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Effect of vitamin D supplementation during pregnancy on neonatal mineral homeostasis and anthropometry of the newborn and infant

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Abstract

Hypovitaminosis D is common in India. In the present prospective partially randomised study of vitamin D (D_3) supplementation during pregnancy, subjects were randomised in the second trimester to receive either one oral dose of 1500 μg vitamin D_3 (group 1, *n* 48) or two doses of 3000 μg vitamin D_3 each in the second and third trimesters (group 2, *n* 49). Maternal 25-hydroxyvitamin D (25(OH)D) at term, cord blood (CB) alkaline phosphatase (ALP), neonatal serum Ca and anthropometry were measured in these subjects and in forty-three non-supplemented mother–infant pairs (usual care). Median maternal 25(OH)D at term was higher in group 2 (58·7, interquartile range (IQR) 38·4–89·4 nmol/l) *v.* group 1 (26·2, IQR 17·7–57·7 nmol/l) and usual-care group (39·2, IQR 21·2–73·4 nmol/l) ($P=0·000$). CB ALP was increased (> 8.02 μkat/l or >480 IU/l) in 66·7 % of the usual-care group *v.* 41·9 % of group 1 and 38·9 % of group 2 ($P=0·03$). Neonatal Ca and CB 25(OH)D did not differ significantly in the three groups. Birth weight, length and head circumference were greater and the anterior fontanelle was smaller in groups 1 and 2 (3·08 and 3·03 kg, 50·3 and 50·1 cm, 34·5 and 34·4 cm, 2·6 and 2·5 cm, respectively) *v.* usual care (2·77 kg, 49·4, 33·6, 3·3 cm; $P=0·000$ for length, head circumference and fontanelle and $P=0·003$ for weight). These differences were still evident at 9 months. We conclude that both 1500 μg and two doses of 3000 μg vitamin D_3 had a beneficial effect on infant anthropometry, the larger dose also improving CB ALP and maternal 25(OH)D.

Key words: Vitamin D supplementation: Pregnancy: Infant anthropometry: Neonatal calcium

Vitamin D deficiency has been reported to be frequent among adolescent girls and pregnant women in India, with approximately 80 % of both urban and rural subjects having serum 25-hydroxyvitamin D (25(OH)D) <50 nmol/l[1,2]. Exposure to the abundant sunlight in India is poor in women because of the traditional modest style of dressing. The resulting consequences to the fetus and the newborn include low cord blood (CB) vitamin D and high alkaline phosphatase (ALP), neonatal hypocalcaemia and poor fetal growth, among others[2,3]. Thus, pharmacological supplementation may be necessary, especially in such vulnerable groups.

Although several studies are available on vitamin D supplementation during pregnancy, its appropriate dose is not clear[4]. In a study by Datta *et al.*[5], 160 pregnant Asian women in the UK were supplemented with a dose of 20 μg/d, which was later increased to 40 μg/d. However, the rise in maternal serum 25(OH)D was from 14·98 nmol/l to only 27·5 nmol/l. Similar results have been found in other studies that administered small daily doses of similar magnitude[6,7], though one study[3] found significantly improved CB 25(OH)D in subjects receiving 25 μg/d when compared with controls. Studies that used larger (stoss) doses have done so only in the third trimester, whereas Ca transfer to the fetus has been shown to occur in the second trimester[8]. Marya *et al.*[9] showed that a large dose of 30 000 μg in the third trimester produced a significant improvement in maternal and cord serum biochemical parameters, as well as in newborn anthropometry. However, measurements were not made for 25(OH)D. Madelenat *et al.*[10] administered a single dose of 2000 μg to fifty-nine pregnant women between 27 and 32 weeks of gestation. Before vitamin D supplementation, 34 % of the women had a 25(OH)D concentration <24·9 nmol/l and 32 % had hypocalcaemia. At delivery, only one woman had low 25(OH)D concentration, whereas 15 % of the

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ALP, alkaline phosphatase; CB, cord blood.

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women showed hypocalcaemia. In India, vitamin D preparations at a dose of 1500 μg are suitable for a directly observed therapy at public health intervention level, on one or more occasions in the whole of pregnancy.

We thus planned to study the efficacy of one dose of 1500 μg cholecalciferol in the second trimester *v.* 3000 μg each in the second and third trimesters, in achieving normal maternal serum 25(OH)D at term, normal calcaemic status of the newborn and its effect, if any, on the anthropometry of the newborn and the infant, and incidence of respiratory tract infections until 9 months of age.

Materials and methods

Subjects

Pregnant women who attended the antenatal clinic in Queen Mary Hospital, Chhatrapati Sahuji Maharaj (formerly King George's) Medical University, Lucknow were enrolled in the present study. The hospital serves women of lower middle and middle socio-economic groups, from urban and rural areas. The sample size required to demonstrate a change in frequency of hypovitaminosis D, from 85% at baseline (known from our previous studies) to 20% at delivery, with 95% CI and a power of 80% is twenty. Based on our previous experience, we expected a dropout rate of 60–70%. Thus, we inducted into the study 300 consecutive women attending our antenatal clinic between 12 and 24 weeks of gestation, who consented to participate. They were randomly assigned (by the use of random number tables) to receive either one oral dose of 1500 μg cholecalciferol at induction into the study (group 1) or 3000 μg cholecalciferol at induction as well as at 28 weeks of gestation (group 2). We chose these doses to represent the smallest supplemental preparation of vitamin D used commonly in our country compared with a dose we believed to be closer to required amounts. All were prescribed 1 g of elemental Ca daily as calcium carbonate without vitamin D. Cholecalciferol was administered under supervision. Subjects were excluded from the study if they were already on Ca or vitamin D supplementation, anticonvulsants, antitubercular treatment or had any medical condition that affected Ca and vitamin D metabolism (including renal and hepatic disease). Consecutive women who were already in third trimester receiving the usual care in the antenatal clinic served as controls. This included the same prescription of Ca as for the subjects receiving vitamin D.

Detailed history and examination were performed with special regard to current and past pregnancies and labour. Socio-economic status was assessed using the Kuppuswamy scale[11]. Clinical features suggestive of osteomalacia (e.g. bone pain, proximal muscle weakness, bone tenderness, fractures) or past history of rickets were examined. Daily intake of Ca was assessed by a FFQ. Sun exposure was calculated, separately for summer and winter, by history of clothing and daily activity pattern (calculated as per Wallace's rule of nine, which allocates 9% of the body surface area to each arm, the front and back of each lower limb, the front and back of the chest, and the abdomen, the head and 1% for the genitalia). Pregnancy-related complications were recorded, including intra-uterine death, cephalopelvic disproportion, caesarean section, non-progression of labour, abnormal lie, premature rupture of membranes, placenta praevia, fetal distress, gestational diabetes and pregnancy-induced hypertension.

Detailed anthropometry of the newborn, including weight, length, head circumference and longest diameter of the anterior fontanelle, was measured at birth and subsequently at 3, 6 and 9 months of age. At each visit, history suggestive of lower respiratory tract infections was examined. Mothers were encouraged to make contact by phone and visit the same team for intercurrent illness and immunisation. Investigators who measured anthropometry of infants and followed them were blinded to the mothers' treatment category.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the institutional ethics committee. Written informed consent was obtained from all subjects at the time of enrolment.

Biochemical analysis

For biochemical analysis, 10 ml of maternal blood collected at induction into the study and again at delivery were immediately transported on ice. Serum or plasma was stored at −70°C for future analysis of serum 25(OH)D and parathyroid hormone, respectively, by RIA/immunoradiometric assay (Diasorin). Serum was also assayed within 24 h for Ca, P and albumin using a fully automated clinical chemistry analyser (RA-XT; Bayer Diagnostics). To exclude placental isoenzyme (stable after heating for 15 min at 65°C), heat-labile ALP was analysed[12]. CB was similarly processed for ALP and 25(OH)D. For CB, the upper limit was taken as twice the adult value of 4·0 μkat/l (240 IU/l)[13]. Serum Ca of the neonate was estimated between 4 and 6 d of life.

Statistical analysis

Statistical analyses were performed using the SPSS Statistical Package (version 12.0; SPSS). Normally distributed continuous variables are expressed as means and standard deviations, and non-parametric variables as medians and interquartile ranges. Proportions were compared using the χ^2 test. The Kruskal–Wallis test was used for multiple group comparisons. Where differences were found to be significant, the Mann–Whitney *U* test was used for *post hoc* comparison between pairs of groups. *P* values expressed are without a Bonferroni correction. Spearman's test was used for correlations. Two-tailed significance at $P<0{\cdot}05$ was considered as significant.

Results

In the second trimester of pregnancy, 299 women were randomised to one of the two treatment arms. A total of forty-three non-supplemented women served as controls (usual-care group). Of the 299 women, ninety-seven delivered in our hospital. At baseline, the subset of ninety-seven were significantly older, had higher BMI and higher dietary Ca intake,

Table 1. Baseline characteristics of women who returned for follow-up *v.* those who did not return
(Mean values and standard deviations)

	Women who did not return for follow-up (*n* 202)		Women who returned for follow-up (*n* 97)		
	Mean	SD	Mean	SD	*P*
Age (years)	25·3	3·8	26·7	4·0	0·006
BMI at enrolment (kg/m^2)	22·4	3·4	23·5	3·6	0·017
Dietary Ca (mg/d)	565	233	634	222	0·015
Sun exposure in winter (min/d)	134	93	139	91	0·53
Sun exposure in summer (min/d)	41	33	35	24	0·27
Serum Ca (mmol/l)	2·3	0·15	2·3	0·18	0·09
Serum P (mmol/l)	1·2	0·2	1·2	0·2	0·18
Serum ALP (μkat/l)	4·38	1·07	4·08	1·45	0·80
Religion					0·62
Hindu	168		83		
Muslim	34		14		
Socio-economic status (*n*)					0·07
Below poverty line	0		1		
Poor	29		5		
Lower middle	113		64		
Middle	52		25		
High	7		2		

ALP, heat-labile alkaline phosphatase (normal 4·0 μkat/l or <240 IU/l).

compared with the 202 women who did not return for follow-up (Table 1). There was no difference in baseline characteristics of the 299 women randomised into two groups, with regard to weight, height, socio-economic status, sunlight exposure, religion, Ca intake, serum Ca and elevated ALP (data not shown). Among the ninety-seven supplemented women and forty-three controls who finally delivered in our hospital, the three groups did not differ significantly in baseline characteristics (Table 2). Maternal height, gestational age at birth and infant's sex also did not differ between the three groups (data not shown). Maternal serum 25(OH)D at delivery was significantly higher in group 2 compared with group 1 and the usual-care group (Table 3). The median time between administration of the second dose of 3000 μg

Table 2. Baseline characteristics of women who were followed up until delivery
(Mean values and standard deviations; median and interquartile ranges)

	Group 1 (*n* 48)*		Group 2 (*n* 49)†		Usual-care group (*n* 43)‡		
	Mean	SD	Mean	SD	Mean	SD	*P*§
Age (years)	26·3	4·2	27·0	3·8	26·2	3·7	0·42
BMI at delivery (kg/m^2)	26·2	3·7	25·5	3·8	25·5	3·4	0·32
Dietary Ca (mg/d)	620	193	648	250	660	158	0·47
Sun exposure in winter (min/d)	138	78	140	103	116	65	0·49
Sun exposure in summer (min/d)	38	27	33	21	31	16	0·62
Serum Ca (mmol/l)	2·3	0·15	2·3	0·2	–	–	0·76
Serum P (mmol/l)	1·2	0·2	1·2	0·2	–	–	0·63
Serum ALP (μkat/l)	3·99	1·26	4·15	1·62	–	–	0·98
Religion							0·81
Hindu	42		41		38		
Muslim	6		8		5		
Socio-economic status (*n*)							0·41
Below poverty line	0		1		0		
Poor	1		4		1		
Lower middle	31		33		27		
Middle	15		10		15		
High	1		1		0		
Serum 25(OH)D (nmol/l)							
Median	31·7		32·0		–	–	0·44
Interquartile range	14·0–57·2		14·5–45·7		–	–	

ALP, heat-labile alkaline phosphatase (normal 4·0 μkat/l or <240 IU/l); 25(OH)D, 25-hydroxyvitamin D.
* Group 1 received only one dose of 1500 μg vitamin D_3 in the second trimester.
† Group 2 received two doses of 3000 μg vitamin D_3 each in the second and third trimesters.
‡ The usual-care group did not receive any supplementation of vitamin D during pregnancy.
§ *P* value for multiple group comparisons by the Kruskal–Wallis test.

vitamin D_3 and delivery in group 2 was 58 d (interquartile range 36·5–70 d). Maternal parathyroid hormone, at delivery showed a moderate inverse correlation with 25(OH)D (r −0·37, P=0·00).

Mean CB ALP and the proportion of subjects with elevated CB ALP were higher in control subjects compared with the supplemented groups (Table 3). Median CB 25(OH)D, as well as the proportion of CB samples with 25(OH)D >25 or 50 nmol/l, did not differ significantly between the three groups. Neonatal Ca, prevalence of hypocalcaemia and numbers of episodes of lower respiratory tract infections were not significantly different between the groups. The anthropometry studies revealed significantly higher birth weight, length and head circumference and a small anterior fontanelle in both supplemented groups, compared with the control group. These differences persisted on follow-up at 3, 6 and 9 months (Table 4). The change in these variables between birth and 9 months was significantly higher in groups 1 and 2 compared with the usual-care group, with respect to length and weight but not to head circumference or anterior fontanelle diameter (Table 4).

The frequency of pregnancy-related complications, including intra-uterine death, pregnancy-induced hypertension, cephalopelvic disproportion, non-progression of labour, caesarean section and placenta praevia, did not differ among the three groups (P=0·347).

Discussion

We found significantly better anthropometry in the newborn in the supplemented groups compared with the control group. These differences were observed to persist until 9 months of age. Infants of supplemented mothers also had improved CB ALP, compared with the controls. Most of the vitamin D supplementation studies in the literature have found favourable outcomes in CB/neonatal Ca or ALP[3,6,7,9,10,14–19]. However, with the doses employed previously, only one study[3] has shown subjects who achieved a maternal 25(OH)D >50 nmol/l at term. In the present study, women supplemented with the higher dose achieved a median 25(OH)D of 58 nmol/l, but only 62% reached >50 nmol/l. However, the present study and the study by Mallet *et al.*[7] did not find improved neonatal Ca in the supplemented groups. The explanation for this lack of improvement in neonatal serum Ca is not clear.

Early studies on vitamin D supplementation in pregnant women have been reported from the UK, mainly on immigrants from the Indian subcontinent[6,14,15]. In these and other studies, women supplemented daily with 25 μg ergocalciferol showed, in comparison with placebo-treated controls, improved maternal serum 25(OH)D and cord or neonatal serum Ca[3,7]. Although these two studies that commented on birth weight did not find improved mean birth weight, the proportion of infants with intra-uterine growth retardation was lower in the supplemented group.

Table 3. Maternal and neonatal biochemical characteristics at delivery
(Mean values and standard deviations; median and interquartile ranges)

Variables	Group 1 (n 48)†	Group 2 (n 48)‡	Usual-care group (n 48)§	P‖
Elevated maternal ALP> 4·0 μkat/l (%)	87·5	70·8	76·2	0·13
Maternal 25(OH)D (nmol/l)				0·000
Median	26·2***	58·7*	39·2	
Interquartile range	17·7–57·7	38·4–89·4	21·2–73·4	
Maternal 25(OH)D > 50 nmol/l (%)	27**	62·5	44·2	0·002
	Group 1 (n 35)	Group 2 (n 39)	Usual-care group (n 41)	
CB ALP (μkat/l)				0·043
Mean	9·10*	8·67	12·36	
SD	7·33	5·04	10·59	
Elevated CB ALP > 8·0 μkat/l (%)	41·9	38·9	66·7	0·031
Cord serum 25(OH)D (nmol/l)				0·27
Median	28·2	24·1	18·5	
Interquartile range	15·1–43·0	12·2–43·9	10·1–29·7	
	Group 1 (n 31)	Group 2 (n 28)	Usual-care group (n 33)	
Neonatal Ca (mmol/l)				0·48
Mean	2·2	2·3	2·2	
SD	0·4	0·4	0·5	
LRTI episodes (n)	2	4	7	0·13

ALP, heat-labile alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; CB, cord blood; LRTI, lower respiratory tract infections.
* Values were significantly different from those of the usual-care group (P<0·05).
** Values were significantly lower number for patients who had serum 25(OH)D levels >50 nmol/l in group 1 compared with group 2 (P<0·01).
*** Median value was significantly different from that of group 2 (P<0·001).
† Group 1 received only one dose of 1500 μg vitamin D_3 in the second trimester.
‡ Group 2 received two doses of 3000 μg vitamin D_3 each in the second and third trimesters.
§ The usual-care group did not receive any supplementation of vitamin D during pregnancy.
‖ P value for multiple group comparisons by the Kruskal–Wallis test; for variables showing significance, *post hoc* comparison between pairs of groups by the Mann–Whitney U test.

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Table 4. Anthropometric characteristics of infants at birth, 3, 6 and 9 months (Mean values and standard deviations)

	Group 1*		Group 2†		Usual-care group‡		
Variables	Mean	SD	Mean	SD	Mean	SD	*P*§
At birth	*n* 36		*n* 35		*n* 38		
OFC (cm)	34·5***	0·9	34·4***	0·6	33·6	0·8	0·000
AF (cm)	2·6***	0·4	2·5***	0·5	3·3	0·5	0·000
Length (cm)	50·3***	0·9	50·1***	0·9	49·4	2·4	0·000
Weight (g)	3·08**	4·06	3·03**	3·85	2·77	3·99	0·003
At 3 months	*n* 31		*n* 33		*n* 35		
OFC (cm)	40·2***	0·6	40·0***	0·7	39·2	0·6	0·000
AF (cm)	1·99***	0·5	1·99***	0·4	2·7	0·5	0·000
Length (cm)	59·8**	1·7	59·9***	1·2	58·6	1·2	0·000
Weight (kg)	5·9***	0·2	5·9***	0·2	5·7	0·2	0·000
At 6 months	*n* 28		*n* 24		*n* 30		
OFC (cm)	41·6***	0·7	41·6***	0·5	40·8	0·7	0·000
AF (cm)	1·4***	0·5	1·4***	0·4	2·0	0·6	0·000
Length (cm)	64·3***	1·9	64·9***	2·1	62·4	1·6	0·000
Weight (kg)	7·2**	0·4	7·3***	0·4	6·8	0·3	0·000
At 9 months	*n* 22		*n* 18		*n* 22		
OFC (cm)	42·9**	0·7	42·4**	2·6	41·8	2·2	0·012
AF (cm)	0·9***	0·4	0·9***	0·3	1·5	0·5	0·000
Length (cm)	69·3**	1·9	69·9***	1·4	67·4	1·7	0·000
Weight (kg)	8·4***	0·6	8·5***	0·5	7·7	0·4	0·000
Difference between birth and 9 months							
OFC (cm)	8·7	1·3	8·8	0·8	8·7	1·2	0·99
AF (cm)	1·7	0·5	1·9	0·3	1·9	0·5	0·27
Length (cm)	19·3**	1·8	20·2**	1·8	18·0	3·3	0·006
Weight (kg)	5·4**	0·7	5·5**	0·7	4·9	0·5	0·003

OFC, occipitofrontal circumference; AF, anterior fontanelle.
Mean values were significantly different from those of the usual-care group: ** $P<0·01$, *** $P<0·001$.
* Group 1 received only one dose of 1500 μg vitamin D_3 in the second trimester.
† Group 2 received two doses of 3000 μg vitamin D_3 each in the second and third trimesters.
‡ The usual-care group did not receive any supplementation of vitamin D during pregnancy.
§ *P* value for multiple group comparisons by the Kruskal–Wallis test; for variables showing significance, *post hoc* comparison between pairs of groups by the Mann–Whitney test.

Furthermore, Brooke *et al.*[14] also reported significantly greater anterior fontanelle size in the control group.

These findings were corroborated and extended by Marya *et al.*[9] from India, who found significantly higher birth weight, length, head and mid-arm circumference, and subscapular and triceps skin folds in the offspring of 100 pregnant women supplemented with two doses of 15 000 μg vitamin D in the third trimester, in comparison with 100 placebo-treated women. In another study by the same authors, supplementation with the aforementioned dose led to a significant improvement in serum Ca, P and ALP in maternal as well as cord sera[16]. Birth weight was also significantly greater and proved more efficacious than a daily dose of 30 μg in the third trimester, which only had a beneficial effect on birth weight but not on maternal or fetal biochemistry. Vitamin D supplementation during pregnancy may have a direct anabolic effect. Alternatively, it may have a more permanent effect through altered fetal programming. Evidence for the latter mechanism has been reported by Javaid *et al.*[20], who found childhood bone size and (total body and lumbar spine) bone mass at 9 years of age to be strongly correlated with maternal serum 25(OH)D at 34 weeks of gestation, as well as with umbilical vein Ca. The correlation for bone mass persisted when adjusted for bone size.

Studies in the non-pregnant state as well as in lactating women have suggested that doses below 50 μg/d are insufficient to maintain serum concentrations of 25(OH)D in the normal range[21–24]. For stoss therapy, doses ranging from 2000 μg, once in pregnancy, to 15 000 μg, twice in the last trimester, have been used[9,10]. Serum 25(OH)D after a single large oral dose of vitamin D has been shown in the non-pregnant state to peak at 1–2 weeks after supplementation and then to start declining[25–27]. Adequate blood levels are not expected to persist beyond 2·5–3 months after a single large oral dose[25,26]. This may be the explanation of the present findings for the lack of a significant difference in CB 25(OH)D between supplemented women and the controls. Further studies may be needed after supplementing pregnant women with cholecalciferol and monitoring serum 25(OH)D concentrations every month. Nevertheless, however, it is noteworthy that both dose regimens had a long-lasting effect on fetal and neonatal anthropometry, as well as on keeping CB ALP significantly lower than in the usual-care group. It is possible that only a modest amount of vitamin D may be needed

during a critical period of pregnancy to bring about some of the effects on the developing fetus.

We chose to supplement from the second trimester for two reasons. Although the third trimester is characterised by the maximum transplacental transfer of Ca, some transfer has been shown to start in the second trimester itself[8]. Furthermore, in our previous studies, we have shown that women were vitamin D deficient, with 34–42% having severe deficiency (serum 25(OH)D < 25 nmol/l)[1,2]. Studies on the rate of increase in serum 25(OH)D after prolonged administration of smaller daily doses have suggested that achievement of steady-state serum levels occurs only after 3 months[21]. Therefore, it is likely that small daily doses may not be suitable for a population that is already moderately to severely deficient.

The present study has limitations. Only ninty-seven of the 299 registered women returned for follow-up. The reason for loss to follow-up was, first, many women returned only for antenatal check-ups, were staying far off from our hospital and preferred to deliver in nearby hospitals. Second, the control group was not a result of the randomisation process, but was a group of women attending the same hospital, whom we could not recruit early enough to give the second trimester medication. Although there was no statistical difference between the groups in a number of biological and biochemical variables, either at registration or at delivery, this is an important limitation to the interpretation of the present results, especially since there was an unexpected trend towards higher median maternal serum 25(OH)D concentration in the usual-care group compared with group 1. Third, we were unable to test for maternal hypercalcaemia due to logistical constraints.

Thus, we conclude that both 1500 μg vitamin D_3 once in the mid-trimester and 3000 μg vitamin D_3 in the mid- and third trimesters improved neonatal anthropometry. The latter dose also decreased neonatal biochemical rickets and partially improved maternal 25(OH)D at term. Further studies are required to determine a minimum effective dose for achieving maternal 25(OH)D > 50 nmol/l while also addressing toxicity concerns.

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