Randomised controlled trial analysing supplementation with 250 versus 500 units of vitamin D3, sun exposure and surrounding factors in breastfed infants

Aris Siafarikas,1–5 Helmut Piazena,6 Uwe Feister,7 Max K Bulsara,5,8 Hans Meffert,9 Volker Hesse1,2

ABSTRACT

Background The rate of non-compliance with vitamin D supplementation is as high as 45%. This is why randomised controlled trials are needed to analyse the response to low doses of vitamin D3.

Objective (1) To compare supplementation with 250 versus 500 units of vitamin D3 and (2) to analyse sun exposure time/ultraviolet B (UVB) exposure during the first 6 weeks of life.

Design 40 breastfed infants (skin photo-types I, II) were recruited in Berlin, Germany (52.5°N), during summer (n=20) and winter (n=20) and randomised into equal groups on either 250 or 500 units of vitamin D3 per day. Outcome measures were: parameters of vitamin D and bone metabolism at delivery and 6 weeks later, sun exposure time, UVB dosimetry and surrounding factors including maternal diet.

Results At delivery 25-hydroxy vitamin D levels were insufficient: 68 (53–83) nmol/l in each group. 6 weeks later levels were sufficient: 139 (114–164) nmol/l on 250 units of vitamin D3 per day and 151 (126–176) nmol/l on 500 units/day. There was no seasonal variation. Daily sun exposure time was 0.4–3.5 h and higher in summer. UVB exposure was 0.01–0.08 minimal erythema dose/day. Calcium levels were within normal.

Conclusions In Berlin, Germany, supplementation with 250 units of vitamin D3 is sufficient for breastfed infants during their first 6 weeks of life in summer and winter. UVB exposure is very low throughout the year.

INTRODUCTION

Humans derive vitamin D by cutaneous synthesis under the influence of sunlight and dietary intake.1 Vitamin D regulates intestinal calcium absorption. It has not only ‘calcitropic’ functions on bone metabolism but also ‘non-calcitropic’ functions on immune mechanisms and cell proliferation. Worldwide the incidence of vitamin D deficiency is rising. This might contribute to an increased prevalence of osteomalacia and rickets as well as increased susceptibility to infectious diseases, and represent a risk factor for the later development of autoimmune disease such as type I diabetes mellitus or multiple sclerosis and some forms of cancer.1–3 Depending on countries and age recommendations for the daily intake range from 200 to 800 units of vitamin D3 with sufficient calcium intake.4–6

In countries lying at high latitude both north and south of the equator skin-derived vitamin D production is insufficient particularly during winter months and vitamin D supplementation is needed.7–9 Newborns and infants are a group at significant risk of vitamin D insufficiency and deficiency.1 4 5 Randomised controlled trials are needed to investigate their response to low doses of vitamin D supplementation and measure the amount of daily sun exposure.5 10

Therefore, we included breastfed infants and their mothers in a study during the first 6 weeks after delivery during summer and winter months in Berlin, Germany, to (1) compare the efficacy of 250 versus 500 units of vitamin D3, (2) quantify sun exposure and (3) analyse surrounding factors including maternal diet.

METHODS

Subjects

Forty infants were recruited after delivery at the Hospital Berlin-Lichtenberg, Germany. Exclusion criteria were vitamin D supplementation during pregnancy, drug abuse, premature delivery and highly pigmented skin (photo-types III and IV according to Fitzpatrick and Bologna11). Infants had to be breast fed. The study was approved by the Ethics Committee of the Charité University.
Hospital, Humboldt University, Berlin, Germany. Written informed consent was obtained from mothers/guardians for every participant.

**Study design**

The study was designed as a prospective randomised controlled trial and registered with the Australian New Zealand Clinical Trials Registry (ANZCTR: ACTRN12609000919213) and WHO (WHO: U1111-1112-2443). Subjects were recruited during an autumn/winter (October until March, n=20) and subsequent spring/summer period (April until September, n=20). Using odd and even numbers taken from opaque envelopes participants were randomised into two subgroups (n=20) on either 250 or 500 units of vitamin D3 as a daily supplement (figure 1). Vitamin D3 was prescribed in tablet form (Vigantoletten 500 IE; Merck Pharma, Darmstadt, Germany). Families received detailed instructions on how to dissolve either one (500 IU) or half a tablet (250 IU) in a spoon and administer the tablet to their child. Each infant was assessed at two time points: at discharge from hospital on day 4 or day 5 after delivery and 6 weeks later.

The outcome measures in this study are described below.

**Clinical signs of rickets**

Subjects were examined for clinical signs of rickets: craniotubes, widened epiphyses, rachitic rosary and deformities of their extremities. Length, bodyweight and head circumference were measured using standardised calibrated equipment.

**Bone metabolism**

Blood was taken from the cubital vein and samples were immediately protected from light. 25-Hydroxy vitamin D (25(OH)D) was analysed using a radioimmunoassay (Biosource, Brussels, Belgium). The assay was designed to measure vitamin D3. The cross reactivity with vitamin D2 was 0.6%. Intra- and inter-assay coefficients of variation were 5.2% and 7.5%, respectively. The laboratory participated in nationwide interlaboratory trials for quality control on a regular basis. We considered 25(OH) D levels below 27.5 nmol/l (11 ng/ml) as vitamin D deficiency, levels between 27.5 and 78 nmol/l (11–31 ng/ml) as vitamin D insufficiency and levels higher than 78 nmol/l (31 ng/ml) as normal. Albumin, alkaline phosphatase, calcium, phosphorus and creatinine were assessed using standard assays. Alkaline phosphatase was measured in microkat/l. Normal values were 3.9–8.7 until day 10 of life and 5.5–12.5 until 6 months of life. The conversion factor into U/l was 60. Urine was collected.

---

**Figure 1** Study flowchart.
from early morning spot urine samples. Calcium, phosphorus and creatinine were analysed using standard methods.

Sun exposure
The infants’ ultraviolet B (UVB) exposure was continuously quantified for 6 weeks after delivery. Dosimeters (VioSpor, Blue Line type III; BioSense, Bornheim, Germany) consisted of a biological UV-sensitive film, a special filter-optic system and the protective dosimeter casing. The highly sensitive DNA molecules of immobilised spores of Bacillus subtilis produce a responsivity profile which corresponds to that of human skin. As the film incorporates a measurement and a calibration zone, the biologically effective dose of each film is determined using a calibration curve. After irradiation the spore film is incubated in a growth medium, and the proteins synthesised during spore germination are stained and evaluated by photometry. Films used in this study integrated the UV radiation effect of UVB range (290–320 nm). The amount of exposure was measured in units of the standardised ‘minimal erythema dose’ (1 MED=250 J/m²). One MED equals the mean amount of UV radiation that causes first degree (‘minimal’) erythema in unadapted human skin of photo-type II. A clip was provided to attach the dosimeter to clothes. The optimal position of the badges on infant’s clothing was attained if both the optical window area of the badge and the infant’s face were parallel. This method was validated under extreme climatic conditions in various groups including children. Meteorological data for the study period including total UV irradiation were provided by the German Meteorological Service. To complete the analysis, questionnaires were handed out covering surrounding factors that influence sun exposure on a daily basis like sunshine or exposures too early in the morning or too late in the afternoon.

Sun exposure and diet
The reported average time of daily sun exposure and UVB exposure as per dosimeter readings was not different between the groups on 250 and 500 units/day (p=0.47 and 0.37, respectively, table 1). The sunshine exposure score was higher in the group on 250 units of vitamin D3 per day as compared with the group on 500 units/day (p=0.048, table 1). Absolute values of UVB exposure were low, ranging from 0.01 to 0.08 MED/day in all groups investigated (p=0.37, table 1).

All infants were breast fed for the time of the study. Analysis of food diaries revealed a balanced mixed diet for all mothers, which met and exceeded the recommended daily intake of 1000–1300 g calcium. Calcium intake was mainly from dairy products like milk, yoghurt and cheese. Mothers did not receive vitamin D or calcium supplements.

RESULTS

Physical examination and anthropometric measures
Physical examination did not reveal signs of rickets in any infant throughout the study period. Length and weight were age-appropriate (table 1).

Biochemistry
At the initial postnatal analysis most infants showed vitamin D insufficiency (table 1). Six weeks later their vitamin D status was significantly improved and within the normal range for all subjects (p<0.0001, table 1). There was no difference between groups (p=0.48).

Calcium and phosphate in serum and urine were within normal limits at all time points in every participant. Calcium levels increased between visits (p=0.0001) and were higher in subjects on 250 units of vitamin D3 per day (p=0.048). Alkaline phosphatase concentrations were within the normal range. However, values increased significantly after 6 weeks independently of dosage of supplementation and season (p<0.05, table 1). Urine calcium excretion and calcium creatinine ratios increased between visits. Phosphate excretion did not change significantly.

DISCUSSION
Knowing that non-compliance with vitamin D supplementation is common, the aim of this study was to analyse the response to low doses of vitamin D3. We demonstrated that the vitamin D status of breastfed infants in Berlin, Germany (52.5°N) could be improved and was sufficient on supplementation with both 250 and 500 units of vitamin D3. UV exposure as measured by personalised UVB dosimetry was too low to stimulate cutaneous vitamin D3 synthesis. Possible explanations were negligible amounts of UVB radiation in the solar spectrum during winter and protective measures including shielding of the infants from given sunshine or exposures too early in the morning or too late in the afternoon.

A recent Cochrane review showed a lack of controlled clinical trials of interventions for the prevention of nutritional rickets in term born children. The recommended minimum intake of vitamin D has not been analysed thoroughly. In our opinion this is important in order to comment on the effects of non-compliance.

After reviewing current recommendations, we chose to compare supplementation with 250 and 500 units/day. Using these doses side effects were not to be expected. According to Ala-Houhala et al20 21 and Holick4 400–600 units are needed for breastfed infants. The European Society for Pediatric Endocrinology, the American Academy of Pediatrics as well as the German, Swiss and Austrian Societies for Nutrition recommend 400 units/day.14 19 23 Pittard et al24 and Backström et al25 demonstrated that supplementation with 250 units of vitamin D3 per day was sufficient in neonates and infants. Most countries with temperate weather conditions do not recommend vitamin D supplementation. However, over the last decade there are several reports on an increased

Arch Dis Child 2011;96:91–95. doi:10.1136/adc.2009.178301 93

Arch Dis Child: first published as 10.1136/adc.2009.178301 on 22 September 2010. Downloaded from http://adc.bmj.com/ on April 8, 2022 by guest. Protected by copyright.
prevalence of vitamin D deficiency even under sunny conditions, demonstrating the need for a minimum amount of vitamin D supplementation. In this study all participants showed improvements in vitamin 25(OH)D levels. The group on 250 units of vitamin D3 achieved changes that were >12 nmol/l, which was previously only reported for supplementation with 400–500 IU of vitamin D3/day. Considering the minimal UVB exposure per day, a daily intake of 200 units of vitamin D was sufficient to demonstrate a significant change in vitamin D levels. Knowing that all infants had sufficient vitamin D supplies in intermediately pigmented breastfed infants (skin phototypes I, II) during both summer and winter. Consequently, these data have to be interpreted with caution. It can be assumed that 400 units of vitamin D3 per day can provide sufficient vitamin D supplies in intermediately pigmented breastfed infants (skin phototypes I, II) during both summer and winter.  

Phosphatase (mmol/l)  
Day 5 2.0 (1.9–2.2)  
6 weeks 2.1 (2.1–2.2)  
Creatinine (µmol/l)  
Day 5 38.4 (29.1–47.6)  
6 weeks 37.7 (31.6–43.8)  
U-calcium (mmol/l)  
Day 5 0.43 (0.1–0.75)  
6 weeks 1.54 (0.82–2.3)*  
U-phosphate (mmol/l)  
Day 5 4.9 (0.85–8.9)  
6 weeks 3.3 (0.3–6.8)  
U-creatinine  
Day 5 4621 (2673–6569)  
6 weeks 769 (337–1201)*  
Ca/creatinine ratio  
Day 5 159 (68–250)  
6 weeks 1823 (1353–4999)*  
Sun exposure/day (h)  
2.1 (1.6–2.6)  
Sun exposure score 2.1 (1.8–2.4)  
UVB exposure (MED/day) 0.05 (0.02–0.08)  

data are presented as mean and 95% CI. P values refer to the comparison of daily supplementation with 250 vs 500 units of vitamin D3.  

Table 1 Demographics, biochemical parameters and sun exposure  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Visit</th>
<th>Supplementation</th>
<th>p Value: 250 vs 500 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>Day 5</td>
<td>3763 (3492–3854)</td>
<td>3584 (3332–3835)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>4768 (4444–5092)*</td>
<td>4915 (3882–4509)*</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>Day 5</td>
<td>52 (51–53)</td>
<td>52 (51–53)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>58 (57–59)</td>
<td>56 (55–57)</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)</td>
<td>Day 5</td>
<td>68 (53–83)</td>
<td>68 (53–83)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>139 (114–164)*</td>
<td>151 (126–176)*</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>Day 5</td>
<td>320 (276–378)</td>
<td>348 (300–396)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>858 (720–996)*</td>
<td>888 (750–1026)*</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>Day 5</td>
<td>2.38 (2.29–2.47)</td>
<td>2.38 (2.29–2.47)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>2.66 (2.58–2.73)*</td>
<td>2.55 (2.47–2.62)*</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>Day 5</td>
<td>2.0 (1.9–2.2)</td>
<td>2.0 (1.9–2.2)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>2.2 (2.1–2.2)</td>
<td>2.1 (2.1–2.2)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>Day 5</td>
<td>38.4 (29.1–47.6)</td>
<td>38.3 (29.3–47.3)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>37.7 (31.6–43.8)</td>
<td>26.8 (19.9–32.2)*</td>
</tr>
<tr>
<td>U-calcium (mmol/l)</td>
<td>Day 5</td>
<td>0.43 (0.1–0.75)</td>
<td>0.55 (0.25–0.86)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>1.54 (0.82–2.3)*</td>
<td>1.45 (0.75–2.15)*</td>
</tr>
<tr>
<td>U-phosphate (mmol/l)</td>
<td>Day 5</td>
<td>4.9 (0.85–8.9)</td>
<td>2.83 (1.0–6.8)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>3.3 (0.3–6.8)</td>
<td>4.8 (1.4–8.2)</td>
</tr>
<tr>
<td>U-creatinine</td>
<td>Day 5</td>
<td>4621 (2673–6569)</td>
<td>5194 (3346–7042)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>769 (337–1201)*</td>
<td>1062 (646–1478)</td>
</tr>
<tr>
<td>Ca/creatinine ratio</td>
<td>Day 5</td>
<td>159 (68–250)</td>
<td>145 (59–231)</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td>1823 (1353–4999)*</td>
<td>3995 (838–6959)*</td>
</tr>
<tr>
<td>Sun exposure/day (h)</td>
<td>2.1 (1.6–2.6)</td>
<td>1.8 (1.3–2.4)</td>
<td></td>
</tr>
<tr>
<td>Sun exposure score</td>
<td>2.1 (1.8–2.4)</td>
<td>1.6 (1.3–1.9)</td>
<td></td>
</tr>
<tr>
<td>UVB exposure (MED/day)</td>
<td>0.05 (0.02–0.08)</td>
<td>0.03 (0.01–0.06)</td>
<td></td>
</tr>
</tbody>
</table>

*Represents a significant difference between time points (day 5 vs 6 weeks, p<0.05). MED, minimal erythema dose; UVB, ultraviolet B.

Calcium levels were within normal in all participants at all time points and increased significantly over 6 weeks time (table 1). All mothers had sufficient calcium intake. We assume that the superior response to supplementation with 250 units of vitamin D per day was influenced by calcium supplies from breast milk that might have been higher than in the group on 500 units of vitamin D3. This is in keeping with other studies reporting that calcium intake can influence vitamin D levels and vitamin D requirements needed to treat metabolic bone disease. Interestingly, maternal calcium intake has no direct impact on the calcium contents of breast milk. However, it may influence maternal skeletal calcium loss during lactation. There are some limitations to the presented study. The study did not include a control group without medication because the importance of vitamin D supplementation during the first 1.5 years of life in Germany could be demonstrated before. The period of observation could not be extended beyond 6 weeks to guarantee compliance with dosimetry. This was sufficient to demonstrate a significant change in vitamin D levels. Knowing that all infants had sufficient vitamin D supplies, the observed increase in alkaline phosphatase during the 6 weeks of observation can be explained by physiologically increased bone turnover. Physiologically decreased creatinine levels are observed soon after delivery. It appears that this was delayed in the group on 250 units of vitamin D3 per day. There is no rational explanation for this and levels are still within normal in both groups. However, this also affects calcium:creatinine ratios. Consequently, these data have to be interpreted with caution. We conclude that in Germany supplementation with 250 units of vitamin D can provide sufficient vitamin D supplies in intermediately pigmented breastfed infants (skin phototypes I, II) during both summer and winter. It can be assumed that 400 units of vitamin D3 per day as recommended by the European Society for Pediatric
Endocrinology and our group allow for sufficient vitamin D intake and occasional non-compliance.

Acknowledgements The study was generously supported by a grant from the Northern German Society of Paediatric and Adolescent Medicine. The authors thank all families for their participation and support.

Funding Northern German Society of Paediatric and Adolescent Medicine.

Competing interests None.

Contributors The authors like to thank Mr K Oehler for his advice and assistance with the biochemical analysis of samples throughout the study.

Ethics approval This study was conducted with the approval of the Ethics Committee, Charité University Hospital, Berlin, Germany.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES