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ORIGINAL ARTICLE

Vitamin D in newborns. A randomised controlled trial comparing daily and single oral bolus vitamin D in infants

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Aim: There are no published data to demonstrate the efficacy of bolus dose vitamin D in newborn infants. The study sought to evaluate this alternative approach of supplementation.

Methods: This single centre, open randomised controlled trial was conducted from August 2013 to May 2014. It compared the efficacy and safety of daily (400 IU) versus a bolus dose (50 000 IU) of cholecalciferol in newborn infants of vitamin D deficient mothers. The primary outcome measure was the rate of 25 hydroxyvitamin D (25OHD) repletion-defined as 25OHD greater than 50 nmol/L. The secondary objective was determining safety using adjusted total serum calcium.

Results: Of 70 eligible infants, 36 received a daily dose and 34 received a single high-dose cholecalciferol. Mean 25OHD in the bolus group (154 nmol/L, 95% confidence interval (CI) 131–177) was higher than the daily group (48 nmol/L, 95% CI 42–54) at 1–2 weeks of age. This was reversed at 3–4 months, (65 nmol/L, 95% CI 59–71) compared with the daily group (81 nmol/L, 95% CI 77–85). More infants in the single bolus group achieved vitamin D repletion (100 vs. 31%) at 1–2 weeks. By 3–4 months, both groups achieved similar vitamin D repletion rates (91 vs. 89%). Mean adjusted total serum calcium in the bolus group were normal at 1–2 weeks (2.73 mmol/L) and 3–4 months (2.55 mmol/L).

Conclusion: Single bolus dosing of 50 000 IU cholecalciferol achieves higher 25OHD repletion rates at 1–2 weeks of age compared with daily dosing, but repletion rates were similar by 3–4 months. There was no hypercalcaemia documented with single bolus dosing in this study.

Key words: 25OHD; cholecalciferol; infant; newborn; vitamin D.

What is already known on this topic

1 Infants of vitamin D deficient mothers are also vitamin D deficient from birth.
2 Daily vitamin D supplementation is universally recommended for at risk newborn infants in the first year of life.
3 Daily supplementation is associated with high rates of non-adherence.

What this paper adds

1 A single bolus dose (50 000 IU) cholecalciferol, achieves better repletion rates earlier but is of similar efficacy by 4 months of age.
2 A single bolus dose (50 000 IU) of cholecalciferol is another practical alternative for infants aged less than 4 months.
3 This raises the possibility of administering ongoing three to four monthly bolus doses throughout the first 12 months of life in exclusively breastfed babies.

Vitamin D deficiency can lead to hypocalcaemic seizures, rickets and gross motor delay during infancy and early childhood.[1–4] Rickets is one of the most common non-communicable diseases in children in the developing world, and has been on the rise in industrialised countries.[5] Associations between vitamin D deficiency, the development of Type I diabetes, multiple sclerosis, the onset of childhood allergies and respiratory tract infections have also been proposed.[6,7]

As fetal vitamin D stores are entirely dependent on maternal vitamin D status, newborns of vitamin D deficient mothers are at risk. There are reports that up to 25% of 'low risk' pregnant women are vitamin D deficient (<50 nmol/l). One in 13 of these women had 25 hydroxyvitamin D (25OHD) <25 nmol/L, which are likely to pose significant risks to their newborn infants.[8] Maternal vitamin D deficiency during pregnancy is associated with impaired lung development and neuro-cognitive difficulties in children.[9] An additional risk factor for vitamin D deficiency in infants is exclusive prolonged breastfeeding. Human milk is a relatively poor source of vitamin D which even from replete women has a vitamin D concentration of only 25 IU/L.[10] Many

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commercial infant formulae contain up to 400 IU vitamin D per litre, which provides the recommended daily intake if an infant were to consume 1000 mL/day.[11,12]

It is recommended that exclusively breastfed infants with at least one other risk factor for low vitamin D should be supplemented with 400 IU daily for at least the first year of life.[12,13] However, non-adherence with daily supplementation is as high as 45%.[14] Consequently, single bolus dosing has been used as an alternative treatment regimen. Whilst this approach has shown to be safe and effective in treating vitamin D deficiency in older children and adolescents,[15,16] it has not been studied in infants less than 6 months of age. The aim of this study was to compare the efficacy and safety of the single bolus dose of vitamin D with the standard of care, daily vitamin D 400 IU in newborn infants.

Methods

Trial design

We conducted a single centre, open-label randomised clinical trial of 70 healthy term infants who were assigned at birth to receive a daily dose of 400 IU or a single dose of 50 000 IU of cholecalciferol. The 50 000 IU dose was calculated as the equivalent of 400 IU multiplied by 120 days (4-month period). The study was undertaken during August 2013 to May 2014 at Sunshine Hospital, St Albans, an outer metropolitan health service located at a latitude of 38°S within Greater Melbourne, Australia.

Study oversight

Ethics approval (HREC 33044B) was obtained from Human Research and Ethics Committees at the Royal Children's Hospital, Melbourne. In cases where prior written consent could not be obtained, provision was made for verbal consent from the parent/guardian followed by delayed written consent. The study was registered with the Australia and New Zealand Clinical Trials Registry in 2013 (ACTRN12613001234707). The full trial protocol can be accessed from the Western Health Centre for Research and Education, Sunshine Hospital, St Albans, Australia. Serious adverse outcomes were reported to the ethics committee at the Royal Children's Hospital and the Western Centre for Health Research and Education.

Population

Pregnant women with vitamin D deficiency were identified using centralised hospital electronic records (Birthing Outcomes Systems) (Andrew Hinterreiter Management Consultant & Technology Services Pty Ltd (MCATS) 1988). Women who had 25OHD concentrations less than 50 nmol/L were screened at their first antenatal visit (8–12 weeks gestation). One month into the study, due to the small numbers of women identified, a study amendment was made to the protocol to increase the 25OHD threshold to less than 75 nmol/L. There is currently no clear consensus for the standard definition of vitamin D deficiency in pregnancy. Current consensus recommendations from the Royal Australian and New Zealand College of Obstetrics and Gynaecology are to provide additional vitamin D supplementation where 25OHD levels in pregnancy are less than 50nmol/L. Positively screened women who were subsequently found to be vitamin D deficient (25OHD less than 50 nmol/L) at more than 34 weeks gestation and who had the intention to exclusively breastfeed were recruited. Women who did not have a documented initial vitamin D concentration but had 25OHD less than 50 nmol/L at more than 34 weeks gestation were also included. Infants were enrolled and randomised at birth if they met the following eligibility criteria: (i) born at 37–42 weeks gestation; (ii) singleton pregnancy and (iii) birthweight appropriate for gestational age according to standardised Centre for Disease Control growth charts.[17] Exclusion criteria were: (i) illicit drug use during pregnancy; (ii) infants requiring resuscitation for more than 10 min at birth; (iii) pre-existing maternal conditions such as type 1 and 2 diabetes mellitus, parathyroid disease, uncontrolled thyroid disease and systemic glucocorticoid/anti-inflammatory or cytotoxic use; (iv) major congenital anomalies and (v) subcutaneous fat necrosis in the newborn.

Randomisation

Randomisation (in random blocks of 2, 4 and 6) was undertaken in a blinded manner. Babies of eligible mothers were randomised at birth using a computer-generated schedule. The allocated treatment arm was kept inside opaque, sealed envelopes, which were numbered sequentially and opened, in numerical order by the study recruiters.

Study treatments

Standard care (the comparator) comprised 400 IU cholecalciferol given in the form of 0.45 mL Pentavite Infant (Bayer Consumer Care, Sydney, NSW, Australia) drops (multivitamin containing vitamin D) daily. The intervention comprised 50 000 IU cholecalciferol in the form of 0.5 mL pure vitamin D_3 powder (PCCA, Houston, TX, USA) dissolved in olive oil (100 000 IU/mL solution) (Advanced pharmaceuticals, West Perth, WA, Australia) made and dispensed by the hospital pharmacy. Both treatments were given orally, the first dose within 48 h of birth. Parents/carers were shown how to draw up and administer 0.45 mL Pentavite. All single doses of 50 000 IU were administered under supervision of midwifery staff, before discharge home.

Endpoints

The primary outcomes were the proportion of infants who were vitamin D replete (25OHD greater than 50 nmol/L). Secondary outcomes were hypercalcaemia, rates of craniotabes, widened epiphyses, rachitic rosary, limb deformities, widened anterior fontanelle and growth and self-reported adherence.

Study procedures

Cord blood was obtained at birth. Venous samples using hand tourniquet were taken from newborn infants. Serum samples underwent batched biochemical analysis at the completion of the last recruited patient. There were no interim analyses.

Information on mode of feeding, hospitalisations, vomiting, abdominal pain and growth parameters were collected using

standardised case report forms at the 1–2 week and 3–4 month follow-up.

Maternal 25OHD were measured by chemiluminescent immunoassay (LIAISON Diasorin 25OHD Assay, Stillwater, MN, USA). 25OHD concentrations in infants were measured by liquid chromatography/tandem mass spectrometry (LC-MS; Shimadzu Nexera UHPLC LC30AD, Canby, OR, USA) connected to AbSciex 5500 MS/MS Qtrap (Foster City, CA, USA). Functional sensitivity was 5 nmol/L. Interassay precision using this technique was 5.71% at 32 nmol/L and 2.61% at 102 nmol/L for 25OHD.

Total calcium and albumin were measured by routine laboratory methods on a Beckman Coulter UniCel DxC 800, Synchron Clinical System (Breakwater, CA, USA). Interassay precision for calcium was 3.86% at 2.05 mmol/L, and for albumin 1.07% at 27 g/L. Where 25OHD values were below the detection limit for the assay, the upper limit of the result was used for data analysis, for example, <5 nmol/L = 5 nmol/L. Albumin-adjusted serum calcium concentrations utilised the following formula: adjusted Ca = total Ca + 0.02(40 − albumin).

Statistical analysis

We calculated that a sample size of 56 would provide 90% power, with a two-sided alpha level of 0.05, to detect a 40% difference in the proportions of vitamin D repletion between the two groups. This is based on reports that 40% of infants prescribed a daily dose of vitamin D were poorly adherent.[18] We therefore assumed that they would have suboptimal vitamin D levels. The single bolus group would achieve repletion rates of 90% and the daily group 50%. Statistical analyses were undertaken by the trial statistician who was blinded to treatment allocation, using IBM SPSS Statistics v20 (SPSS Inc, Chicago, IL, USA). All predetermined analyses were performed according to intention to treat principle.

Results

Study patients

Of 276 women who were vitamin D insufficient (25OHD less than 75 nmol/L) on initial antenatal screen, 145 were considered eligible. The 72 who had 25OHD levels greater than 50 nmol/L at more than 34 weeks gestation were subsequently excluded. Fifty-nine women did not have vitamin D concentrations assayed at more than 34 weeks gestation. The remainder did not have the intention to breastfeed, met exclusion criteria, or were 'missed' opportunities and were not recruited at birth (Fig. 1). Seventy women of various ethnicities (see Table S1, Supporting Information) were recruited at birth. Thirteen of these women did not have documented 25OHD concentrations at the first antenatal visit but had vitamin D deficiency at greater than 34 weeks gestation. Thirty-six babies were randomised to the daily dosing and 34 to the single bolus dose.

Vitamin D

Maternal 25OHD at more than 34 weeks gestation were in the mild to severely deficient range (<12.5–49 nmol/L). Cord 25OHD were similar to maternal concentrations (daily group 32 nmol/L vs. single bolus group 33 nmol/L) (Table 1).

At 1–2 weeks, mean 25OHD concentrations were higher (154 nmol/L, 95% confidence interval (CI) 131–177) in the single bolus compared with the daily group (48 nmol/L, 95% CI 42–54) ($P < 0.001$) (Fig. 2). The reverse was seen at 3–4 months, where mean 25OHD were lower in the bolus (65 nmol/L, 95% CI 59–71) compared with the daily group (81 nmol/L, 95% CI 77–85) ($P = 0.008$). At 1–2 weeks, all (100%) newborn infants receiving single high bolus dose were vitamin D replete compared with (31%) in the daily group (relative risk (RR) 2.8, 95% CI 1.7–4.6, $P < 0.001$). Similar repletion rates were achieved in both groups by 4 months (daily 91% vs. bolus 89%) (RR 0.97, 95% CI 0.80–1.2, $P = 0.78$) (Table 2).

Two newborn infants in the bolus group had elevated 25OHD greater than 250 nmol/L (277 and 310 nmol/L) at 1–2 weeks. No infant had 25OHD greater than 250 nmol/L at 3–4 months.

Adjusted calcium

Adjusted serum calcium concentrations at 1–2 weeks was statistically different between groups. The bolus group achieved lower mean adjusted serum calcium (2.73 mmol/L) compared with the daily group (2.81 mmol/L) ($P = 0.005$) (Table 2). The highest adjusted serum calcium in the daily group was 3.06 mmol/L compared with 2.93 mmol/L in the bolus group.

At 3–4 months, mean adjusted serum calcium concentrations were similar between the groups (daily 2.58 mmol/L vs. bolus 2.55 mmol/L) ($P = 0.24$). Adjusted serum calcium in the bolus group ranged from 2.41 to 2.70 and 2.49 to 2.84 in the daily group. All serum calcium concentrations measured were within the normal age-appropriate reference interval.

Clinical parameters

At birth, three babies in the daily group and four babies in the bolus group had clinical signs of vitamin D deficiency; craniotabes and widened anterior fontanelle ($P = 0.70$). By 3–4 months, craniotabes had resolved in all babies. One in each group had widened anterior fontanelle. No babies were identified with rachitic rosary, widened epiphyses or limb deformities.

Only three newborns (one daily, two bolus) at 1–2 weeks, and four newborn infants (one daily, three bolus) at 3–4 months reported infrequent vomiting. There were three adverse events, presumed to be unrelated, during the course of the study. Two infants required phototherapy for neonatal jaundice. Another was admitted on three occasions during the first month of life for the investigation and management of presumed sepsis, laryngospasm and poor feeding.

Adherence

Reported adherence at the 3–4 month follow-up visit was poor with only eight (31%) babies reporting daily adherence. Seven (27%) reported missing 'days', five (19%) reported missing 'weeks' and five (15%) reported missing months. Our accountability log of final reconciliation is not included as there was a poor rate of return (15/32) and reported spillage of Pentavite.

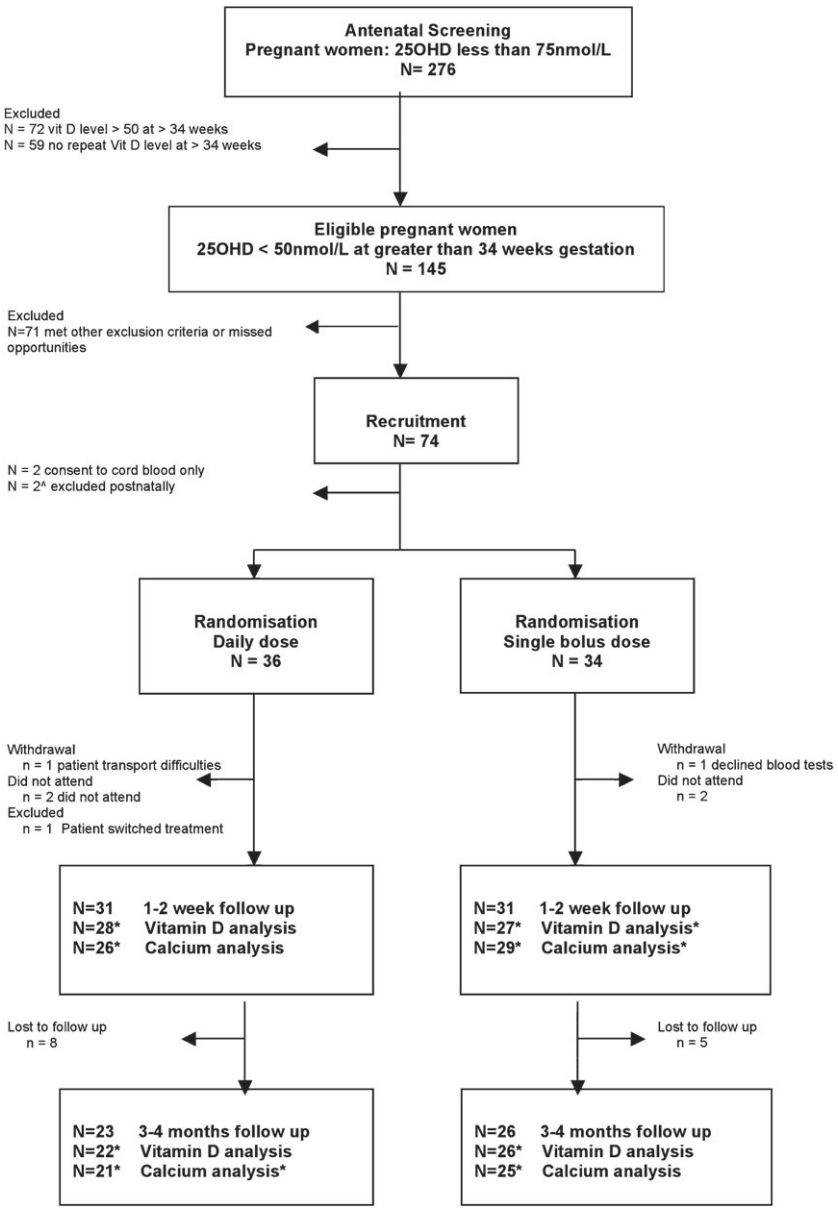

Fig. 1 Screening, randomisation and follow-up. *Insufficient serum sample; ^fetal arrhythmia and decision to formula feed from birth; lost to follow-up, uncontactable and unable to be traced; did not attend, maternal illness, other.

Mode of feeding

Exclusive breastfeeding rates in both groups dropped significantly by 3–4 months. Seven (29%) in the daily group and 12 (44%) in the bolus group were exclusively breastfed. The rates of mixed feeding were 12 (46%) in the daily group and 6 (22%) in the bolus group. The rates of exclusive formula feeding were 5 (19%) in the daily group and 8 (29%) in the bolus group. There were no significant differences between the modes of feeding ($P = 0.139$).

Discussion

To our knowledge, this is the first randomised controlled trial to compare the alternative treatment regimen (50 000 IU) to the standard daily dose in newborn infants. A single bolus dose achieved higher 25OHD at 1–2 weeks, but the reverse was seen at 3–4 months of age. Vitamin D repletion rates were higher by 65% in the single bolus group. Infants in this group were three times more likely to be vitamin D replete by 2 weeks. By 4 months, repletion rates were in excess of 88% in both groups. Two newborn infants who received single oral bolus dosing had elevated 25OHD greater than 250 nmol/L at 1–2 weeks but none had vitamin D toxicity (defined as greater than 350 nmol/L). We propose that this may reflect the metabolic pathway of vitamin D inactivation. Following production in the skin from ultraviolet B light, or oral ingestion, vitamin D undergoes a series of hydroxylation processes, to form 25OHD (the form measured in vitamin D assays to assess vitamin D status) and then to the biologically active form of $1,25(OH)_2D$. Both 25OHD and $1,25(OH)_2D$ are inactivated by 24 hydroxylase to form $24,25(OH)_2D$ and $1,24,25(OH)_3D$, respectively. Our hypothesis is that when this system is

Table 1 Maternal and neonatal baseline characteristics†

Maternal	Daily vitamin D (*n* = 36)	Bolus vitamin D (*n* = 34)
Age (years), mean (SD)	27 (4.2)	27 (5.3)
Median (IQR)	27 (5.4)	27 (6.3)
BMI, mean (SD)	28 (7.4)	30 (8.2)
Smoking status, *n* (%)		
Yes	3 (8.6)	6 (17.6)
No	32 (91.4)	28 (82.3)
Skin pigment, *n* (%)		
Light-olive	18 (50)	17 (50)
Dark	18 (50)	17 (50)
Veiled, *n* (%)	2 (5)	2 (6)
First 25OHD (nmol/L), mean (SD)	41 (16.4)	37 (14.9)
Median (IQR)	43 (23)	38 (23)
Gestation (weeks), mean (SD)	24 (5.5)	25 (5.0)
Median (IQR)	26 (7.6)	26 (4.3)
Vitamin D supplementation, *n* (%)		
Yes	33 (91.7)	29 (85.3)
No	1 (2.8)	3 (8.8)
Unknown	2 (5.6)	2 (5.9)
Second 25OHD (nmol/L), mean (SD)	34 (11.9)	29 (14.5)
Median (IQR)	35 (20)	31 (27)
Gestation (wks), mean (SD)	36 (1.4)	36 (1.5)
Median (IQR)	36 (1.15)	36 (1.15)
Newborn infant		
Gestation at birth (weeks)		
Mean (SD)	39 (1.1)	39 (1.2)
Median (IQR)	40 (1.2)	39 (1.3)
Growth parameters, mean (SD)		
Weight (g)	3541 (392)	3468 (536)
HC (cm)	35 (1.4)	34 (1.9)
Length (cm)	51 (1.6)	50 (2.3)
Mode of delivery, *n* (%)		
Normal vaginal	23 (63.9)	21 (61.8)
Instrumental	6 (16.7)	4 (11.8)
Caesarean section	7 (19.4)	9 (26.5)
Apgar scores, median (IQR)		
1 min	9 (1)	9 (1)
5 min	9 (0)	9 (0)
Resuscitation, *n* (%)		
Nil	33 (91.7)	28 (82.4)
<10 min	3 (8.3)	6 (17.6)
Cord blood (*n*)	33	29
Vitamin D_3 (nmol/L), mean (SD)	32 (13.6)	33 (19.3)
Vitamin D_2 (nmol/L), mean (SD)	1.1 (0.8)	0.6 (0.6)
Corrected Ca (mmol/L), mean (SD)	2.72 (0.1)	2.76 (0.2)

†There were no statistically significant ($P < 0.05$) results. BMI, body mass index; Ca, calcium; HC, head circumference; IQR, interquartile range; SD, standard deviation.

exposed to a high dose of vitamin D, metabolism to its inactive form is upregulated. This may be an explanation for the substantial decline in 25OHD seen from 1–2 weeks to 4 months with bolus dosing. Confirmation of this would require quantifying 1,25OHD and 24,25OHD via mass spectrometry, which was not practically feasible for this study.

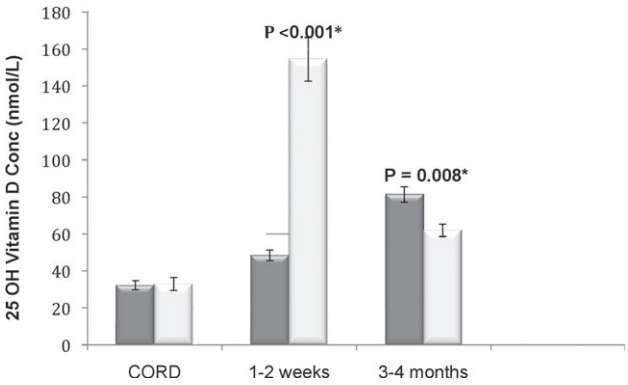

Fig. 2 Vitamin D concentration at different time points. Error bars represent 95% confidence intervals for the standard error of the mean. (■), Daily; (■), bolus.

There was no evidence of clinically significant hypercalcaemia in the single bolus group at either time point. More importantly, despite half to one-third of newborn infants going on to have mixed or exclusive formula feeding (an additional source of vitamin D), none of them had hypercalcaemia at 3–4 months of age. At 1–2 weeks, adjusted serum calcium concentrations were statistically higher in the daily group, however, this was unlikely to be clinically significant as both were within the normal age-appropriate reference range. Seven newborn infants in the daily group, and two newborn infants in the bolus had elevated adjusted serum calcium concentrations of greater than 2.85 mmol/L at 1–2 weeks. Only one of these newborn infants (bolus group) had a 25OHD level greater than 250 nmol/L (319 nmol/L) coinciding with a hypercalcaemia (2.88 mmol/L). These are unlikely to be due to haemolysis as free-flowing samples were taken by venepuncture. It is well recognised that the first few weeks of life is a time of significant hormonal changes in calcium homeostasis as parathyroid hormone secretion increases and parathyroid hormone-related protein decreases.[19,20] This is likely to be the underlying cause of greater variation in adjusted serum calcium concentrations at this time. Whilst there are a few case reports of severe hypercalcaemia in grossly excessive (300 000 IU) vitamin D overdose,[21–23] cholecalciferol doses used in these studies far exceeded those in our study. Excessive daily dosing such as 1600 IU vitamin D given to breastfed infants of vitamin D deficient mothers[24] can also cause elevated 25OHD greater than 250 nmol/L.

This study was conducted in an ethnically diverse region in Melbourne, Australia, over winter 2013 to autumn 2014. Seasonal effects were not addressed as the study was only conducted between August and May. Regular sun exposure is not recommended in infants due to long-term risk of developing skin malignancies[12] and is typically minimal in this cohort. We did not document sun exposure. We consider that this is not a significant confounding factor for vitamin D status in this age group. This is a small study with a moderate drop out rate (~25%). Due to funding limitations, we were unable to recruit larger numbers to compensate for the moderate drop out. In a substantial proportion of infants up to 1 year of age, 25OHD can exist as C_3 epimer. We acknowledge recommendations that the levels of epimer

Table 2 Vitamin D repletion status and corrected calcium

	Daily vitamin D (n = 31)	Bolus vitamin D (n = 31)	P-value
1–2 weeks			
Age (days), mean (SD)	8 (1.3)	8 (1.8)	0.72
Weight (g), mean (SD)	3510 (363)	3497 (548)	0.79
HC (cm), mean (SD)	35.7 (1.4)	35.5 (1.6)	0.67
Vitamin D status, n (%) replete (>50 nmol/L)	10/28 (35.7)	27/27 (100)	<0.001*
Corrected calcium (mmol/L), mean (SD)	2.81 (0.1)	2.73 (0.07)	0.005*
3–4 months (n)	23	26	
Age (months), mean (SD)	3.8 (0.4)	3.9 (0.3)	0.39
Weight (g), mean (SD)	6963 (732)	6534 (905)	0.08
HC (cm), mean (SD)	41.2 (1.4)	41.3 (1.6)	0.91
Length (cm), mean (SD)	63.8 (2.2)	61.7 (2.6)	0.64
Vitamin D status, n (%) replete (>50 nmol/L)	20/22 (90.9)	23/26 (88.5)	0.78
Corrected calcium (mmol/L), mean (SD)	2.58 (0.08)	2.56 (0.07)	0.36

*P < 0.05. HC, head circumference; SD, standard deviation.

should be determined to allow for differentiated assessment of vitamin D status, however, our mass spectrometry techniques were not available for epimer assays at the time of the study. Adherence rates with daily dosing were lower (33%) than that previously reported in Swiss infants.[18] Reliance on parents/carers giving accurate doses of small volumes (0.45 mL Pentavite), and supervision of the single bolus dose given in hospital could be potential factors in poor adherence and influence the outcome. However, the marked difference in 25OHD concentrations at 1–2 weeks is unlikely to be explained completely by these factors. Most poorly adherent infants were on mixed formula/breastfeeding regimens (9/12) and consequently received some vitamin D in their formula feeds. The Vitamin D supplement is unhydroxylated, so is not measured in the assay used. Mixed or formula feeding and the effect of possibly taking some Vitamin D supplements several days prior to the 3–4 month blood test may explain why daily vitamin D levels were increasing in the daily group despite a 33% non-adherence rate.

The strength of this study is the biochemical analysis of vitamin D using tandem mass spectrometry. Previous studies have shown marked variability in serum 25OHD measurements between laboratories.[25–27] Assay variation confounds the diagnosis of hypovitaminosis D. This has led to the adoption of tandem mass spectrometry, as the 'gold standard' for measuring serum 25OHD.[28] Previous studies using vitamin D radioimmunoassay or chemiluminescent assay reported cord 25OHD concentrations at 65% of maternal levels,[29] however, cord 25OHD concentrations measured by liquid chromatography - mass spectrometry (LC-MS) in this study were remarkably comparable with maternal vitamin D, measured by chemiluminescent immunoassay.

Conclusion

This study shows that a single bolus dose of cholecalciferol is a safe and effective alternative to daily vitamin D dosing for healthy breastfed newborn infants of vitamin D deficient mothers. Larger, multicentered randomised clinical trials are required to support our findings, however, this study raises the question of whether ongoing four monthly single bolus dosing of cholecalciferol could be used for up to 12 months of age, as a vitamin D supplementation regimen in exclusively breastfed infants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Ethnicity of pregnant women
Table S2. Data for figure: 25 hydroxy vitamin D concentrations at different time points