1·55	0·19	1·55, 1·58	0·068
P		<0·0001			0·008			<0·0001		
Serum ALP (U/l)										
Baseline	274·5	101·3	263·3, 285·8	277·2	108·7	264·8, 289·5	272·4	119·9	259·0, 285·9	0·867
Post-supplementation	258·1	85·6	248·6, 267·6	260·7	83·0	251·3, 270·0	256·7	102·8	245·3, 268·1	0·861
P		<0·0001			<0·0001			<0·0001		
UCaCrR (mg/mg)										
Baseline										0·108
Median	0·0261			0·0219			0·0204			
Range	0·001–0·130			0·003–0·125			0·001–0·152			
Post-supplementation										0·703
Median	0·0337			0·0315			0·0345			
Range	0·001, 0·184			0·002, 0·245			0·002, 0·212			
P		<0·0001			<0·0001			<0·0001		

study by Lewis *et al.*[22] in vitamin D-sufficient subjects. The rise, however, in serum 25(OH)D post-supplementation with 1000 IU in the present study was not only significantly higher than in studies carried out with vitamin D-sufficient subjects (14·5 and 12·5 nmol/l)[17,22] but also significantly higher in studies undertaken with VDD subjects (15·5 and 17·3 nmol/l)[19,20]. This is possibly due the fact that the baseline serum 25(OH)D levels in the present study subjects was markedly lower than all the studies quoted above. The other possible explanation is the differences in the BMI of subjects as the serum 25(OH)D response is dependent on the vitamin D dose per unit of weight[7]. The mean BMI of subjects in the present study was markedly lower than that reported in other studies[17,19,20,22].

The overall increase of 4·5 (SD 2·5) nmol/l in serum 25(OH)D per 100 IU of vitamin D supplementation in the present study was significantly greater than that of 1·8±2·5 nmol/l reported in literature[30]. It is well known that the increment in serum 25(OH)D levels after vitamin D supplementation is inversely correlated with dose per unit weight[7], baseline serum 25(OH)D levels[19,26], and dose and duration of the study[16], which was also observed in present study explains the higher increase in serum 25(OH)D per 100 IU of vitamin D supplementation. Interestingly, a study among fifty-six vitamin D-sufficient children with mean baseline serum 25(OH)D of 72·3 (SD 17·5) nmol/l did not show any further increase in serum 25(OH)D with 1000 IU supplementation for 11 weeks[23]. This finding suggests that our bodies adapt to an increase in serum 25(OH)D as per their requirement following supplementation with vitamin D. Other supplementation studies performed in children with VDD are not comparable as vitamin D supplementation was carried out either weekly[25], or fortnightly[18] and monthly doses[18,26].

The results of our study showed that 2000 IU/d of vitamin D were required to achieve the serum 25(OH)D levels of ≥50 nmol/l in 94 % of participants. This observation was consistent with what was reported by Rajakumar *et al.*[35] who showed that 2098 IU of vitamin D/d were needed to maintain serum 25(OH)D levels at 50 nmol/l in 97·5 % of US children. A systematic review and meta-analysis from Middle East and North Africa (NENA region) also suggested that a daily dose of 1000–2000 IU of vitamin D will be required to obtain serum 25(OH)D levels of >50 nmol/l in the majority of the paediatric population[27]. These observations raise doubts about the adequacy of current recommendations of 400 IU/d by ICMR or 600 IU/d by IAP and IOM for Indian children and adolescents.

The possible explanations as to why 15·9 % subjects in the present study did not achieve the desired levels of ≥50 nmol/l could be higher baseline BMI, lower baseline serum 25(OH)D and higher baseline PTH levels in these study subjects when compared with those became vitamin D sufficient. These children may require either higher supplemental dose of vitamin D or longer duration of supplementation to respond and normalise serum 25(OH)D as has also been observed by several other workers[16,25,36].

A significant decrease in serum PTH levels as well as decline in the prevalence of secondary hyperparathyroidism was also reported in a study from Middle East[21] and in one of our earlier studies where the decline in secondary hyperparathyroidism was reported from 50 to 7·1 % when VDD children were supplemented with 60 000 IU/month for a period of 6 months[37]. All these studies had subjects with VDD and high baseline serum PTH levels. In contrast, no significant decrease in serum PTH was recorded in studies carried out in subjects without VDD[17,19,20,23] as these studies had lower serum PTH levels

when compared with present study. The fact that the mean decrease in serum PTH was not statistically significant among the three groups, suggests that decrease in serum PTH is not dose dependent as also noticed in other study[22]. Since the mean decrease in serum PTH was significantly higher in participants with severe VDD and those with secondary hyperparathyroidism; it may be hypothesised that children and adolescents in the current study truly represented vitamin D deficiency as opposed to those from west who either did not truly have vitamin D deficiency or had subclinical VDD[38]. Those studies were probably conducted to raise serum 25(OH)D levels to >75 nmol/l to derive controversial extra-skeletal benefits particularly in paediatric population[39].

Persistence of secondary hyperparathyroidism in 8·4 % subjects despite serum levels of 25(OH)D being ≥50 nmol/l may be indicative of either persistent low dietary intake of Ca in them or inability of parathyroid gland to return to it's normal functioning within 6 months despite achieving adequate levels of serum 25(OH)D. This is similar to what is seen with serum thyroid-stimulating hormone levels remaining suppressed for months despite patient being in remission in Grave's disease with normal T3 and T4 levels. Possibility of primary hyperparathyroidism is ruled out as none of these subjects had hypercalcaemia[39].

In this study, though serum levels of Ca showed statistically significant decline following vitamin D supplementation with 600 and 1000 IU of vitamin D/d, the levels were still numerically within normal limits. Whether this decrease has any clinical relevance is questionable. Hyperparathyroidism is associated with increase in serum Ca, ALP and decrease in phosphates; hence, improvement in secondary hyperparathyroidism post-supplementation may have led to decrease in serum Ca, ALP and increase in serum phosphates. Significant decrease in serum ALP levels and increase in serum phosphates levels post-supplementation has also been observed in one of our earlier reports[26]. Some other studies have reported no change in serum Ca, phosphates and ALP levels[17,23].

The UCaCrR has shown a wide variation ranging from 0·024 to 0·44 in various geographic areas[40–47]. Two early studies from India showed a mean ratio of 0·155 and 0·299, respectively[48,49]. The median value noted in the present study was 0·022 (0·0003–0·152), which significantly increased to 0·032 (0·001–0·250) post supplementation. The change in UCaCrR in children following vitamin D_3 supplementation has not been studied earlier, however, there are conflicting reports in adults[50,51]. Though hypercalcaemia and hypercalciuria always remains a possibility with vitamin D supplementation as reported by Talib *et al.*[19] in three children following supplementation, there was no case of hypercalcaemia and only two cases of hypercalciuria were detected in the present study. It is also known that hypercalciuria and hypercalcaemia are unrelated with dose and duration of vitamin D supplementation[19,52].

The main strength of our study was a large cohort of schoolchildren undertaken for daily vitamin D supplementation and evaluation of UCaCrR to detect hypercalciuria which has been done for the first time in Indian children. We did not advise any change in life style, which can be an important confounding factor and may affect the results of vitamin D intervention. However, exposure to sun[11,12] remains an important part of management of both symptomatic and asymptomatic VDD subjects. Possible weaknesses were inability to carry out individual randomisation and evaluate bone formation and resorption markers.

Conclusion

Supplementation of vitamin D with all three daily doses of vitamin D_3 (600, 1000, 2000 IU) resulted in significant increase in the serum 25(OH)D levels in schoolchildren with VDD. Children seem to benefit maximum with the daily dose of 2000 IU/d with 94 % achieving serum levels of ≥50 nmol/l following supplementation. The rise in serum 25(OH)D was inversely proportional to age, BMI and serum 25(OH)D levels. Whether daily allowance of 400 IU as recommended by ICMR or 600 IU by IAP and IOM, would suffice in children and adolescents with VDD to achieve serum levels of ≥50 nmol/l, remains debatable. Further studies are required to be undertaken before revising the earlier proposed RDA by ICMR, IAP and IOM.

Acknowledgements

The authors gratefully acknowledge USV Private Limited, India, for providing vitamin D_3 capsules for the trial. The authors thank Mr D. H. Pai Panandiker, Chairman, and Ms Rekha Sinha, CEO, International Life Sciences Institute (India) for sponsoring the project. The authors highly appreciate the support of the principals, parents and children for participating in this trial. The authors would like to put on record our appreciation for the help rendered by Ms Pamela Marwaha, Dr S. K. Mathur and Ms Neeru Gandhi of Society for Endocrine Health for Elderly, Adolescents and Children for their valuable contribution towards this project.

Nestle R & D, India has provided funds for the study. The funding organisation played no role in the study design; in the collection of data; in the writing of the report; or in the decision to submit the report for publication.

R. K. M. and G. S. – conceptualising the study, clinical evaluation and preparation of manuscript. M. K. G. – designing the study, analysis of data and preparation of manuscript. N. G. – laboratory evaluation of hormones. A. M. – designing the study and preparation of manuscript. N. D. – biochemical evaluation of samples. M. K. – sample size calculations and statistical analysis. M. A. G. – recruitment and clinical evaluation of the subjects. A. N., P. A., A. S., A. C. and R. K. M. – execution of project including sample and data collection, supervision of supplementation in all schools, sample collection and data entry.

The authors declare that there are no conflicts of interest.

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