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

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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.

What is already known on the topic

- Vitamin D deficiency is highly prevalent in countries lying at high latitude.
- Non-compliance with vitamin D supplementation is high.

What this study adds

- Supplementation with 250 units/day can be sufficient for breastfed newborns in Berlin, Germany, during summer and wintertime.
- UVB exposure during the first 6 weeks of life is minimal throughout the year and unlikely to support cutaneous vitamin D production.

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] Compliance with vitamin D supplementation is as low as 45%.[8 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 Bolognia[11]). Infants had to be breast fed. The study was approved by the Ethics Committee of the Charité University

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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).[12] 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: craniotabes, 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.[13] 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.[14]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

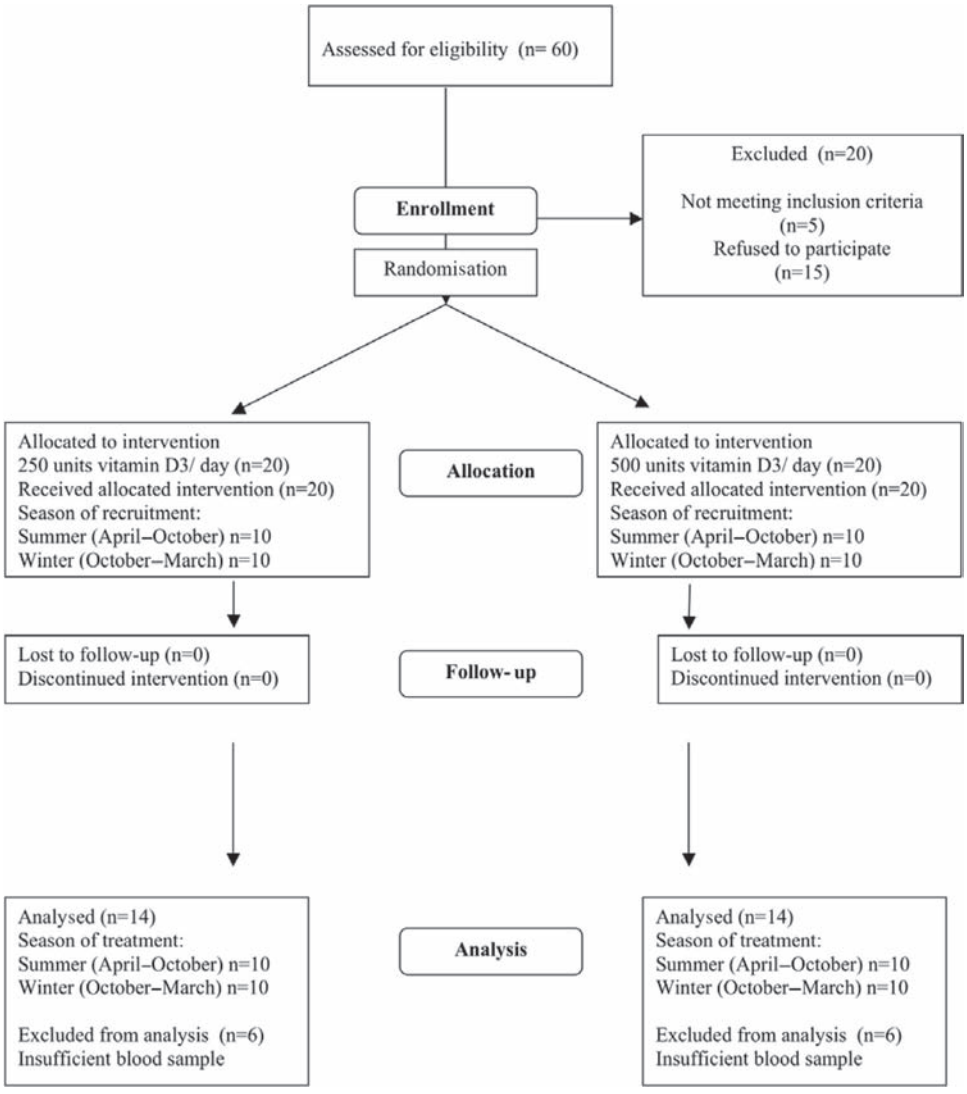

Figure 1 Study flowchart.

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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. 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.[15] Films used in this study integrated the UV radiation effect of the UVB range (290–320 nm). The amount of exposure was measured in units of the standardised 'minimal erythema dose' (1 MED=250 J/m^2). 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.[15–17] 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 sun protection, clothing or type of baby carrier. This information was transformed into a validated score system ('sunshine exposure score'[18]). Higher scores were equivalent to stronger sun exposure.

Nutrition

Mothers were asked to keep a food diary for every day of the study period in a semiopen form. We directly asked for number of breastfeeds, approximate amounts of dairy intake as represented by milk, cheese and yoghurt in grams. Mothers added meat, vegetable and protein intake in an open form. Dietary contents were analysed using a computer-based program of the German Society for Nutrition (DGE, please see online supplement).

Statistical analysis

Using 20 children in each group we were able to detect a change of at least 30 nmol/l in 25(OH)D levels. To demonstrate significance a sample size of 3.3 per group was needed to achieve a test power of 80% and a p value of 0.05.

Data were analysed using a generalised linear mixed model with repeated measures controlling for season (SPSS software, V.17). Significance was set at a level of $p<0.05$.

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.

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.[19] Calcium intake was mainly from dairy products like milk, yoghurt and cheese. Mothers did not receive vitamin D or calcium supplements.

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.[5] The required 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.[20 21] According to Ala-Houhala *et al*[22] and Holick[1] 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 al*[24] and Backström *et al*[25] 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

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Table 1 Demographics, biochemical parameters and sun exposure

Parameter	Visit	Supplementation 250 IU/day (n=14)	Supplementation 500 IU/day (n=14)	p Value: 250 vs 500 IU
Gestation (weeks)		39 (39–40)	39 (39–40)	0.63
Weight (g)	Day 5	3763 (3492–3854)	3584 (3332–3835)	0.32
	6 weeks	4768 (4444–5092)*	4195 (3882–4509)*	0.02
Length (cm)	Day 5	52 (51–53)	52 (51–53)	0.69
	6 weeks	58 (57–59)	56 (55–57)	0.02
25(OH)D (nmol/l)	Day 5	68 (53–83)	68 (53–83)	0.99
	6 weeks	139 (114–164)*	151 (126–176)*	0.48
Alkaline phosphatase (U/l)	Day 5	320 (276–378)	348 (300–396)	0.48
	6 weeks	858 (720–996)*	888 (750–1026)*	0.78
Calcium (mmol/l)	Day 5	2.38 (2.29–2.47)	2.38 (2.29–2.47)	0.99
	6 weeks	2.66 (2.58–2.73)*	2.55 (2.47–2.62)*	0.048
Phosphate (mmol/l)	Day 5	2.0 (1.9–2.2)	2.0 (1.9–2.2)	0.85
	6 weeks	2.2 (2.1–2.2)	2.1 (2.1–2.2)	0.33
Creatinine (μmol/l)	Day 5	38.4 (29.1–47.6)	38.3 (29.3–47.3)	0.1
	6 weeks	37.7 (31.6–43.8)	26.8 (19.9–32.2)*	0.01
U-calcium (mmol/l)	Day 5	0.43 (0.1–0.75)	0.55 (0.25–0.86)	0.6
	6 weeks	1.54 (0.82–2.3)*	1.45 (0.75–2.15)*	0.85
U-phosphate (mmol/l)	Day 5	4.9 (0.85–8.9)	2.83 (1.0–6.8)	0.46
	6 weeks	3.3 (0.3–6.8)	4.8 (1.4–8.2)	0.52
U-creatinine	Day 5	4621 (2673–6569)	5194 (3346–7042)	0.67
	6 weeks	769 (337–1201)*	1062 (646–1478)*	0.32
Ca/creatinine ratio	Day 5	159 (68–250)	145 (59–231)	0.82
	6 weeks	1823 (1353–4999)*	3898 (838–6959)*	0.34
Sun exposure/day (h)		2.1 (1.6–2.6)	1.8 (1.3–2.4)	0.47
Sun exposure score		2.1 (1.8–2.4)	1.6 (1.3–1.9)	0.048
UVB exposure (MED/day)		0.05 (0.02–0.08)	0.03 (0.01–0.06)	0.37

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

prevalence of vitamin D deficiency even under sunny conditions, demonstrating the need for a minimum amount of vitamin D supplementation.[26]

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.[10 27] Considering the minimal UVB exposure of participants any contribution by UV-stimulated cutaneous vitamin D3 synthesis is unlikely (table 1). In contrast to our findings Wolpowitz and Gilchrest[7] reported that in Boston, Massachusetts, USA (42.4°N), incorporating 5 min of sun exposure per day, a daily intake of 200 units of vitamin D was sufficient to prevent deficiency in June. Moreover, Holick[28] recommended a daily UV exposure of 25% of personal MED on face, hands and arms or on hands and legs to generate sufficient vitamin D3 concentrations. One MED of UV radiation has the potential to induce cutaneous production of 1200 units of vitamin D from each square metre of body surface area.[29] However, both doses and area of UV skin exposures have to be smaller to prevent acute and chronic skin damages such as sunburn and skin cancer.[1]

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.[6] 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.[25 30] Interestingly, maternal calcium intake has no direct impact on the calcium contents of breast milk.[31 32] However, it may influence maternal skeletal calcium loss during lactation.[33]

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.[19] 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.[34]

Physiologically decreased creatinine levels are observed soon after delivery.[35] 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 photo-types 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

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Endocrinology[4] and our group[36] allow for sufficient vitamin D intake and occasional non-compliance.

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Competing interests None.

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

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